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Original Research Article

Antimicrobial and anti-inflammatory activities of *Annona muricata* L. (Annonaceae) formulated as an herbal toothpaste

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Abstract

Purpose: The major drawback with many orthodox conventional types of toothpaste is their side effects and sometimes resistance associated with their use. It is on this basis that we investigated the anti-inflammatory and antimicrobial activities of the leaves of *Annona muricata* L. (Annonaceae) formulated as herbal toothpaste.
Methods: Phytochemical and chromatographic screenings were investigated using standard procedures. Anti-inflammatory evaluation was carried out using egg albumin. The antimicrobial evaluation of extract of *A. muricata* and formulated herbal toothpaste was by agar well diffusion method against selected microorganisms using ciprofloxacin and nystatin as positive controls. The sensory and physicochemical properties of the formulated herbal toothpaste were evaluated.
Results: Phytochemical screening of *A. muricata* revealed the presence of tannins, cardiac glycosides and alkaloids. The anti-inflammatory activity was dose dependent. There was inhibition of activity by the crude extract and formulated herbal toothpaste containing *A. muricata* against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Candida albicans.* The *A. muricata* formulated herbal toothpaste was sweet and pleasantly smelling with good foaming ability and had a pH range of 7.24-7.92.
Conclusion: *A. muricata* possess chemical constituents which could be responsible for the observed anti-inflammatory and antimicrobial properties. These constituents when present in the formulation of toothpastes could result in more therapeutic effectiveness and reduction in resistance.

Keywords: *Annona muricata*, anti-inflammatory, antimicrobial, toothpaste

Introduction

The importance of the tooth in the human body cannot be over-emphasized, hence teeth being unhealthy could lead to digestive problems and malnutrition [1]. In other to make the tooth healthy and functional, activities of cariogenic microorganisms need to be reduced to the barest minimum. This could help to prevent diseases associated with oro-dental infections and dental caries [1].

Microorganisms such as *Streptococcus mutans, Streptococcus mitis, Staphylococcus aureus* and *Candida albicans* amongst others have been implicated in dental diseases [1]. Oral hygiene is an important aspect of human health and different modalities are offered for the treatment of dental and oral diseases. Toothpaste is used to promote oral hygiene [2] and standard toothpaste formulas contain a combination of Fluoride and detergents, which contributes to the efficacy of biofilm control [3].

There has been challenging issues with the available modern chemically based toothpastes, despite their effectiveness in combating cariogenic microbes. One of such is the resistance of these cariogenic microorganisms to commonly used antibiotics [4,5].

It has been postulated that the addition of different antimicrobial agents enhances reduction, control and prevention of the accumulation of cariogenic and periodontopathogenic microorganisms [6]. Also, modern technology has led to the production of branded toothpastes with the incorporation of synthetic antimicrobial agents such as chlorhexidine, triclosan and fluoride [7].

The use of these constituents have been very effective but with some drawbacks; Fluorinated toothpaste, apart from not been recommended for children below 6 years of age, causes pigmentation of teeth and weakening of enamel, while chlorhexidine, a chlorophenyl bisbiguanide causes pigmentation of dental, mouth and tongue environment with altered sense of taste, irritation and oral dryness, scaling of gingival and negative effects in ingestion [8,9].

These draw back are the driving force for the formulation of toothpaste from medicinal plants that have established antimicrobial activities. *Annona muricata* L. is specie of the genus Annona of the Custard apple tree family, Annonaceae that has edible fruits. The fruit is called *Soursop, Graviola, Guanabana* due to its slightly acidic taste when ripe [10]. *A. muricata* is native to the Caribbean and Central America, but is now widely cultivated throughout the world [10].

The leaves and stems of Soursop show active cytotoxicity against Cancer cells, due to bioactive compounds called acetogenins. In addition to its anticancer properties, it has been used to treat infections, cough, weight loss, herpes, inflammation of the nose and throat [11]. Other secondary plant metabolites present in *A. muricata* are the alkaloids: annonamine, coreximine and reticuline [12] and phenolics: caffeoylqunic acid, quercetin, gentisic acid and gallic acid [13].

Literature is replicate with the antimicrobial activities of *A. muricata*, but there are no reported studies on the formulation of toothpaste using the crude extract of the plant. It was in this regard that this research work was aimed at providing scientific information on the anti-inflammatory and antimicrobial activities of *A. muricata* and assessed the antimicrobial activity of the formulated toothpaste using the ethanol extract of the plant against selected pathogenic microorganisms.

Materials

All chemicals used in this study were of analytical grades and used as supplied by local vendors. The leaves of *Annona muricata* Linn (Annonaceae) were collected from the medicinal garden of Madonna University, Elele Campus, Rivers State, Nigeria. The plants were authenticated by the curator at the Department of Pharmacognosy, Faculty of Pharmacy of the institution by comparison with voucher specimens in the herbarium with the number MAU/057. The fresh leaves were air-dried for 72 hours and powdered using an electric mill.

Methods

Phytochemical screening

Screening for secondary plant metabolites was carried out according to standard methods [14-17]. These involved chemical tests for alkaloids (alkaloidal salts and bases); tannins (true and psuedotannins); cardiac, saponins, anthracene and cyanogenetic glycosides. Thin layer chromatography of the crude extract of *A. muricata* on silica gel-G, activated by heating at 110°C for 30 minutes was developed with the various solvent systems, viewed under UV light and sprayed with Dragendorff's spray reagent and R_f values calculated.

Paper chromatography using the ascending method and Whatman No.3 mm was developed with the solvent system n-butanol, acetic acid and water in the ratios of 12:3:5 and 4:1:5, examined in daylight and under UV light at 25 nm, sprayed with ferric chloride until colours developed and R_f values calculated.

Preparation of plant extract

The dried leaves of *A. muricata* (0.96 kg) were extracted with 95% ethanol (5×2L), using maceration method. The solvent was evaporated under reduced pressure using rotary evaporator, which yielded an extract of 162 g which was stored in a refrigerator at 4°C until needed for use.

Determination of anti-inflammatory activity

The method of Okoli and Akah [18] was used to evaluate the anti-inflammatory activity of the crude extract of *A. muricata*. Thirty-five (35) adult Wistar rats of both sexes were used. They

were divided into 7 groups (A-G) of 5 animals per group, left to acclimatize for four weeks. The rats were starved for 12 hours prior to the anti-inflammatory experiment but allowed access to water. Access to feed and water was allowed during the experiment.

The crude extract of *A. muricata* and aspirin were separately administered intraperitoneally. Group A served as negative control and received normal saline while Groups B-F received 500, 250, 125, 62.5 and 31.3 mg/kg of the crude extract of *A. muricata* while Group G served as the positive control and received 100 mg of aspirin. The animals were left for 30 minutes, thereafter 1ml of fresh egg albumin was injected into the sub-plantar of the right hind paw of each of the rats.

Using a Vernier caliper, the diameter of the hind paw was measured at 30 minutes' interval and readings were taken at 30, 60, 90, 120, 150 and 180 minutes, respectively. Percentage inflammation and percentage inhibition of inflammation were calculated using the formula

.

$$\% \text{ inflammation} = \frac{Ct \times 100}{Co} \quad \ldots. (1)$$

$$\% \text{ inhibition of inflammation} = \frac{Co - Ct \times 100}{Co} \quad ..(2)$$

Where Ct is inflammation at the dose

Co is inflammation at normal saline

Formulation of herbal toothpaste

The herbal toothpaste was formulated using the ethanol extract of *A. muricata* as the active ingredient and other none bioactive ingredients as given in Table 1.

Table 1: Composition of formulated toothpaste

Ingredients	Material used	Quantity
API	Crude extract of *A. muricata*	5 g
Abrasive	Calcium carbonate	30 g
Humectants	Glycerin	28 g
Forming agent	Sodium lauryl sulphate	1.5 g
Sweetner	Saccharin	2 g
Binder	Acacia	5 g
Flavour	Peppermint oil	2 g
Water	Distilled water	25 ml

Acacia (5.0 g) was transferred into a clean mortar. Distilled water was added with continuous trituration till gel was formed. Glycerin was added to the gel. Saccharin (2.0 g) and the remaining quantity of water were mixed in a separate beaker and added to the gel. Calcium carbonate was added slowly to the mixture and mixed well.

The extract (5.0 g) was incorporated with vigorous mixing. The peppermint oil was added and sodium lauryl sulphate was dissolved in minimum quantity of water and added to the above mixture. The product was weighed and packed in an air-tight container. A formulation that contains no extract was similarly prepared [19].

Determination of organoleptic properties of the formulated toothpaste

The organoleptic properties such a taste, colour, odour and the texture of the formulated toothpaste and toothpaste base were determined using the respective sense organs [20].

Determination of foaming ability of the formulated toothpaste

The formulated herbal toothpaste (5.0 g) was placed in a 100 ml glass beaker, 10 ml of water added; covered with a foil and allowed to stand for 30 minutes. Beaker content was stirred and slurry transferred to a 250 ml graduated measuring cylinder. More water was added to the 50 ml point and content maintained at 30°C stirring using a glass rod to ensure a uniform suspension. The cylinder was stoppered and shaken 12 times, allowed to stand for 5 minutes and the volume of foam with water (V_1) and water alone (V_2) was taken. Four replicates were determined and the average calculated [21].

$$\text{Foaming power} = V_1 - V_2 \quad \ldots. (3)$$

V_1 = volume in ml of foam with water.

V_2 = volume in ml of water only.

Determination of the pH of the toothpaste

The herbal toothpaste (5.0 g) was placed in a 150 ml glass beaker and 45 ml of freshly boiled, cooled water was added. It was stirred to make a thorough suspension. The pH was determined

using a pH meter (Oaklon pH meter model 1100). The pH meter was set at 7.0 at room temperature and the electrode immersed into toothpaste suspension. Four replicate determinations were made and average calculated [22].

Determination of moisture

The herbal toothpaste (5.0 g) was placed in an evaporating dish, dried in an oven at 105°C until the weight remained constant and noted as W_1. Four replicate determinations were made and average calculated [23].

Percentage loss by mass: $(W_0\text{-}W_1/W_0)\times100$....(4)

Determination of spreadability

The herbal toothpaste (1.0 g) was placed on a glass plate of 10 x 10 cm size and covered with another glass plate of the same size. A weight of 1.0 kg was placed on top of the glass plate and allowed to stand for 10 minutes and removed. The diameter of the spread on the plate was measured and the mean of four values was taken [23].

Antimicrobial Assay

Clinical strains of five bacteria (*Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis*) and one yeast (*Candida albicans*) were used for the antimicrobial assay. The organisms were obtained from the microbiology laboratory of Madonna University Teaching Hospital (MUTH) in Elele, Rivers State. The purity of the culture prior to use was confirmed by conventional, cultural, morphological and biochemical methods by the chief laboratory technologist, *Mr. Charles Akwuroha* at the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Madonna University. The *bacteria* and *fungus* cultures were maintained in nutrient agar and Sabouraud dextrose agar respectively at 4°C.

Preparation of inoculum

An overnight culture was used for the preparation of microbial suspension with a turbidity equivalent to that of 0.5 McFarland's standard.

Agar well diffusion method

The media were prepared and sterilized at 121°C for 15 minutes. About 30 ml Nutrient agar was seeded with bacteria culture and allowed to solidify and on each plate, wells of 10 mm in diameter were made. The formulated toothpaste was adjusted to 20 mg/ml and the open wells were filled with different concentrations of the herbal toothpaste ranging from 6.25 mg/ml to 100 mg/ml and ciprofloxacin (Reference standard) and incubated at 37°C for 24 hours. For antifungal assay, Sabouraud agar was used in place of nutrient agar and the medium incubated at 28°C for 48 hours, with nystatin as the reference standard. All tests were carried out in triplicates. The inhibition zone diameters were measured [24]. The experiment was repeated using the crude ethanol extract of *A. muricata*.

Statistical analysis

All the data were expressed as mean ± SEM (standard error of mean). p-values of < 0.05 were considered statistically significant.

Results

Phytochemical screening

The results of the phytochemical screening of the leaves of *A. muricata* (Table 2) revealed the presence of alkaloidal salts and bases, as extractions by polar and none-polar solvents showed the presence of precipitates with the various alkaloidal reagents. Tannins were present as true (hydrolysable and condensed tannins) and as pseudotannins. Only cardiac glycosides were confirmed among the glycosidal experiments.

Chromatographic screening

Thin-Layer Chromatography to establish chromatographic fingerprints using various solvent systems and detecting with Dragendorff (Table 3a) showed two spots (with Rf values of 0.79 and 0.88) which gave reddish brown colour with Dragendorff, with the solvent system chloroform:methanol (1:9)

Table 2: Phytochemical constituents of *A. muricata* leaves

Classes of secondary metabolites	Inferences
Alkaloidal salts	+
Alkaloidal bases	+
True tannins	+
Pseudo tannins	+
Anthracene derivatives	-
Saponins glycosides	-
Cardiac glycosides	+
Cyanogenetic glycosides	-

Key: - = absent; + = present

Table 3a: Thin-layer Chromatography of ethanol extract of the leaves of *A. muricata*

Solvent systems	No. of spots	Colour in daylight	Colour in UV	Colour after spraying with Dragendorff	R_f values
N-butanol:Acetic acid:Water (12:3:5)	-	Colourless	Colourless	Colourless	-
Acetic acid:Water:Ammonia (90:7:3)	-	Colourless	Colourless	Colourless	-
Chloroform:Methanol:Hexane (3:2:1)	1	Colourless	Light green	Reddish brown	0.71
Methanol: Ammonia (9: 1)	1	Colourless	Light green	Reddish brown	0.69
Methanol: Ammonia (1: 9)	1	Colourless	Light green	Reddish brown	0.48
Chloroform: Methanol (3:7)	1	Colourless	Light green	Reddish brown	0.82
Chloroform: Methanol (1:9)	2	Colourless	Light green	Reddish brown	0. 88 & 0.79
Chloroform: Methanol (9:1)	1	Colourless	Light green	Reddish brown	0.82
Methanol:Butanol (5:5)	-	Colourless	Colourless	Colourless	-

Table 3b: Paper Chromatography of the ethanol crude extract of *A. muricata*

Solvent systems	No. of spots	Colour in daylight	Colour in UV	Colour after spraying with Dragendorff	R_f values
N-butanol:Acetic acid:Water (12:3:5)	1	Colourless	Light fluorescent green	Blue black	0.27
N-butanol:Acetic acid:Water (4:1:5)	1	Colourless	Light fluorescent green	Blue black	0.69

Anti-inflammatory activity

Table 4 shows the effects of the crude extract of *A. muricata* and the standard drug (aspirin) on the inflammation of the hind paw oedema at time intervals of 30 minutes for 3 hours. Percentage inhibition of inflammation by the crude extract was dose-dependent and increased with time.

The crude extract showed anti-inflammatory activity at all doses and the percentage inhibition at the dose range of 125, 250 and 500 mg/ml were comparable to that of aspirin. At the dose of 500 mg, the activity was higher than that of the reference drug at all times, except at 180 min.

Inflammation of hind paw (oedema) by the various doses of *A. muricata* and aspirin were significant all through the period of assay $p < 0.05$ compared to normal saline (Table 4).

Percentage Inflammation of hind paw (oedema) by the various doses of *A. muricata* and aspirin were significant all through the period of assay $p < 0.05$ compared to normal saline (Figure 1a).

Percentage inhibition of Inflammation by the various doses of *A. muricata* and aspirin were significant all through the period of assay $p < 0.05$ compared to normal saline (Figure 1b).

Table 4: Results of average inflammation of hind paw (oedema) in diameter (mm)

Doses	30 min	60 min	90 min	120 min	150 min	180 min
500 mg/ml	5.30 ± 0.08	5.11 ± 0.04	4.92 ± 0.04	4.68 ± 0.24	4.36 ± 0.00	4.18 ± 0.44
250 mg/ml	5.31 ± 0.04	5.20 ± 0.18	5.01 ± 0.04	4.81 ± 0.64	4.32 ± 0.00	4.18 ± 0.00
125 mg/ml	5.42 ± 0.08	5.21 ± 0.08	5.02 ± 0.00	4.86 ± 0.34	4.63 ± 0.02	4.44 ± 0.02
62.5 mg/ml	5.64 ± 0.32	5.41 ± 0.08	5.21 ± 0.16	5.03 ± 0.18	4.87 ± 0.14	4.66 ± 0.00
31.3 mg/ml	5.80 ± 0.06	5.63 ± 0.38	5.41 ± 0.94	5.12 ± 0.66	4.92 ± 0.26	4.78 ± 0.02
15.6 mg/ml	6.05 ± 0.42	5.89 ± 0.08	5.55 ± 0.32	5.27 ± 0.00	5.11 ± 0.44	5.01 ± 0.76
Normal saline	7.12 ± 0.03	7.04 ± 0.11	6.91 ± 0.02	6.83 ± 0.07	6.31 ± 0.01	6.00 ± 0.07
Aspirin (100 mg)	5.95 ± 0.07	5.33 ± 0.04	5.17 ± 0.12	4.86 ± 0.14	4.37 ± 0.02	4.01 ± 0.08

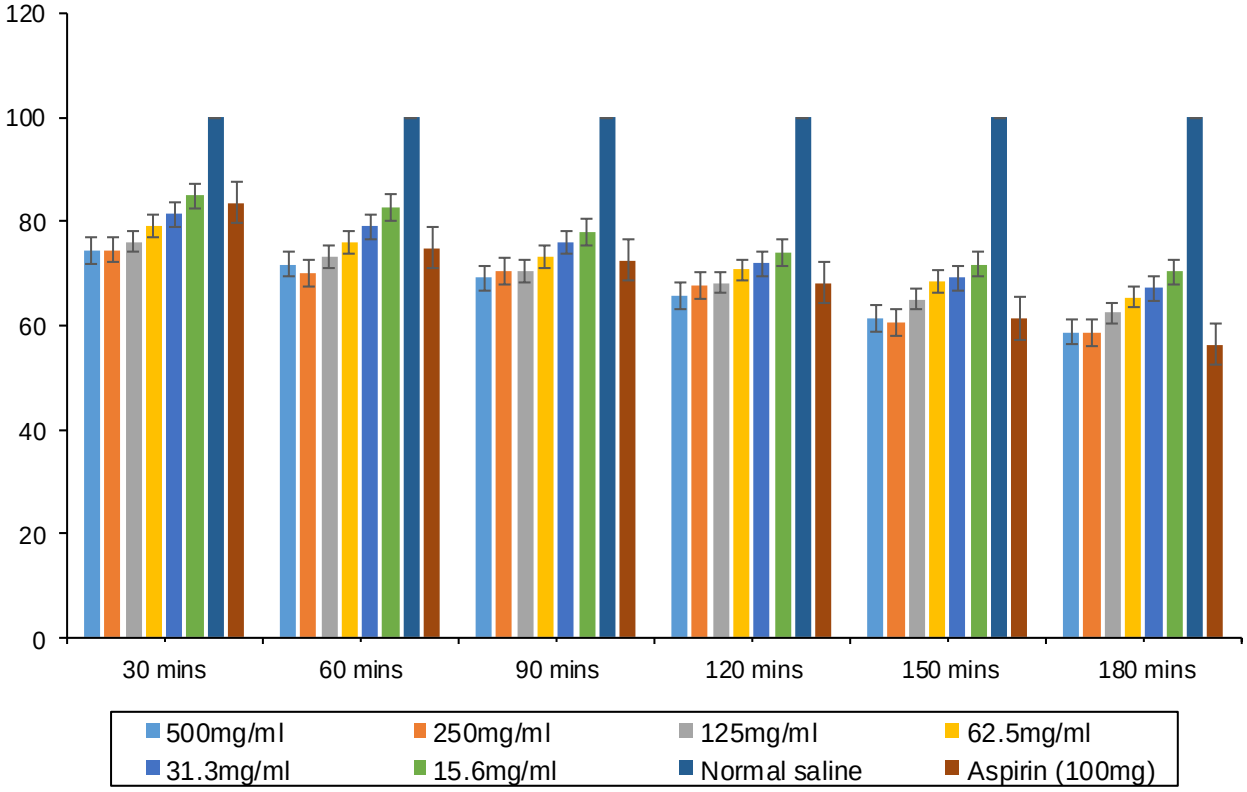

Figure 1a: A graph of percentage inflammation against time in minutes

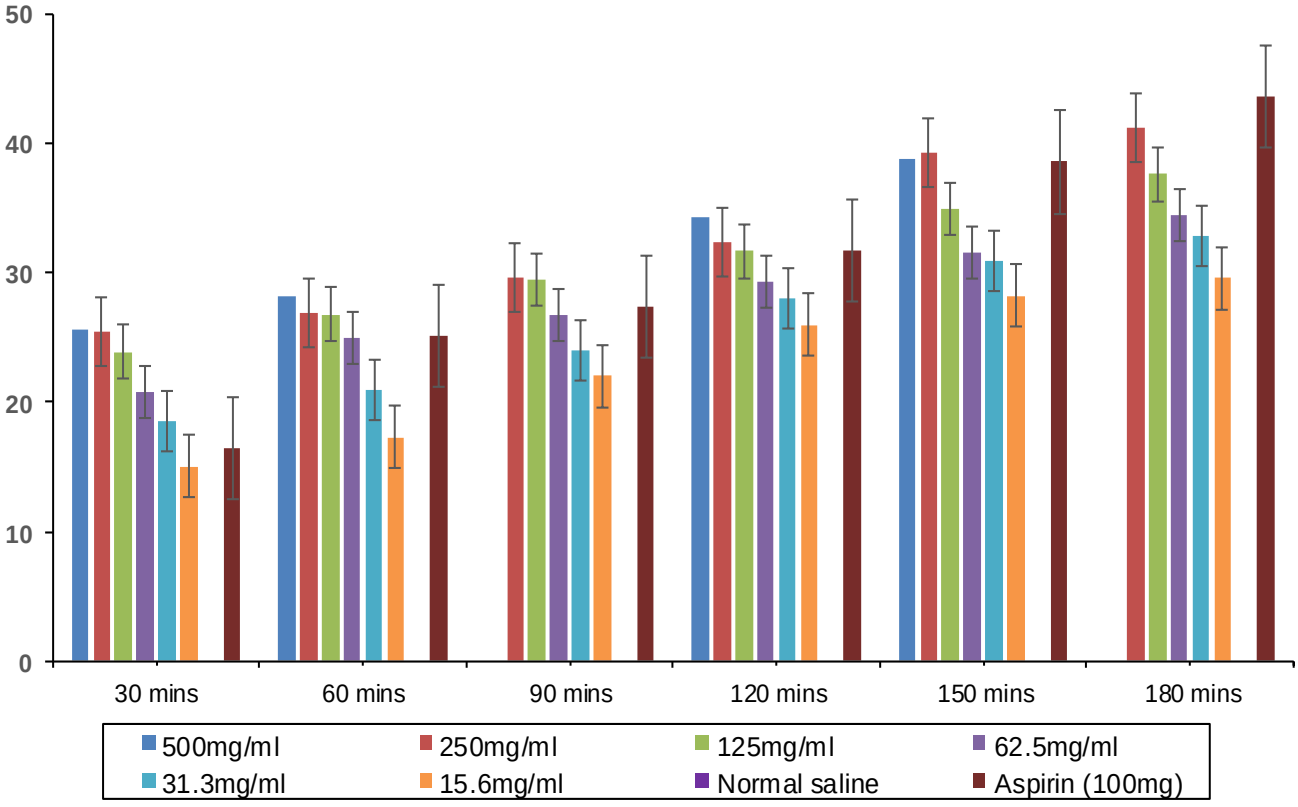

Figure 1b: A graph of percentage inhibition of inflammation against time in minutes

Physicochemical properties

The toothpaste base, commercial toothpaste and the formulated herbal toothpaste (FHT) were evaluated for physicochemical properties (Table 5). The FHT was smooth; paste-like; fragrance was pleasant, similar features with the commercial toothpaste. Taste was sweet and minty, with a higher pH range of 7.84-7.92. There was no significant difference between the FHT and the commercial toothpaste in foaming capacity, but the FHT showed a much lower moisture content and spreadability value.

Antimicrobial activity

The antimicrobial evaluation of the FHT against selected microorganisms (Table 6b) showed broad spectrum of activity. FHT was effective against Gram negative and Gram positive bacteria as well as against *C. albicans*. There was no activity against *P. aeruginosa*. The FHT had higher antimicrobial activity when compared to the crude extract of *A. muricata* (Tables 6a and 6b). The highest antimicrobial activity was recorded by the FHT against *B. subtilis* (23 ± 1.55). Conversely, the least antimicrobial activity was recorded by the crude extract of *A. muricata* against *B. subtilis* (5.5 ± 0.04).

Table 5: Physiochemical properties of the formulated herbal toothpaste, commercial herbal toothpaste, and the toothpaste base

Test/samples	Toothpaste base	Commercial toothpaste	Herbal toothpaste formulation
Appearance	Paste-like	Paste-like	Paste-like
Color	White	Aqua green	Dark brown
Taste	Sweet	Sweet	Sweet and minty
Odour	Pleasant	Pleasant	Pleasant
Texture	Smooth	Smooth	Smooth
Foaming capacity (cm)	49 ± 0.55	57 ± 1.28	57 ± 0.04
pH	6.74 – 6.98	7.50 - 7.77	7.84-7.92
Moisture	29.14 ± 0.25	31.75 ± 1.82	27.22 ± 0.03
Spreadability (cm)	6.01 ± 1.64	6.98 ± 1.42	5.87 ± 0.52

Table 6a: Antimicrobial activity of *Annona muricata* ethanol crude extract

Test organisms	Diameter of zones of inhibition (mm)							
	6.25 mg/ml	12.25 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	Cp (10 µg/ml)	Nys	DMSO
P. aeruginosa	G	G	G	G	G	26.0 ± 1.50	ND	G
S. aureus	G	G	8 ± 0.17	14 ± 0.50	15 ± 1.44	20.0 ± 1.04	ND	G
K. pneumonia	G	6 ± 0.54	9 ± 1.48	12 ± 0.72	14 ± 1.34	17.5 ± 0.42	ND	G
B. subtilis	5.5 ± 0.04	9 ± 1.22	15 ± 1.62	19 ± 1.44	20.5 ± 0.71	24.5 ± 0.25	ND	G
E. coli	6 ± 1.25	10.5 ± 0.34	13 ± 0.45	16 ± 0.04	22.75 ± 0.01	30.0 ± 0.02	ND	G
C. albicans	G	5 ± 1.25	8.5 ± 0.05	12 ± 1.10	15.5 ± 1.50	ND	17.5 ± 0.42	G

Values are expressed as mean ± SEM, (G) indicates no inhibition zone; (ND) indicates not determined; (Cp) indicates Ciprofloxacin; (Nys) indicates Nystatin. n=4

Table 6b: Antimicrobial activity of the formulated herbal toothpaste and toothpaste base

Test organisms	Diameter of zones of inhibition (mm)							
	6.25 mg/ml	12.25 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	Toothpaste base	Cp (10 µg/ml)	Nys
P. aeruginosa	G	G	G	G	G	G	26.0 ± 1.50	ND
S. aureus	G	12 ± 1.58	13.5 ± 1.64	18 ± 0.88	21.75 ± 0.05	G	20.0 ± 1.04	ND
K. pneumonia	6 ± 1.43	7 ± 0.28	14 ± 1.12	15.5 ± 1.10	16.5 ± 0.06	G	17.5 ± 0.42	ND
B. subtilis	15.5 ± 0.07	18 ± 0.16	18.5 ± 0.78	22.5 ± 0.28	23 ± 1.55	6 ± 0.24	24.5 ± 0.25	ND
E. coli	13.5 ± 0.22	15.0 ± 0.09	17.5 ± 0.12	20 ± 1.84	21.75 ± 1.6	5 ± 1.56	30.0 ± 0.02	ND
C. albicans	10 ± 0.65	11.5 ± 1.35	13 ± 1.45	16.5 ± 1.43	19.5 ± 0.71	6 ± 1.15	ND	17.5 ± 0.42

Values are expressed as mean ± SEM, (G) indicates no inhibition zone; (ND) indicates not determined; (Cp) indicates Ciprofloxacin; (Nys) indicates Nystatin. n=4

Discussion

The extraction technique is a crucial stage because it will not only determine how the extract is obtained, but the polarity of the constituents that will be present. The percentage yield of 16. 86% obtained from the ethanol extraction of the powdered leaves of *A. muricata* (500 g) was high when compared to values obtained with the maceration method with other medicinal plants [25,26]. The maceration method which was used for the extraction process uses simple materials and very suitable for thermolabile plant materials, since it does not involve the use of heat [27].

Phytochemical screening of *A. muricata* revealed the presence of alkaloids, tannins and cardiac glycosides. These three secondary plant metabolites (alkaloids, tannins and cardiac glycosides) had earlier been reported to be present in the leaves of *A. muricata* [28]. Alkaloids are known for their biological activities such as anti-oxidant, muscle relaxant property, antimicrobial, anti-cancer and anti-diabetic activities [29]. Tanins have shown potential antiviral, antibacterial and anti-parasitic effects [30]. The observed anti-inflammatory and antimicrobial activities could be as a result of secondary metabolites present in the plant material.

Anti-inflammatory data for the leaf extract of *A. muricata* indicated reduction in the induced edema which was significant ($P<0.05$). The extract suppressed inflammation, and as the dose increased, the percentage inflammation decreased per time interval. It showed that the crude extract of the leaves administered at different dose concentrations demonstrated positive anti-inflammatory activity that was dose dependent. The ethanol leaf extract of *A. muricata* had earlier been reported to possess both anti-inflammatory and anti-arthritic activities [31], using xylene-induced ear edema in mice and complete Freund's adjuvant (CFA)-induced arthritis in rats.

The results of the sensory and physical evaluation of the toothpaste (Table 4) showed that they were not only smooth, but smelled pleasantly and tasted sweetly. These can be attributed to the presence peppermint oil and saccharin (served as flavouring agent and sweetener respectively). These attributes could enhance consumer acceptability.

Toothpaste having good foaming ability tends to provide good cleansing effects on the teeth. In other words, foaming ability is a measure of the cleansing power of the toothpaste, which can be affected by the presence of surfactants (i.e. sodium lauryl sulphate). Sodium lauryl sulphate produces foam that lowers the surface tension of the surface film on the tooth, thereby suspending and removing debris [32]. The foamability of the toothpaste can assist the cleaning ability of toothpaste because the foam is expected to help remove oil, food debris, microbe and unwanted particles in the mouth [33]. The formulated toothpaste containing *A. muricata* had the same foaming ability with the commercial toothpaste, indicating that both had equal cleansing strength.

Spreadability measures the extent of the area that the toothpaste can spread e.g. on the teeth, gum, gum lines and other areas, and the extent of penetration into the affected tooth and gum [32]. The results of the spreadability showed that the formulated herbal toothpaste was less than that of the toothpaste base and commercial toothpaste. This could be due to the interactions of the secondary plant metabolites present in the plant.

The reported pH range was 7.24 ± 1.92 indicating alkalinity. Neutral to mild alkaline pH of the toothpaste helps prevent acid mineralization of the teeth [34]. The pH of the formulated herbal toothpaste, which was higher than the toothpaste base could have been influenced by the presence of alkaloidal salts and bases (forms salts on reacting with the acid), resulting in an alkaline range. Development of dental diseases may be prevented when the teeth is kept at an alkaline range compared to an acidic range [33].

The antimicrobial activity of plant extracts is majorly linked to the presence of phytochemicals. The formulated toothpaste, containing the crude extract of *A. muricata* showed antimicrobial activities against the gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*); gram negative bacteria (*Escherichia coli*) and against the fungus *Candida albicans*. These inhibitions give credence to the fact that *Annona muricata* leaf extract exhibit antibacterial and antifungal activities [28,35] and hence can be used for the treatment of various illnesses caused by bacteria and fungi. The formulated toothpaste had a higher activity than the toothpaste base because

of the presence of the secondary plant metabolites present in the plant. The higher antimicrobial activity of the formulated herbal toothpaste compared to the crude extract could be due to the antimicrobial effects of some of the other constituents like peppermint oil and sodium lauryl sulphate [36-38].

Conclusion

Annona muricata possesses important chemical constituents which anti-inflammatory and antimicrobial properties. The crude extract of *A. muricata* as well as the formulated herbal toothpaste showed pronounced inhibitory activities against cariogenic organisms. This implies that bioactive constituents of *A. muricata* could be useful starting materials for the production of herbal toothpaste that could help to reduce the incidence(s) of dental diseases.

Conflict of Interest

No conflict of interest is associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. AAT conceived, designed and supervised the study. AAT and OLU collected, analyzed the data and prepared the manuscript. All the authors read and approved the final draft submitted.

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