



# FORMULATION, PHYSICOCHEMICAL CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF HERBAL TOOTHPASTE CONTAINING NEEM (AZADRIRACHTA INDICA) AND OCIMUM GRATISSIUM LEAF EXTRACTS

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**ABSTRACT:** Herbal Toothpaste is a toothpaste formulated with medicinal plants, herbs, or their extracts as one or more active ingredients to help clean the teeth and maintain oral health. *The growing demand for natural and effective oral hygiene products has driven the development of herbal formulations. This study investigates the formulation and evaluation of herbal toothpaste using Neem (Azadirachta indica) and Basil (Ocimum gratissimum) leaves, both known for their antibacterial, antifungal, and anti-inflammatory properties. The active compounds were extracted from Neem and Basil leaves and combined with standard toothpaste ingredients to produce a stable formulation. Methodological evaluations included determining the toothpaste's pH (8.53), moisture content (66.20%), spreadability (5.5 cm/s), and viscosity at varying speeds. Antimicrobial activity against Streptococcus mutans and Candida albicans was assessed using the agar diffusion method, while antioxidant potential was measured through DPPH radical scavenging. Sensory analysis was conducted on 40 participants to evaluate taste, texture, odor, and appearance. Results demonstrated the toothpaste's smooth texture, optimal flow, and effective oral hygiene properties, showing significant antibacterial and antioxidant activity. Compared to commercial toothpaste, this herbal alternative offers a chemical-free solution with reduced side effects, such as oral sensitivity and irritation. The study concludes that Neem and Basil-based toothpaste is a viable, eco-friendly option for maintaining oral health. Further research is recommended to explore its commercial potential and long-term benefits.*

**Keywords:** Antibacterial activity, Antioxidant activity, Basil, Eco-friendly, Herbal toothpaste, Moisture content, Natural formulation, Neem, Oral hygiene, pH balance, Spreadability, Viscosity.

## 1.0 INTRODUCTION

Herbal toothpaste is a type of toothpaste formulated with extracts from medicinal plants and other natural ingredients to help clean teeth, maintain oral hygiene, and promote healthy gums. Unlike conventional toothpaste, which may rely mainly on synthetic ingredients, herbal toothpaste contains plant-derived ingredients such as neem, basil, clove, miswak, and peppermint. In summary, herbal toothpaste is a plant-based oral-care preparation designed to clean teeth and support oral hygiene using selected herbal ingredients alongside suitable formulation components. Medicinal plants are traditionally used worldwide as remedies for the treatment of various diseases that can cause dental diseases like dental caries, bleeding and dental halitosis and other body diseases including asthma, gastrointestinal symptoms, skin disorders, respiratory and urinary problems, and hepatic and cardiovascular disease (Tian, 2021). These plants synthesize a diverse array of chemicals and biologically active compounds that are important for them to survive and flourish in the natural environment, including dental disorder protection and other part of the body protective functions with respect to abiotic stresses derived from temperature, water status and mineral nutrient supply and to bacteria, virus, and fungi (Vardhini and Anjum, 2019).

According to World Health Organization (2020) more than 80% of the world's population relies on plants medicine for their primary healthcare needs. Use of plant medicines in Africa in which Nigeria is included represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat dental chronic diseases as well as infectious diseases

(Ammara, 2018). Many developing countries like Nigeria depend on plant resources mainly for herbal medicines, food, forage, construction of dwellings, making household implements, sleeping mats, and for fire and shade as well as used in the treatment of chronic infectious diseases due to its chemical ingredients present in them (Ammara, 2018).

Also, plants represent a rich source of chemical and antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Chandram *et al.*, 2019). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties (Srivastav *et al.*, 2022). Plants are believed to be important source of new chemical substances with potential therapeutic effects on dental treatment through the production of herbal toothpaste and tooth powder for oral health care (Basha, 2019).

Oral health refers to the health of the teeth, gums, and the entire oral-facial system that allows us to smile, speak, and chew. Some of the most common diseases that impact our oral health include cavities (tooth decay), gum (periodontal) disease, caries, oral bleeding, and oral cancer. The most prevalent oral diseases include dental caries (tooth decay), periodontal (gum) disease, tooth loss, cancers of the lips and oral cavity. More than 3.5 billion people suffer from oral diseases (WHO, 2020) in African continents alone, without any notable improvement of the situation between 1990 and 2019. According to Paul (2023), untreated dental caries in permanent teeth is the single most prevalent condition globally, affecting 2.3 billion people. Severe periodontal disease, a major cause of total tooth loss, is estimated to affect 267 million people, particularly older people (WHO, 2020). Oral health is essential to good health and well-being and this can only be achieved with the use of effective toothpaste or tooth powder (Basha, 2019).

Toothpaste is a paste or gel used with a toothbrush to maintain and improve oral health and aesthetics. Since their introduction several thousand years ago, toothpaste formulations have evolved considerably from suspensions of crushed egg shells or ashes of complex formulations with often more than 20- 30 ingredients. It is believed to be the ancient Egyptians where the first toothpaste who developed a dental cream around 3,000 - 5,000 BC, which contained powdered ashes from oxen hooves, myrrh, egg shells and pumice, primarily with the aim to remove debris from teeth (Van, 2019). Since then many scholars and scientists in African countries have developed toothpaste from synthetic chemicals and plants called herbal toothpaste (Van, 2019).

However, herbal toothpaste is a type of toothpaste made from natural ingredients derived from plants and herbs. It is formulated to provide oral hygiene and dental care using botanical extracts, essential oils, and other natural substances (Ganesh *et al.*, 2019). Herbal toothpaste is often preferred by those seeking alternatives to conventional toothpaste, which may contain artificial ingredients and chemicals. The ingredients in herbal toothpaste can vary widely but often include plants oil like Neem, clove, peppermint, basil, turmeric and tea tree oil, which are known for their antibacterial, antifungal, and anti-inflammatory properties. These natural ingredients are believed to help fight plaque, prevent cavities, and promote healthy gums (Ganesh *et al.*, 2019).

There are a lot of herbal products available in the market and herbal toothpaste is also one of the products. Toothpaste is the common product in our family and we hardly care to know about the product which we are using (Paul, 2023). Herbal toothpaste is having advantages over synthetic products. Many herbal toothpaste formulations are free from artificial colors, flavors, and preservatives, making them a popular choice among individuals looking for natural and eco- friendly oral care products (Paul, 2023). However, it is important to note that herbal toothpaste may not contain a mineral that is commonly added to conventional toothpaste to prevent tooth decay. Toothpaste, as an irreplaceable agent in effective home care system, is a gel or paste dentifrice used with a toothbrush as an accessory to clean and maintain health of teeth in order to enhance oral hygiene (Paul, 2023).

Herbal toothpaste comes in many flavors and helps to leave mouth and breath feeling fresh after brushing. It can also make any scents from strong-flavored foods like garlic or onion. Herbal toothpaste made from neem leaves, basil, tea trees, mint trees and turmeric leaves often resolves with good oral hygiene, such as longer and more frequent brushing and flossing and an antiseptic mouth wash can help. Most of the synthetic tooth paste contain fluoride or sodium lauryl sulfate which cause adverse effect to tooth. So, to avoid such effect formulated herbal based tooth paste, nowadays natural sources remain attractive primarily when compared to the synthetic one which are devoid of side effect, minimum number of ingredients (Malgi *et al.*, 2022).

Similarly, using the plant leaves of neem and basil for toothpaste, the toothpaste has strength, stability, cleanness, excellent foaming, taste, odour, antimicrobial and chemical capability to control and prevent tooth decay, caries, bleeding in the teeth, and other diseases that can attack teeth. As a result of this, the present study focusses on the formulation and evaluation of herbal toothpaste using neem leaves (*Azadirachta indica*) and Basil leaves (*Ocimum gratissimum*) (Paul, 2023).

Many modern toothpastes do not adequately address overall oral hygiene and the prevention of common dental conditions such as halitosis, tooth decay, dental caries, and gum bleeding. Additionally, some commercial toothpastes contain synthetic detergents, which have been linked to painful oral ulcers, also known as canker sores or aphthous ulcers (Tonzetich, 2021). Another significant concern is tooth sensitivity, where individuals

experience discomfort or pain when consuming hot, cold, or sweet foods. This sensitivity often results from enamel erosion, which exposes the underlying dentin layer. Certain ingredients found in commercial toothpastes, such as baking soda and highly salted compounds, may further aggravate tooth sensitivity.

Given these concerns, there is a growing need for safer, natural alternatives in oral care products. Herbal-based formulations have gained attention due to their potential antibacterial, anti-inflammatory, and soothing properties. This study aims to formulate and evaluate a herbal toothpaste using Neem (*Azadirachta indica*) and Basil (*Ocimum gratissimum*), which are known for their medicinal benefits in oral health. The goal is to develop an effective toothpaste that minimizes adverse effects while promoting overall dental hygiene.

This study will benefit individuals suffering from dental diseases by providing insights into the management, prevention, and potential treatment of conditions such as tooth decay and halitosis using a cost-effective, herbal-based toothpaste formulated with Neem (*Azadirachta indica*) and Basil (*Ocimum gratissimum*). Since these natural ingredients are readily available, the study promotes an accessible and affordable alternative for oral hygiene.

Additionally, this research will be valuable to professional dental practitioners by offering a natural formulation that may complement existing dental treatments. It will also benefit individuals who frequently rely on self-remedying oral health treatments by providing a scientifically evaluated herbal toothpaste, potentially reducing the risks associated with harmful or ineffective self-medication practices.

The justification for this study stems from the increasing demand for natural and safe alternatives in personal care products, particularly in the domain of oral hygiene. Herbal toothpaste formulations that utilize natural ingredients, such as *Neem* and *Basil* leaves, are gaining traction due to their numerous health benefits and fewer adverse effects compared to chemical-based products. The aim of the study is to formulate and evaluate the physicochemical properties and antimicrobial activities of herbal toothpaste produced.

## 2.0 MATERIALS AND METHODS

### 2.1 MATERIALS

#### 2.1.1 Plants

The plant material used are: neem leaves (*Azadirachta indica*); and basil leaves (*Ocimum gratissimum*)

#### 2.1.2 Organism

While, the organism used are: *Streptococcus mutans*; and *Candida albicans*

#### 2.1.3 Reagents and Apparatus

The equipment and reagents required and used for this study are shown in the Table 2.1 below:

**Table 2.1: Equipment and Reagents**

| S/N | Equipment          | Reagents                       |
|-----|--------------------|--------------------------------|
| 1   | pH meter           | Distilled water                |
| 2   | Measuring Cylinder | Buffer                         |
| 3   | Stirrer            | Sodium Lauryl Sulphate (SLS)   |
| 4   | Weighing apparatus | Sorbitol                       |
| 5   | Graduated cylinder | Calcium carbonate              |
| 6   | Beaker             | Calcium methyl cellulose (CMC) |
| 7   | Viscometer         | Silica                         |
| 8   | Oven               | Sodium Benzoate                |
| 9   | Desiccator         | Sodium Tripolyphosphate (STPP) |
| 10  | Stop watch         | Colorant                       |
| 11  | Glass slide        | Glycerin                       |
| 12  | Spatula            | Citric Acid                    |
| 13  | Ruler              | Peppermint                     |
| 14  | Homogenizer        | Saccharine                     |
| 15  | Sterile swabs      | Sodium bicarbonate             |
| 16  | Spectrophotometer  | Starch substrate               |

|    |                           |                                                |
|----|---------------------------|------------------------------------------------|
| 17 | Incubator                 | Amylase assay buffer                           |
| 18 | Antibiotic disc dispenser | DNSA (3,5 – Dinitrosalicylic acid) Reagent     |
| 19 | Vortex mixer              | Casein substrate                               |
| 20 | Agar plate                | Tris-HCL Buffer                                |
| 21 | Micro centrifuge tube     | TCA (Trichloroacetic acid)                     |
| 22 | Conical flask             | DPPH (2,2 – Diphenyl-1-Picrylhydrazyl) Reagent |
| 23 | Filter paper              | Ascorbic acid                                  |
| 24 | Centrifuge                | Methanol                                       |
| 25 | Autoclave                 | FRAP Reagent                                   |
| 26 | Petri Dish                | Folin-Ciocalteu Reagent                        |
| 27 | Glass rod                 | Tannic acid                                    |
| 28 |                           | Mueller Hinton Agar                            |

#### 2.1.4 Study Area and Population

The study area is the Biochemistry Department, School of Biochemical Science, Federal Polytechnic, Ede campus environment of Ede North Local Government, Osun State, Nigeria. The study population comprised of 40 selected participants include staff and students randomly from the said department and institution.

#### 2.1.5 Demographic Information of Participants

A total of 40 participants were selected as mentioned above and demographic data of participants and their views on sample tested were collected.

#### 2.1.6 Data Collection Method

Data were collected from 40 selected participants for the study using questionnaire method to collect data for the study.

#### 2.1.7 Preparation of Evaluation Sheet

40 evaluation sheets were prepared to record the information collected from 40 participants for the study and the sheet was attached to the questionnaire and sent to each participant for data collection.

#### 2.1.8 Collection and Identification of Plant Species

Fresh Basil (*Ocimum gratissimum*) and Neem (*Azadirachta indica*) leaves that were discovered growing spontaneously on the ground in C.A.C Grammar School, Odo-Eja Area, Ede, Osun State, were plucked separately into a new polythene bag and was brought into Biochemical Science Laboratory, Federal Polytechnic, Ede for further analysis.



Fig. 2.1: Basil leaves preparation



**Fig. 2.2: Neem leaves preparation**

### 2.1.9 FORMULATION OF TOOTHPASTE INGREDIENT

The formulation ingredients are depicted in Table 2.2.

**Table 2.2. Formulation of herbal toothpaste**

| S/N | Name of Ingredients for Production | Quantity |
|-----|------------------------------------|----------|
| 1   | Neem leaves extract                | 100ml    |
| 2   | Basil leaves extract               | 100ml    |
| 3   | Sodium Lauryl Sulphate (SLS)       | 62.5g    |
| 4   | Sorbitol                           | 50ml     |
| 5   | Calcium carbonate                  | 125g     |
| 6   | Calcium methyl cellulose (CMC)     | 125g     |
| 7   | Silica                             | 62.5g    |
| 8   | Sodium Benzoate                    | 125g     |
| 9   | Sodium Tripolyphosphate (STPP)     | 62.5g    |
| 10  | Sodium bicarbonate                 | 125g     |
| 11  | Glycerin                           | 50ml     |
| 12  | Citric Acid                        | 125g     |
| 13  | Peppermint                         | 100ml    |
| 14  | Saccharine                         | 8g       |
| 15  | Sodium bicarbonate                 | 125g     |
| 16  | Clove oil                          | 20ml     |
| 17  | Peppermint                         | 100ml    |

### 2.1.10 PREPARATION OF EXTRACTS

#### 2.1.10.1 EXTRACTION PROCEDURE

The Neem and Basil leaves sample were taken and washed in order to take out impurities from them. The Neem and Basil leaves were squeezed to produce a filtrate and the mixture was strained through a filter to remove solids and collect the filtrate.

#### 2.1.10.2 PREPARATION AND PRODUCTION OF HERBAL TOOTHPASTE

100ml of each filtrate of Neem and Basil leaves was measured and 800ml of distilled water was added to the filtrate to make it up to 1 liter. 125g of calcium methyl cellulose (CMC) was also measured. The calcium methyl cellulose (CMC) was added slowly to the Basil and Neem leaves filtrate while stirring continuously to prevent clumping. It was allowed to hydrate for 30mins until it formed a smooth, gel-like solution. In a separate container, the following dry ingredient were combined.

- Calcium carbonate - 125g
- Silica - 62.5g
- Sodium Bicarbonate - 125g
- Sodium TripolyPhosphate - 62.5g
- Sodium Lauryl Sulphate (SLS) - 62.5g

- Sodium Benzoate - 125g
- Citric Acid - 125g
- Saccharin - 8g

All the dry ingredients were stirred together to ensure even distribution. The dry ingredients were added slowly into the calcium methyl cellulose (CMC) – Basil – Neem mixture while stirring continuously until the dry powder were fully incorporated and no lumps remained. The essential oils listed below were added and mixed thoroughly.

- Glycerin - 50ml
- Sorbitol - 50ml
- Peppermint - 100ml
- Clove oil - 20ml

In addition, colourant was added and mixed well. Once the toothpaste was smooth and well mixed, it was transferred into a toothpaste tube and stored in a cool dry place.



**Fig 2.3: Herbal Toothpaste**

## 2.2 EXPERIMENTAL ANALYSIS

The following analysis were observed: pH; Moisture Content; Viscosity Measurement; Spreadability Test; Thermal Stability; Antibiotic Susceptibility Test; Antioxidant Activity Assay; Foamability Test; Sensory Evaluation; Grittiness Evaluation; Amylase Activity Test; Protease Activity Test; Minerals Contents; and Total Phenolic Content

## 3.0 RESULT AND DISCUSSION

### 3.1 RESULTS

#### 3.2 Physicochemical properties

##### 3.2.1 chemical and physical characteristics

**table 3.1: Chemical and Physical Characteristics of Toothpaste**

| Test                | Observations   |                |          |                |                                                     |
|---------------------|----------------|----------------|----------|----------------|-----------------------------------------------------|
| Parameters          | Sample 1       | Sample 2       | Sample 3 | MD             | Analysis                                            |
| pH                  | 8.53           | 8.54           | 8.52     | 8.53           | $\frac{W_3 - W_1}{W_2 - W_1} \times 100\%$          |
| Moisture Content    | 66.20%         | 66.10%         | 66.30%   | 66.20%         | $S = 500 \times \frac{5.5}{300} = 9.2 \text{ cm/s}$ |
| Spread-ability (S)  | 5.4 cm/s       | 5.6 cm/s       | 5.5 cm/s | 5.5 cm/s       |                                                     |
| Viscosity (cp) 25°C | Speed<br>10rpm | Speed<br>20rpm |          | Speed<br>30rpm | Mean                                                |
|                     | 46568          | 37290          |          | 28556          | 37471.33                                            |

**Note: MD = Mean Diameter**

### 3.2.1.1 pH Value

The herbal toothpaste had an average pH of 8.53, indicating an alkaline nature. Alkaline pH is advantageous as it helps neutralize acids in the mouth, reducing the risk of tooth decay and enamel erosion. This aligns with findings by Kadam (2021), who reported similar pH values for herbal formulations.

### 3.2.1.2 Moisture Content

The moisture content consistency in the 3 samples subjected to test were 66.20%, 66.10% and 66.30% with mean value of 66.20%. Hence, the moisture content was 66.20%. The moisture content of Toothpaste was very good.

### 3.2.1.3 Spreadability

The spreadability of the herbal toothpaste was measured and calculated to be 5.5 cm/s. This shows that the toothpaste spreadability was good because of its higher spreadability of 5.5cm per second.

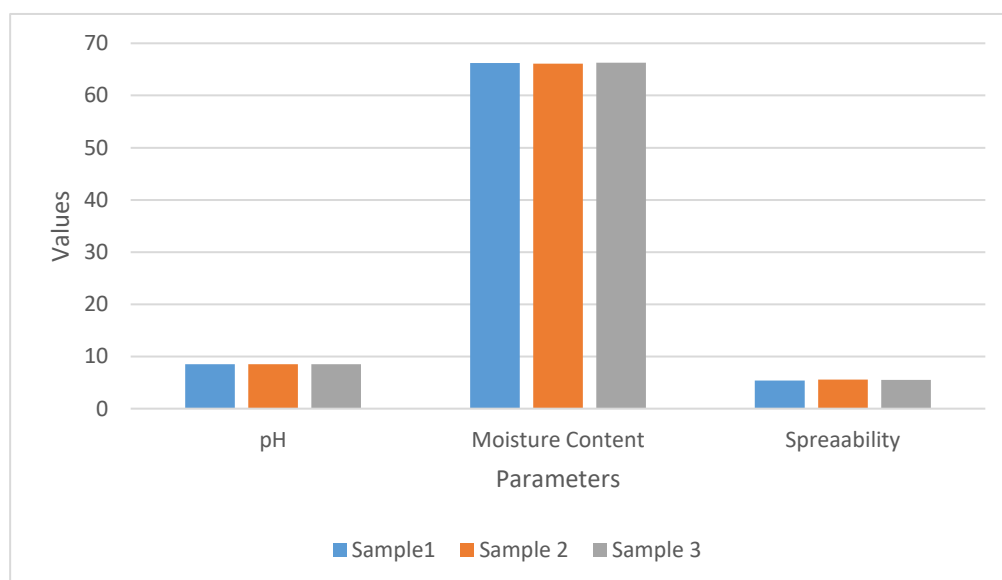


Fig.: 3.1 Moisture Content

### 3.2.1.4 Viscosity

The viscosity activity of the toothpaste formulated at 3 different speeds and 25°C temperature were determined. The toothpaste samples contained 46568 at 10rpm, 37290 at 20 rpm and 28556 at 30 rpm. The average mean value was 37471.33 for the 3 different speeds, this means the toothpaste has an excellent viscosity activity.



Fig. 3.2: Viscosity of the Toothpaste

The viscosity of the toothpastes as measured by different spindle speeds at room temperature was shown in Fig. 3.2. The results showed that viscosity significantly decreased as the spindle speed increased.

### 3.2.2 Thermal Stability

It can be seen in Table 4.4 below, at 25°C / 60% of temperature / humidity, the toothpaste colour remained olive green while pH was 8.1 and spreadability was 7.2cm. At 30° C / 65% of temperature / humidity, the toothpaste

colour, pH, and spreadability remained unchanged (no change in the value). But at 40°C / 75%, only colour and pH remain unchanged while spreadability reduced slightly to 7.15cm.

**Table 3.2: Thermal Stability and Shelf-Life of Toothpaste**

| Temperature/Humidity | Appearance  | Ph  | Spreadability |
|----------------------|-------------|-----|---------------|
| 25°C / 60%           | Olive Green | 8.1 | 7.2cm         |
| 30° C / 65%          | Olive Green | 8.1 | 7.2cm         |
| 40°C / 75%           | Olive Green | 8.1 | 7.15cm        |

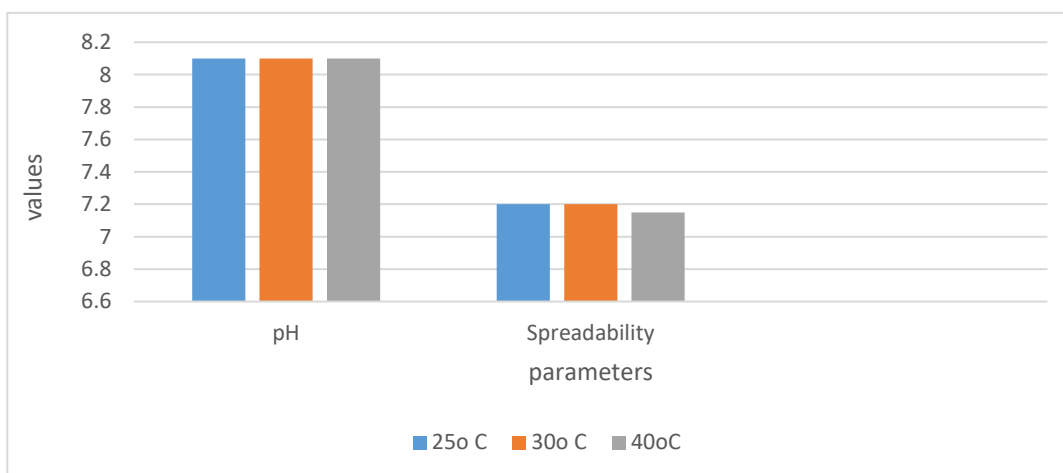


**Fig. 3.3: Spreadability**

**Table 3.3: Thermal Stability Analysis**

| Conditions                 | Observations        |                     |                     |                         |                   |
|----------------------------|---------------------|---------------------|---------------------|-------------------------|-------------------|
| Temperature                | Colour              | Odour               | Texture             | Phase Separation        | Result            |
| Room Temperature (Control) | No changes observed | No changes observed | No changes observed | No changes observed     | Stable            |
| High Temperature (40°C)    | No changes observed | No changes observed | No changes observed | Slight phase separation | Moderately Stable |
| Low Temperature (4°C)      | No changes observed | No changes observed | No changes observed | No changes observed     | Stable            |

The toothpaste samples produced into different temperature at room temperature, 40°C and 4°C respectively. There were no changes in colour, texture and odour of the samples in 3 different temperatures (i.e. room temperature, 40°C and 4°C). Only sample treated or stored at 40°C temperature has slight phase separation while samples at room temperature and 4°C have no phase separation changes.

### 3.3 Biological Activities

#### 3.3.1 Antimicrobial Activity

**Table 3.4: Antimicrobial Activity (*Streptococcus mutans*. And *Candida abican*)**

| Test                          | Zone of Inhibition (mm) |             |             |           |
|-------------------------------|-------------------------|-------------|-------------|-----------|
| Samples                       | Test 1 (mm)             | Test 2 (mm) | Test 3 (mm) | Mean Dev. |
| <i>Streptococcus mutans</i> . | 10.6                    | 10.2        | 10.8        | 10.5      |
| <i>Candida abican</i>         | 16.4                    | 15.9        | 16.2        | 16.2      |

In this study the antimicrobial activity of formulated herbal Toothpaste was investigated against Gram positive bacteria (*Streptococcus mutans* and *Candida abican*). The zone of inhibition against selected bacterial pathogens against formulated herbal Toothpaste showed the zone of inhibition of *Streptococcus mutans* to be 10.6 mm, 10.2mm and 10.8 mm with average mean value of 10.5mm as shown in **Table 3.4** above while the zone of

inhibition of *Candida albicans* were found to be 16.4mm, 15.9mm and 16.2mm respectively with average mean value of 16.2mm. This result shows that the clear area around indicates where the microorganisms are inhibited sensitive (S) large zone of inhibition than 20 mm susceptibility resistance. Small or absence zone of inhibition less than 10mm indicates resistance. Intermediate, zone of inhibition between 11-19 mm indicates intermediate susceptibility.

### 3.3.2 Anti-Oxidant Activity

**Table 3.5: Anti-oxidant Activity of Toothpaste**

| Test                                   | Observations |          |          |           |
|----------------------------------------|--------------|----------|----------|-----------|
| Parameters                             | Sample 1     | Sample 2 | Sample 3 | Mean Dev. |
| DPPH (%)                               | 44.73        | 44.74    | 44.74    | 44.736    |
| FRAP ( $\mu\text{MFe}^{+2}/\text{g}$ ) | 3.58         | 3.57     | 3.59     | 3.58      |

Mean Dev. = Mean Deviation

The anti-oxidant activity of the toothpaste formulated were determined. The toothpaste samples contained Dipalmitoyl-Sn-Glycerol-3-Phosphoglycerol (DPPH) of 44.73%, 44.74% and 44.74% for the 3 samples. The average mean value of DPPH was 44.736%, this means the toothpaste has an excellent anti-oxidizing activity as shown in Table 4.4 below and this makes the product have anti-fungal activity. Also, the Ferric Reducing Antioxidant Power (FRAP) anti-oxidize properties were found to be  $3.58 \mu\text{MFe}^{+2}/\text{g}$ .

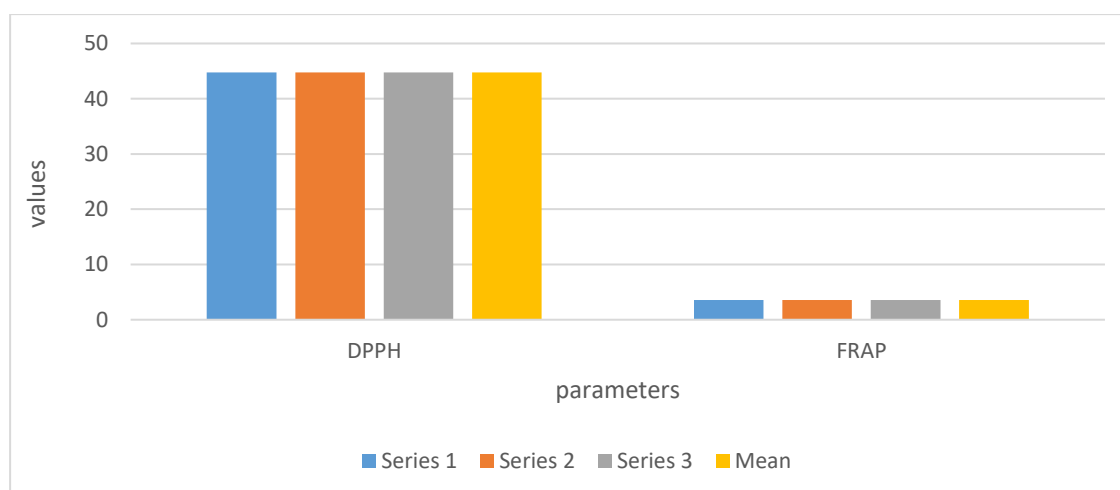


**Fig 3.4: DPPH and FRAP of Herbal Toothpaste**

**Table 3.6: Enzymatic activity of the Herbal Toothpaste**

| Test                    | Observations |          |          |          |
|-------------------------|--------------|----------|----------|----------|
| Parameters              | Sample 1     | Sample 2 | Sample 3 | Analysis |
| Amylase Activity (U/g)  | Sample 1     | Sample 2 | Sample 3 | Mean     |
|                         | 0.47         | 0.46     | 0.48     | 0.47     |
| Protease Activity (U/g) | Sample 1     | Sample 2 | Sample 3 | Mean     |
|                         | 0.009        | 0.008    | 0.010    | 0.009    |

Note: MD = Mean Diameter;

### 3.3.3 Amylase Activity

The amylase activity of the toothpaste formulated was determined and discovered that the toothpaste samples contained 0.47U/g, 0.46 U/g and 0.48U/g. The average mean value was 0.47U/g, this means the toothpaste has a good amylase activity.

### 3.3.4 Protease Activity

The protease activity of the toothpaste formulated was determined. The toothpaste samples contained 0.009U/g, 0.008 U/g and 0.010U/g. The average mean value was 0.009U/g, this means the toothpaste has an excellent protease activity.

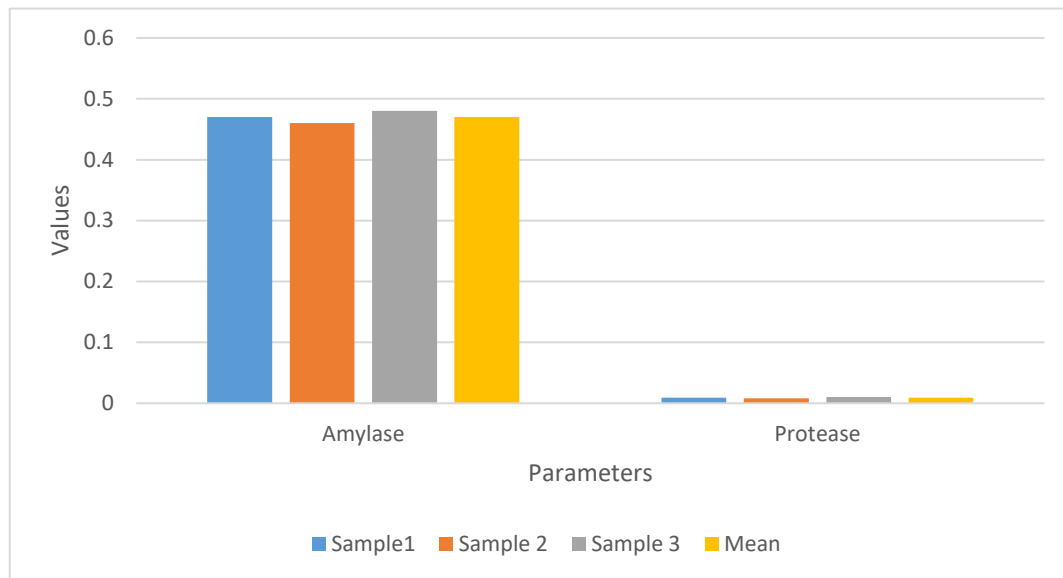


**Fig. 3.5: Protease Activity**

## 3.4 Sensory and Physical Evaluations

### 3.4.1 Profile of the Participants

**Table 3.7: Participant's Profile**

| Profile                | Categories   | Frequency |     | Percentage (%) |  |
|------------------------|--------------|-----------|-----|----------------|--|
| Sex                    | Female       | 19        |     | 47.5           |  |
|                        | Male         | 21        |     | 52.5           |  |
|                        | Total        | 40        | 100 |                |  |
| Age                    | 18- 27 years | 18        |     | 45.0           |  |
|                        | 28-37 years  | 20        |     | 50.0           |  |
|                        | 38-47 years  | 2         |     | 5.0            |  |
|                        | Total        | 40        | 100 |                |  |
| Status                 | Single       | 23        |     | 57.5           |  |
|                        | Married      | 17        |     | 42.5           |  |
|                        | Total        | 40        | 100 |                |  |
| Academic Qualification | OND          | 13        |     | 32.5           |  |
|                        | HND          | 15        |     | 37.5           |  |
|                        | B.Sc         | 9         |     | 22.5           |  |
|                        | Master       | 2         |     | 5.0            |  |
|                        | Total        | 40        |     | 100            |  |

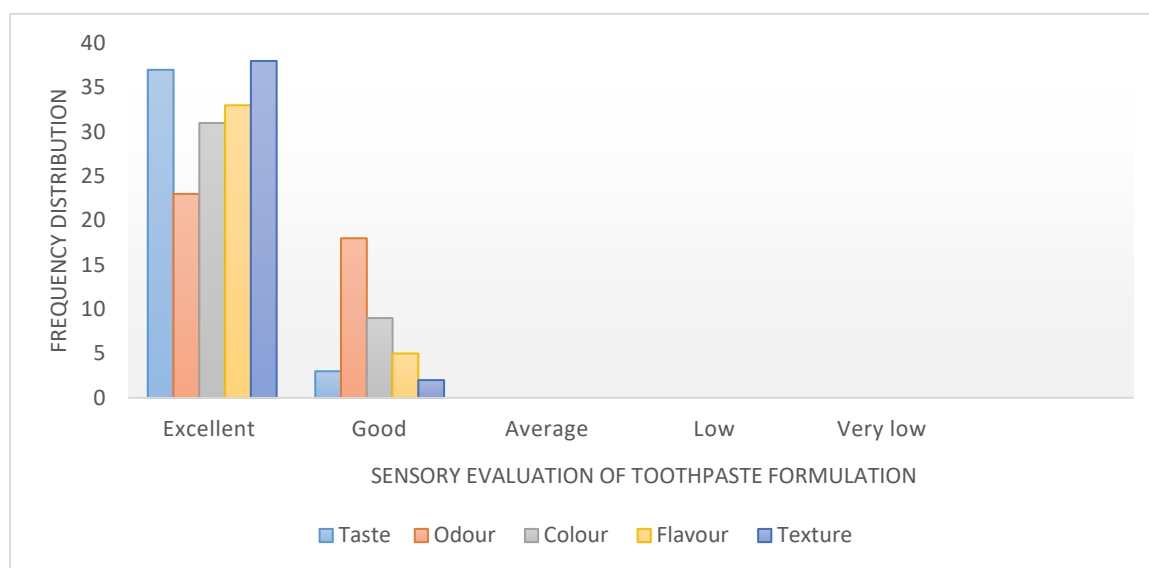
**Source:** Field survey (2024).

In table 3.7 above, it can be seen that 52.5% of the participants were male while 47.5% were female. Meanwhile, 45 % of the participants' age was fell between 18 – 27 years, 50% of them have age between 28 – 37 years while 5.0% of the participant's fell between 38-47 years. Likewise, 57.5% participants are single while 42.5% are female. Also, 32.5% of the participants are OND undergraduate students, 37.5% are HND undergraduate students, 22.5% are Bachelor Degree holders and 5% of them are Master holders.

**Table 3.8: Sensory Evaluation**

| Sensory Characteristics of Herbal Toothpaste |                   | Excellent<br>F % |      | Good<br>F % |      | Average<br>F % |     | Low<br>F % |     | Very low<br>F % |     |
|----------------------------------------------|-------------------|------------------|------|-------------|------|----------------|-----|------------|-----|-----------------|-----|
| Taste                                        | Minty Sweet       | 37               | 92.5 | 3           | 7.5  | 0              | 0.0 | 0          | 0.0 | 0               | 0.0 |
| Odour                                        | Mindy Sweet Aroma | 23               | 57.5 | 18          | 42.5 | 0              | 0.0 | 0          | 0.0 | 0               | 0.0 |
| Colour                                       | Olive Green       | 31               | 77.5 | 9           | 22.5 | 0              | 0.0 | 0          | 0.0 | 0               | 0.0 |
| Flavour                                      | Sweet & Fine      | 35               | 87.5 | 5           | 12.5 | 0              | 0.0 | 0          | 0.0 | 0               | 0.0 |
| Texture                                      | Fine paste        | 38               | 95.0 | 2           | 5.0  | 0              | 0.0 | 0          | 0.0 | 0               | 0.0 |
| Appearance                                   | Paste             | 39               | 97.5 | 1           | 2.5  | 0              | 0.0 | 0          | 0.0 | 0               | 0.0 |

**Key: F = Frequency; % = Percentage**

**Fig. 3.6: Sensory Evaluation of Toothpaste**

The taste of the toothpaste produced was excellently of astringent and minty sweet flavor according to 90% of the participant's observations, while the odour was a good minty sweet aroma, the colour was olive green with sweet and fine flavor and texture based on the participants' observation. The appearance was pasting form as indicated in the Table 4.8. In summary, the herbal toothpaste produce has astringent and sweet taste, minty sweet aroma, olive green colour, and sweet fine flavor and texture.

**Table 3.9: Physical Evaluation of Herbal Toothpaste**

| Test                   | Observations |          |          |        |          |
|------------------------|--------------|----------|----------|--------|----------|
| Parameters             | Sample 1     | Sample 2 | Sample 3 | MD     | Analysis |
| Foamability (ml/100ml) | Sample 1     | Sample 2 | Sample 3 | Mean   |          |
|                        | 12.5         | 12.4     | 12.6     | 12.5   |          |
| Grittiness             | Sample 1     | Sample 2 | Sample 3 | Result |          |
|                        | Absent       | Absent   | Absent   | Absent |          |

**Note: MD = Mean Diameter;**

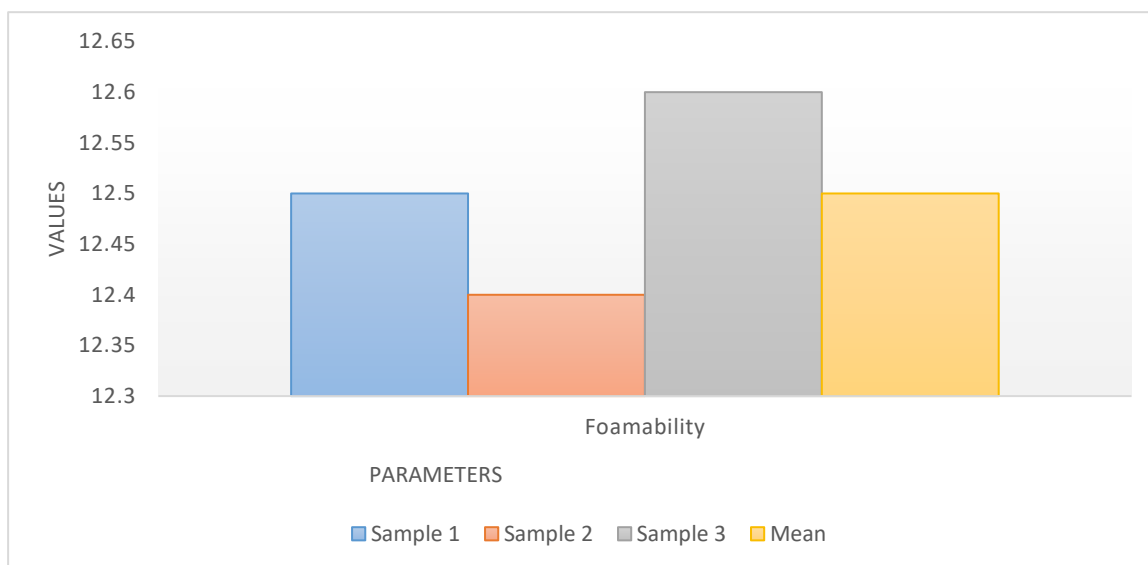


**Fig. 3.7: Foamability**

### 3.4.2 Foamability

The foamability of toothpaste were 12.5 ml/100ml, 12.4 ml/100ml, and 12.6 ml/100ml for the three samples having average mean value of 12.5 ml/100ml which show good foamability power of the toothpaste produced.

### 3.4.3 Grittiness

The grittiness test was performed to evaluate the presence of abrasive particles on toothpaste formulated which can cause discomfort or damage the teeth and gums. There was no grittiness in the Toothpaste produced as the samples subjected to test indicate absent of grittiness particles. This result indicates that the toothpaste formulated has undergone proper mixing of materials used during the production. The gritty matter test helps to ascertain the presence of solid in the formulation. This is important as regular usage of formulation as gritty matter can wear off the teeth enamel and cause injury to skin. Gritty matters were absent in all tested toothpastes.

## 3.5 Minerals Composition and Total Phenolic Contents

### 3.5.1 Minerals Analysis

**Table 3.10: Minerals Analysis**

| Test           | Minerals             |                     |                   |                       |                      |
|----------------|----------------------|---------------------|-------------------|-----------------------|----------------------|
| Samples        | Calcium<br>(mg/100g) | Cupper<br>(mg/100g) | Lead<br>(mg/100g) | Chromium<br>(mg/100g) | Cadmium<br>(mg/100g) |
| Sample 1       | 3873.00              | 0.00                | 0.00              | 0.00                  | 0.00                 |
| Sample 2       | 3874.00              | 0.00                | 0.00              | 0.00                  | 0.00                 |
| Average (Mean) | 3873.50              | 0.00                | 0.00              | 0.00                  | 0.00                 |

**Mean Dev. = Mean Deviation**

The presence of minerals elements in the toothpaste formulated were determined. The toothpaste samples contained Calcium (Ca) with 3873.50 mg/100g. There was no presence of other elements like Cupper (Cu), Lead (Pb), Chromium (Cr) and Cadmium (Cd). The presence of calcium in the sample indicates that the toothpaste was good for teeth by improving the energy of the teeth.

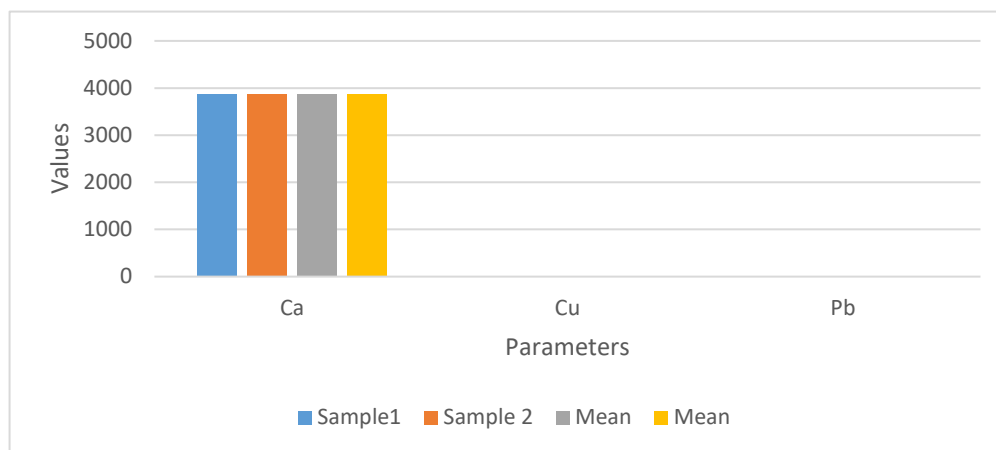


**Fig. 3.8: Mineral Analysis of the Herbal Toothpaste**

### 3.5.2 Total Phenolic Content

**Table 3.11: Total phenolic content of the Herbal Toothpaste**

| Sample 1 | Sample 2 | Sample 3 | Mean |
|----------|----------|----------|------|
| 0.06     | 0.07     | 0.05     | 0.06 |

There was presence of total phenolic compound in the toothpaste formulated. The samples subjected to test have 0.06, 0.07 and 0.05mgTAE/g total phenolic compound. The average mean value calculated was 0.06mgTAE/g. This shows that toothpaste formulated has moderate total phenolic compound.

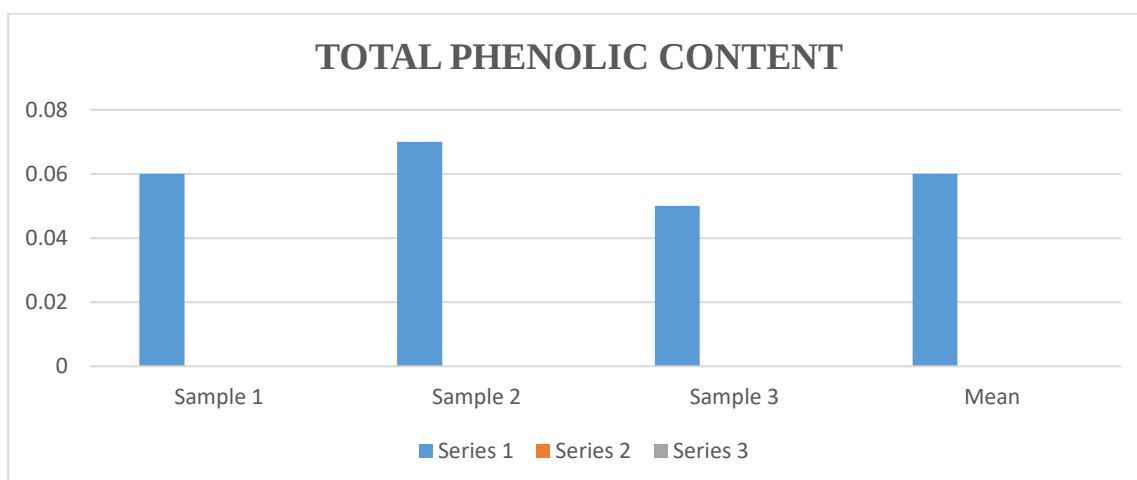


**Fig 3.9: Total phenolic content of the Herbal Toothpaste**

## 3.2 DISCUSSION

### 3.2.1 Physicochemical Properties

The formulation and evaluation of herbal toothpaste using Neem leaves (*Azadirachta indica*) and Basil leaves (*Ocimum gratissimum*) provided significant insights into the physical and chemical properties of the product. The study successfully demonstrated the viability of using these natural ingredients in toothpaste formulation, highlighting their effectiveness in maintaining oral hygiene and preventing common dental ailments.

The pH of the formulated herbal toothpaste was found to be 8.53 on average. This indicates that the toothpaste is alkaline, which is beneficial in neutralizing acidic conditions in the mouth that contribute to tooth decay. Previous studies have suggested that an alkaline pH in toothpaste can help reduce enamel erosion and promote oral health (Kadam, 2021). Compared to commercial toothpastes, which often have a pH ranging from neutral to slightly acidic, the herbal toothpaste in this study presents an advantage in maintaining a stable oral environment.

The moisture content of the toothpaste samples ranged between 66.10% and 66.30%, with a mean value of 66.20%. Moisture content is a crucial factor in toothpaste formulation as it affects the stability, shelf life, and microbial susceptibility of the product. A well-balanced moisture content ensures that the toothpaste retains its desired consistency and does not dry out too quickly. The consistency observed in the moisture content across different samples also signifies a well-formulated product with good homogeneity, which is crucial for user satisfaction and effectiveness.

The viscosity of the toothpaste was analyzed at different spindle speeds (10 rpm, 20 rpm, and 30 rpm) at 25°C, with mean values recorded as 46,568 cP at 10 rpm, 37,290 cP at 20 rpm, and 28,556 cP at 30 rpm, respectively. The average viscosity value of 37,471.33 cP indicates that the toothpaste possesses a suitable thickness, ensuring that it adheres to the toothbrush while being easy to dispense and apply. Viscosity plays a crucial role in the formulation of toothpaste as it affects its flow behavior and usability. The results obtained in this study are comparable to those of Wade (2017), who reported similar viscosity values in herbal toothpaste formulations.

The spreadability of the herbal toothpaste was measured at 5.5 cm/s. Spreadability is an important parameter that determines the ease of application and distribution of toothpaste on the toothbrush and teeth. The higher spreadability observed in this study suggests that the toothpaste is easy to apply, ensuring even coverage on the dental surfaces. This characteristic is vital for the user experience and adherence to regular brushing habits. The findings align with previous research by Li et al. (2018), which indicated that herbal toothpaste formulations with good spreadability are more preferred by consumers.

It can be seen in Table 4.2, at 25°C / 60% of temperature / humidity, the toothpaste colour remained green while pH was 8.53 and spread-ability was 5.5 cm/s. At 30° C / 65% of temperature / humidity, the toothpaste colour, pH, and spread-ability remained unchanged (no change in the value). But at 40°C / 75%, only colour and pH remain unchanged while spread-ability reduced slightly to 5.15cm. Wade (2017) study finding also found herbal toothpaste formulated with good thermal stability when tested at 30°C / 90%.

The results from this study indicate that the formulated herbal toothpaste possesses desirable properties comparable to, and in some cases superior to, conventional synthetic toothpaste. Synthetic toothpaste often contains fluoride, sodium lauryl sulfate (SLS), and other chemical ingredients that may cause adverse effects such as irritation and hypersensitivity. The herbal toothpaste in this study, however, relied on the antimicrobial and anti-inflammatory properties of Neem and Basil extracts, making it a safer and more natural alternative for oral hygiene.

Neem and Basil leaves are widely recognized for their medicinal properties. Neem is known for its antibacterial, antifungal, and anti-inflammatory effects, making it effective in preventing plaque buildup, cavities, and gum diseases (Gupta, 2019). Basil, on the other hand, possesses antimicrobial properties that contribute to fresh breath and overall oral health (Barsto, 2018). The inclusion of these natural ingredients in the toothpaste formulation not only enhances its efficacy but also aligns with the growing consumer preference for herbal and organic dental care products.

Given the promising results obtained from this study, there is a strong potential for the commercialization of herbal toothpaste formulated with Neem and Basil extracts. The increasing demand for natural and chemical-free oral care products suggests that such formulations could gain widespread consumer acceptance. Furthermore, the cost-effectiveness of using locally sourced herbal ingredients makes this product a viable alternative, especially in regions where access to commercial oral hygiene products is limited.

### **3.2.2 Biological Activities**

The result of antimicrobial activity of formulated herbal toothpaste exhibited the significant inhibition against the tested bacteria and observed higher activity against *Streptococcus* sp. than *Candida albicans* bacteria and *Staphylococcus* sp. Hence, it was confirmed that the prepared herbal toothpaste possess the great antibacterial potential and it could be applicable for the bacteria causing oral infection. The study by Sodan (2020) which discovered that the herbal toothpaste produced was resistance to *Escherichia* sp., and *Staphylococcus* sp. and has ability to prevent oral infection and this study finding was found to be similar. According to a study by Kadam (2021), *Escherichia* sp., was found to be sensitive to anti-microbial activity. The prepared product was efficient against the *Streptococcus* sp., according to a study by Kumar and Navaratnam (2018).

The anti-oxidant activity of the toothpaste formulated were determined. The toothpaste samples contained DPPH of 44.73%, 44.74% and 44.74% for the 3 samples. The average mean value of DPPH was 44.736%, this means the toothpaste has an excellent anti-oxidizing activity as shows in Table 4.3 below and this make the product has anti-fungi activity. This finding was similar to the Dessler (2016) study finding who discovered that herbal toothpaste formulated contained anti-oxidize properties.

The finding of the study in Table 4.4 showed that the toothpaste sample produced was stable in colour, odour, phase separation, and texture at room temperature, 40°C and 4°C respectively. Only sample treated or stored with high temperature (i.e. at 40°C) was slightly change of phase separation. Therefore, the toothpaste was stable and has excellent thermal stability. Similarly, Wale (2017) study's finding observed that herbal toothpaste formulated with neems leaves has thermal stability at temperature between -50°C to 50°C and excellent self-efficacy while Vankudavathu et al. (2023) established the fact that herbal toothpaste formulated has a good thermal stability at high, room and low temperature up -10°C and 60°C respectively

### 3.2.3 Enzymatic Activity

The amylase and protease activity of the toothpaste formulated was determined and discovered that the toothpaste samples contained 0.47U/g, 0.46 U/g and 0.48U/g. The average mean value was 0.47U/g, this means the toothpaste has a good amylase activity, while the protease activity of the toothpaste formulated was determined. The toothpaste samples contained 0.009U/g, 0.008 U/g and 0.010U/g. The average mean value was 0.009U/g, this means the toothpaste has an excellent protease activity. This finding was supported by Gupta (2019) who stated that herbal toothpaste formulated should contain protease not greater than 0.5U/g.

### 3.2.4 Physical and Sensory Properties

The sensory evaluation of the formulated herbal toothpaste containing Neem (*Azadirachta indica*) and Basil (*Ocimum Grassitimum*) leaves was conducted to assess its acceptability based on taste, color, odor, texture, foaming ability, and grittiness. Sensory evaluation plays a vital role in consumer preference and product quality assessment, ensuring that the toothpaste is both effective and pleasant to use. The color of the formulated toothpaste was found to be olive green, which is likely due to the presence of natural pigments in Neem and Basil leaves. Consumers often associate green color with herbal and natural products, which could be a key marketing advantage. The odor of the toothpaste was evaluated as having a minty-sweet aroma, attributed to the presence of peppermint and basil extracts. A pleasant odor is essential in oral care products as it enhances freshness and promotes regular use.

The taste of the toothpaste was rated highly by participants, with the majority describing it as astringent and sweet, which is typical of herbal formulations. This can be linked to the presence of Neem, Basil, and peppermint extracts, which contribute to both antimicrobial properties and a refreshing aftertaste. Previous studies by Salam (2018) and Zheng (2018) support the findings that natural sweetening agents, such as stevia and peppermint, contribute to an enhanced taste experience in herbal toothpaste.

The texture and consistency of the toothpaste were also examined. The herbal toothpaste had a smooth, fine texture, indicating that the formulation was well-balanced and free from large abrasive particles. The smoothness of toothpaste is an important factor in user experience, as gritty or uneven textures can be uncomfortable and may lead to reduced consumer preference. Foaming ability is a key characteristic in toothpaste as it influences spreadability, cleansing efficiency, and consumer perception. The foaming capacity of the herbal toothpaste was determined through a standardized method, yielding a foamability range of 12.4 to 12.6 ml per 100 ml of sample, with an average value of 12.5 ml per 100 ml. This indicates good foaming potential comparable to commercial herbal toothpaste brands.

Sodium Lauryl Sulfate (SLS) is commonly used as a foaming agent in conventional toothpaste, but in herbal formulations, foaming can be achieved using plant-derived surfactants. Studies by Williams (2020) and Sodan (2020) show that herbal formulations with natural foaming agents can maintain good foaming properties while reducing the risk of irritation associated with synthetic chemicals. The results of this study align with these findings, proving that the herbal toothpaste had sufficient foamability without the use of synthetic surfactants.

Grittiness is a crucial factor in assessing the abrasiveness of a toothpaste formulation. While mild abrasiveness is necessary to remove plaque and stains, excessive abrasiveness can cause enamel erosion and gum irritation. The grittiness test conducted on the herbal toothpaste indicated the absence of abrasive particles, confirming that the formulation was safe for regular use. The absence of grittiness suggests that the Calcium Carbonate and Silica used in the formulation were properly incorporated and well-balanced, ensuring an effective yet gentle cleansing action.

A study by Lam (2018) on herbal toothpaste formulated with Turmeric also reported similar results, where the toothpaste exhibited good cleansing action without excessive abrasiveness. Furthermore, Gupta (2019) highlighted that herbal toothpaste formulations should aim for minimal abrasiveness to prevent long-term dental damage, supporting the findings of this research.

Modern synthetic toothpaste often contains fluoride, artificial preservatives, and chemical abrasives, which may cause adverse effects such as tooth sensitivity, gum irritation, and allergic reactions. In contrast, herbal toothpaste formulations offer a natural and safer alternative, as they contain antimicrobial, anti-inflammatory, and healing properties derived from plant extracts.

The use of Neem and Basil in the toothpaste formulation has been supported by various studies, including Sofowora (2019), which highlighted Neem's antibacterial effects against oral pathogens. Similarly, Paul (2023)

reported that Basil possesses antifungal and plaque-reducing properties, making it a beneficial ingredient for herbal toothpaste formulations.

The sensory evaluation results suggest that the herbal toothpaste formulated in this study meets consumer expectations in terms of taste, odor, texture, foamability, and grittiness. Additionally, the absence of synthetic chemicals makes it a safer alternative to conventional toothpaste, reducing the risks of chemical-related oral health issues.

### **3.2.5 Mineral Composition and Total Phenolic Content**

There was presence of total phenolic compound in the toothpaste formulate. The samples subjected to test have 0.06, 0.07 and 0.05mgTAE/g total phenolic compound. The average mean value calculated was 0.06mgTAE/g. This shows that toothpaste formulated has moderate total phenolic compound. The result was contradiction to the study finding of Smith et al. (2018) who found total phenolic compound in herbal toothpaste to be 2.17mg/TAE which was greater than this study result.

The mineral analysis of the formulated herbal toothpaste revealed a significant presence of calcium (Ca) at 3873.50 mg/100g, while heavy metals such as copper (Cu), lead (Pb), chromium (Cr), and cadmium (Cd) were undetectable. Calcium is an essential mineral for maintaining dental health, primarily due to its role in the remineralization of tooth enamel and strengthening of teeth (Enax & Eppe, 2018). The high calcium content observed in this study suggests that the toothpaste may aid in enhancing enamel repair and promoting stronger teeth, which aligns with the findings of previous studies on calcium-based toothpastes (Najibfard et al., 2011).

The absence of heavy metals like lead, cadmium, and chromium is a positive indicator of the safety and quality of the formulated toothpaste. Heavy metal contamination in oral care products can pose serious health risks, including toxicity and long-term systemic effects (Health Canada, 2022). The non-detection of these elements suggests that the raw materials and formulation process used in this study meet safety standards and are free from harmful contaminants.

## **4.0 CONCLUSION AND RECOMMENDATIONS**

### **4.1 CONCLUSION**

The formulation and evaluation of herbal toothpaste using neem (*Azadirachta indica*) and basil (*Ocimum basilicum*) leaves demonstrated the potential of these plants as effective natural ingredients for oral care. The results showed that the toothpaste possessed excellent physicochemical properties, including optimal pH, moisture content, viscosity, spreadability, and thermal stability, all of which contribute to the product's overall quality and usability.

The biological activity of the toothpaste was evident in its strong antimicrobial and antioxidant properties, highlighting the effectiveness of neem and basil's bioactive compounds in combating oral pathogens and protecting oral tissues from oxidative stress. The enzymatic activity tests confirmed the presence of amylase and protease, further supporting the toothpaste's ability to break down food particles and proteins, enhancing oral hygiene.

Mineral analysis revealed a high concentration of calcium (3873.50 mg/100g), which is essential for strengthening teeth and aiding enamel remineralization. The absence of toxic heavy metals like copper (Cu), lead (Pb), chromium (Cr), and cadmium (Cd) emphasizes the safety of the formulated product. The total phenolic content also indicated the toothpaste's antioxidant potential, adding to its health benefits.

Sensory evaluation of the toothpaste showed positive feedback from participants in terms of texture, taste, color, freshness, foamability, and ease of use. The smooth texture with minimal grittiness and the pleasant herbal flavor enhanced user satisfaction, making the product appealing and comfortable for daily use.

The findings suggest that the formulated herbal toothpaste possesses excellent chemical and physical properties, making it a safe and effective alternative to conventional synthetic toothpaste. The study further concludes that herbal toothpaste is a viable, cost-effective, and natural oral hygiene product with no observed adverse effects.

### **4.2 RECOMMENDATIONS**

Based on the findings and conclusions of this study, the following recommendations are made:

- Regular use of herbal toothpaste should be encouraged, as it has antimicrobial properties that help control bacteria responsible for dental plaque and gingivitis.
- The formulated toothpaste should be recommended for public use due to its beneficial nutrient profile, which supports overall oral health by providing essential vitamins and minerals necessary for maintaining strong teeth and healthy gums.
- Future studies should focus on the long-term effects of herbal toothpaste on oral health, including its

effectiveness in preventing dental diseases such as halitosis, cavities, and gum bleeding.

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