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Research Article

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Antioxidant and Phytochemical Properties of Some Brands of Dental Formulations sold in Owerri, Imo State, Nigeria

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Abstract

Oral health has a significant impact on overall quality of life and wellbeing. Dental problems are mostly due to microbial infections. Three major types of dental problems are dental plagues, dental caries and periodontal diseases. The tooth is a very sensitive and important part of the human body. It performs very important function in the overall wellbeing of an individual. Dental problems are on the increase and some people are of the opinion that most of the toothpaste sold in the markets has little or no effect in easing or stopping these dental problems and prefer to use chewing sticks and charcoal. This study investigates the antioxidant and phytochemical properties of different brands of dental formulations. Fifteen dental formulations consisting of eight toothpaste, four mouth wash/rinse and three herbal powers were screened using standard methods. Descriptive cross-sectional design was adopted and questionnaire used to determine choice, preference and frequency of usage that made the selection of the different brands screened. Terpernoid is heavily present in all the samples followed by saponin, alkaloid and glycoside. The phytochemical is in the range of 0.01 – 8.35. The presence of phytochemicals is an added advantage to the dental formulations. Phytochemicals have been reported to exert strong antibacterial activity against several microbes associated with oral diseases. Sample A had the lowest antioxidant activity (12.39±0.01) while sample J had the highest antioxidant activity (60.72±0.32). Antioxidant protects the body from oxygen scavenging bacteria. Herbal formulations proved most effective compared to others.

Keywords: phytochemicals, antioxidants, Dental Formulations

Introduction

The tooth is a very sensitive and important part of the human body. It performs very important function in the overall wellbeing of an individual. Toothpaste is a dentifrice that improves the aesthetic appearance and health of the teeth. It is commonly found and sold in flexible tubes or sachets for the promotion of oral hygiene, removal of dental plagues and food debris from the mouth as well as elimination of halitosis from the mouth. Toothpaste is being classified as drugs

not cosmetics. As drug it contains an active ingredient, which helps it to achieve the effects the consumer desires (Yigit*et al.*, 2008). It is important to note that most of the cleaning is done by the mechanical use of the toothbrush onto which the toothpaste is pasted on and not just the toothpaste alone. The mouth is where food digestion starts such as protein, vitamins, carbohydrates etc. it is usually the first point of contact of food or any edible substance to the body (Deng *et al.*, 2009).

Oral health has a significant impact on overall quality of life and wellbeing. Dental problems are mostly due to microbial infections and there are three major types of dental problems: dental plagues, dental caries and periodontal diseases (Prasanth, 2011). Plagues have also been linked to gingivitis, dental carries as well as periodontal diseases. Most toothpaste sold in the market are natural toothpaste as they do not contain triclosan/ fluoride. They usually contain natural ingredients such as sodium chloride, and plant extracts like lemon, rosemary, sage and myrrh (Mali *et al.*, 2012).

Oral disorders are on the rise every now and then, therefore, global demand for effective and cost-efficient preventative and treatment products has grown (Wu *et al.*, 2001). Dental caries has become a major concern to the health sector in the world because of diseases associated with it (Botelho *et al*., 2007).

The modern chemical-based toothpaste and mouthwash have been effective in combating cariogenic microbes. However, the major challenging issue is the resistance of these cariogenic microorganisms to some commonly used antibiotics such as penicillin, chloramphenicol, clindamycin, ampicillin and other antimicrobial chemicals (Bhattacharya *et al*., 2003). These drawbacks are the driving forces that cause the need for the formulation of toothpaste from medicinal and edible plants containing natural antimicrobial agents. Nature is endowed with herbs for human use but the world out of a desire for modern development has overlooked this potent provision of the ‘herbs’ that contains secondary metabolites (Kolapo*et al*., 2009).

This study reports on antioxidant and phytochemical properties of some brands of toothpaste, dental powder and mouth rinse.

Materials and Methods

Distribution of Questionnaires

Questionnaires were distributed to those consented to participate. Sampling of question on the effects of different toothpaste, dental powder and mouth rinse stating choice and preference, frequency of usage as well as quantity used in each application were filled appropriately. Their response determined the choice of toothpaste and other herbal dental formula used in this study. Sample of the questionnaire is given below:

Section A: Demographic data

A. Gender (A) Male (B) Female
B. Age Bracket (A) 20 – 30 (B) 40 – 50 (C) 50 and above
C. Preferred toothpaste brand (A) Close up (B) Colgate (C) Pepsodent (D) longrich Toothpaste (E) Macleans (F) oral – B (G) Sensodyne
D. Preferred dental powder…….
E. Preferred Mouth Rinse…….
F. How often do you brush (A) once daily (B) morning and nights (thrice a day) (C) after every meal (D) twice a week (E) not at all
G. Why do you prefer the toothpaste brand you chose
H. Have you ever tried a different brand? Yes or No
I. Does your preferred toothpaste brand give you a lasting fresh breath?
J. Is your preferred toothpaste brand hot / spicy/calm/soothing /refreshing? Tick any one
K. What do you look out for when purchasing your toothpaste (A) low price (B) fluoride free (C) contain fluoride (D) child friendly flavor (E) no flavor (F) no sugar (G) well know

brand (H) colour (I) anti – bacterial agent (J) abrasives (for removal of plagues) (K) others. Tick any one

L. Do you have any sensitivity to any of the ingredients in your regular toothpaste (A) Yes (B) No if yes, please specify
M. Have you and your dentist discussed the toothpaste that you are currently using? Yes or No
N. Have you heard the phrase "spit, don't rinse"? (A) Yes (B) No
O. What do you think results in healthier teeth? (A) The number of times one brush his teeth (B) the manner/thoroughness in which one brushes his teeth? Tick any one
P. Do you enjoy brushing your teeth (A) No (B) Yes

Have you ever had a side – effect from using a particular toothpaste brand? (A) No (B) Yes if yes (Please specify)……

Brands of Toothpaste, Mouth Wash and Dental Powder

Fifteen dental formula comprising eight (8) chemical toothpaste, three (3) herbal dental powder and four (4) oral mouth were investigated for their phytochemical and antioxidant potentials. For ethical consideration, the brands were coded A- O.

Sample names	Sample codes
CLUP	A
MYMY	B
DRCHDP	C
OBT	D
COLP	E
PEPT	F
SENDT	G
MYMYT	H
OBMW	I
DHP	J
COLMW	K
MEDXMW	L
MEDXWMW	M
DADP	N
CCTP	O

Determination of phytochemical properties of brands of Toothpaste, Dental Powder and Mouth rinse (wash)

Preparation of Samples for Phytochemical Analysis

Twenty (10) grams of toothpaste and dental powder were weighed using a digital weighing balance and poured into different 250 ml conical flasks containing 50 mls of sterile distilled water in a ratio of 1:5 (w/v).

The conical flask was agitated vigorous on an electronic shaker for 30-60 minutes and allowed to stand for 18-24 h.

Qualitative and Quantitative Phytochemical Screening

The toothpaste, mouth rinse and dental powder were analyzed for the presence of Alkaloids, Saponins, Tannins, Cardiac Glycoside, Anthraquinones, Steroid, terpenoid and flavonoids, according to standard methods (Edori*et al*., 2019)

Screening for alkaloids - The Wangner's test was used for identification of Alkaloids in the samples (toothpaste, mouth rinse and dental powder). A few drops of 1% HCL was added to 3 ml of the extracts in a test tube and the HCL mixture was heated for 15 minutes, allowed to cool and 2 drops of Wagner reagent was dropped into the mixture and observed for a reddish brown precipitate which indicates the presence of Alkaloid (Edori *et al*., 2019).

Screening for Saponins- To examine for the presence of saponins, the frothing test was performed. To 1 ml of sample taken into a test tube, 9 ml of distilled water was added and shaken vigorously for 15 seconds and extracts was allowed to stand for 10 minutes. Formation of stable foam (1 cm) indicates the presence of saponins (Thilagavathi *et al*., 2015).

Screening for tannins - Test for tannins was done by adding 10% of freshly prepared potassium hydroxide (KOH) to 3 ml of the sample (toothpaste, mouth rinse and dental powder) in $Mn(OH)_2$ and observed for dirty white precipitate, which authenticates the presence of tannins (Edori *et al*., 2019).

Screening for steroids - To screen for steroid 3 drops of concentrated H_2SO_4 was added to 3 ml of the sample (toothpaste, mouth rinse and dental powder) and when a red colour is formed, it indicates the presence of steroid (Edori *et al*., 2019).

Screening for flavonoids - Two grams (2 g) of the sample (toothpaste, mouth rinse and dental powder), completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone in water bath. The mixture was filtered while still hot and the filtrate cooled before use.

Screening for Anthraquinones- This was carried out by Bontrager's test method. 0.5 g of the sample (toothpaste, mouth rinse and dental powder) was put into dry test tube followed by 5 ml chloroform and shaken for 5 min. Extracts was filtered and the filtrate shaken with an equal volume of 100% ammonia solution. A pink violet or red colour in the ammoniacal layer (lower layer) indicates the presence of free anthraquinones (Maria *et al*., 2018).

Screening for cardiac glycoside - To 10 ml of concentrated H_2SO_4 was added 3 ml of the sample (toothpaste, mouth rinse and dental powder) and the mixture heated for fifteen minutes in boiling water bath. Thereafter, 10 cm^3 of Fehling's solution was added to it while still boiling and observed for a brick red colour which is an indication of the presence of glycosides (Edori, *et al*., 2019).

Determination the Antioxidant activities of some brands of Toothpaste, Dental Powder and Mouth Rinse

The radical scavenging activity of different extracts was determined by using 2, 2-diphenyl-1-picrylhydrazyl **(**DPPH) assay according to Chang *et al*. (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm.

Reagent preparation

0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml of ethanol.

Working procedure

Different volumes (2 – 20 µl) of plant extracts were made up to 40 µl with DMSO and 2.96 ml DPPH (0.1 mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3 ml of DPPH was taken as control. The % radical scavenging activity of the plant extracts was calculated using the following formula,

%RSA = (Abs Control – Abs Sample/ Abs Control) × 100

Where, RSA is the Radical Scavenging Activity; *Abs control* is the absorbance of DPPH radical + ethanol; *Abs sample* is the absorbance of DPPH radical + paste extract.

Results

Table 1 shows the result of the qualitative phytochemical analysis of the dental formulations while Table 2 shows that of the quantitative phytochemical analysis of the samples. From the results obtained, most of the dental formulation contains phytochemicals. Anthraquinone was absent in sample C, L, N and O. Tannin was absent in sample I, K and L. Flavonoid was absent in Sample E and L. Steroids was absent in Sample L only.

Table 3 illustrates the results for the radical scavenging activity (%RSA) of the oral extracts for the antioxidant assay. From the result, it is clear that all samples possess antioxidant properties. The highest %RSA was shown by sample J while the lowest was shown by sample A.

Table 1
Qualitative Phytochemical Composition of some Dental Formula

Sample code	SAP	ALK	TAN	FLA	TEP	ANT	GLY	STE
A	++	+	+++	+	+++	+	+++	+
B	+++	+++	+++	+++	+++	+	+++	+
C	+++	+++	+++	+	+++	-	+++	++
D	++	+++	+++	+++	+++	+++	+	++
E	+++	++	+++	-	+++	++	+++	+++
F	+++	+	+++	++	+++	++	+++	+
G	+++	++	+++	++	+++	+++	+++	+
H	+++	+++	+++	+++	+++	+++	+++	+++
I	+++	+++	-	+++	+++	+	+++	+++
J	+++	+++	+	+++	+++	+++	+++	+++
K	+++	+++	-	+++	+++	+	+++	+++
L	+++	+++	-	-	+++	-	+	-
M	+++	+++	+++	+++	+++	+	+++	+
N	+++	+++	+++	+	+++	-	+++	+++
O	++	+++	+++	+++	+++	-	+++	+++

SAP, Saponin; AKL, Alkaloid; TAN, Tannin; FLA, Flavonoid; TEP, Terpenoid; ANT, Anthraquinone; GLY, Glycosides; STE, Steroids; +, Low; ++, Moderate; +++, High;-, not detected

Table 2
Quantitative Phytochemical Composition of some Dental Formula(n=2)

Sample code	SAP	ALK	TAN	FLA	TEP	ANT	GLY	STE
A	3.29±0.01	1.70±0.05	5.44±0.03	1.26±0.03	7.19±0.01	1.02±0.01	8.24±0.05	1.15±0.05
B	8.06±0.01	6.11±0.01	5.27±0.01	5.03±0.01	6.07±0.01	0.18±0.01	7.48±0.03	0.33±0.01
C	6.10±0.05	5.03±0.01	6.06±0.03	0.99±0.05	5.05±0.01	0.11±0.02	7.92±0.01	2.95±0.01
D	7.10±0.01	5.04±0.05	5.96±0.02	5.05±0.01	3.27±0.01	7.12±0.01	1.26±0.01	3.15±0.05
E	6.60±0.04	2.06±0.01	6.78±0.01	0.14±0.05	3.96±0.05	3.07±0.01	6.06±0.01	6.52±0.01
F	7.73±0.01	1.27±0.01	6.22±0.01	2.38±0.05	4.23±0.01	3.06±0.02	6.94±0.03	1.99±0.05
G	7.06±0.05	2.38±0.01	6.47±0.02	1.93±0.01	6.20±0.05	4.10±0.01	7.11±0.01	2.06±0.01
H	6.05±0.01	6.05±0.05	6.04±0.01	4.49±0.01	4.94±0.01	4.48±0.01	6.27±0.01	6.07±0.01
I	7.05±0.01	5.05±0.01	0.10±0.01	5.11±0.02	3.39±0.01	0.21±0.03	7.94±0.03	7.18±0.01
J	6.01±0.03	5.06±0.01	1.05±0.01	5.04±0.01	5.53±0.05	7.03±0.01	5.45±0.00	6.01±0.03
K	6.84±0.01	7.06±0.02	0.08±0.01	5.93±0.01	4.06±0.01	0.51±0.03	6.03±0.01	5.64±0.01
L	7.16±0.01	6.07±0.01	0.02±0.02	0.13±0.02	6.32±0.01	0.01±0.00	1.31±0.01	0.03±0.01
M	7.05±0.05	6.09±0.02	6.80±0.01	4.20±0.01	4.55±0.03	0.64±0.03	8.06±0.01	2.10±0.01
N	8.53±0.01	6.92±0.01	6.93±0.01	0.03±0.01	5.26±0.01	0.02±0.01	5.38±0.01	5.58±0.03
O	3.33±0.01	5.27±0.04	5.95±0.01	3.28±0.01	5.05±0.03	0.01±0.02	5.06±0.01	4.43±0.02

SAP, Saponin; AKL, Alkaloid; TAN, Tannin; FLA, Flavonoid; TEP, Terpenoid; ANT, Anthraquinon; GLY, Glycocides; STE, Steroids

Table 3
Antioxidant Composition of some Dental Formula (n=3)

Sample Code	Absorbance	% RSA Values
A	2.628±0.02	12.39±0.01
B	1.871±0.01	37.63±0.012
C	1.506±0.01	50.21±0.03
D	1.573±0.03	52.44±0.04
E	1.604±0.02	53.47±0.021
F	1.590±0.02	53.01±0.023
G	1.489±0.01	49.64±0.043
H	1.652±0.01	55.07±0.01
I	1.682±0.01	56.07±0.021
J	1.823±0.03	60.72±0.032
K	1.691±0.01	56.36±0.04
L	1.695±0.01	56.49±0.02
M	1.600±0.05	53.33±0.03
N	1.600±0.01	53.34±0.03
O	1.600±0.02	53.33±0.023

***RSA, radical scavenging activity;* Ads control; 3.000; SD±; Standard Deviation from mean values**

Discussion and Conclusion

Oral microorganisms, including bacteria, archaea, fungi, viruses, and protozoa, are closely associated with oral disease processes. It has been demonstrated that the diversity of the oral microbiome in children increases after they acquire their initial colonizing microorganisms (Gomez & Nelson, 2017). Dental caries has caused the destruction of the superficial dental structure, wearing of the enamel and removal of the tooth by releasing acid due to metabolic activities of cariogenic bacteria on the remnant food in the mouth (Botelho *et al*., 2007). Microbes such as *Streptococcus mutans*, *Staphylococcus aureus*, *Streptococcus mitis*and *Candida albicans* havebeen implicated in dental diseases (Bhattacharya *et al*.,2003; Akhtar &Bhakuni, 2004).

The advent of modern technology has brought in branded toothpastes and mouthwashes with the incorporation of synthetic antimicrobial agents such as chlorhexidine, triclosan, and fluoride (Zauri-Arite *et al*., 2001; Maripandi *et al*., 2011).

Phytochemicals are biologically active organic substances found in plants used by humans as food, which may be beneficial for health, but for which no specific human deficiency disorder has been identified. Phytochemicals are recognized as bioactive components in traditional herbal medicines used in the formulation of toothpaste and herbal formulations (Yigit, Aktas & Ayyildiz, 2008).

All dental formulations (Samples A - O) contains phytochemicals and exhibited antioxidant properties. The importance of bioactive compounds like alkaloids, phenolics, flavonoids and some other secondary metabolites have been reported by Kala *et al*. (2010). The presence of these phytochemicals is an added advantage to the dental formulations. These phytochemicals have been reported to exert strong antibacterial activity against several microbes associated with oral diseases (Adeyemi *et al*., 2004). Terpernoid is heavily present in all the samples followed by saponin, alkaloid and glycoside. The presence of these phytochemicals ranges from 0.01 – 8.35.

Sample A had the lowest antioxidant activity (12.39±0.01) while sample J had the highest antioxidant activity (60.72±0.32). Antioxidants have the ability to scavenge free radicals in the human body and have been suggested to contribute to the protective effect of plant-based foods on diseases (Stanner and Weichselbaum 2013). Herbal formulations proved most effective compared to others. This could be as a result of the natural herbs added as components or additives of the final products as was reported by Yigit, Aktas & Ayyildiz (2008).

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