

COMPARATIVE DETERMINATION OF ANTIMICROBIAL ACTIVITIES AND PHYTOCHEMICAL SCREENING ON SOME LOCAL HERBS (NEEM, GUAVA, LIME) USED AS CHEWING STICKS IN LAFIA –NASARAWA STATE, NIGERIA.

DENNIS D.Y; AND SAMUEL K.E

Department of Science Laboratory Technology, Nasarawa State Polytechnic- Lafia, Nigeria.

ABSTRACT

The comparative determination of antimicrobial and phytochemical screening of Neem, Guava, Orange stems extract was investigated. The samples were obtained by cutting the stems of Neem, Orange and Guava, it was then oven dried and the dried samples were powdered using manual blender and a fine powder was obtained. The powdered sample of the three plants were subjected to phytochemical screening, testing for presence of tannin, flavonoid, saponins, hydroxymethyl anthraquinone and reducing sugar according to the standard method. Neem, Guava and Orange extracts revealed that ethanol extract contains the following: saponins, flavonoid. Neem extract showed inhibition/growth for staphylococcus aureus. Orange extract showed inhibition on gram positive bacteria which is staphylococcus aureus

Introduction:

In many traditional cultures, there are no plastic-bristle brushes, rather, the use of herbs chewing sticks for retiring dental problems are common. The cleansing efficacy of chewing sticks is attributed to the mechanical effects of its fibres release of beneficial chemicals or a combination of both.

The use of chewing sticks has been documented since ancient times. This kind of tooth brushing has been used by the Babylonians some 7000 years ago (Almas, *et al.* 2004).

Humans have sought cures for diseases in nature since ancient times and even recently, the use of herbal medicine in dietary supplements, energy drinks,

rather than gram negative, while Guava extract showed inhibition activities against bacteria that are gram positive. The phytochemical screening revealed these antimicrobial activities in them. It is also in the light of this knowledge that most families in Lafia and some parts of our country Nigeria preferred the use of these chewing sticks rather than the conventional toothpaste sold for the treatment of mouth odour, infections & discoloured teeth. In this work, it is recommended that, the general public could use these herbal plants for the above mentioned correction and for its affordability, but with caution when being used due to the presence of tannins & saponins which have adverse effect on human health.

Keywords: *Comparative; Antimicrobial Activity; Phytochemical; Screening; Herbs.*

m ultivitamins, massage, and weight loss products has gained popularity (Petrovska, 2012). These uses broadened the field of herbal medicine and also increased its credibility.

The field of dentistry also has begun to exploit herbal properties for the purpose of relieving tooth pain, gum inflammation, and canker sores (Dileep, 2013). However, it is of utmost importance to understand the interactions of plant extracts with the body, which is important in dental growth.

Chewing sticks are twigs or roots of certain plants that are chewed until one end is frayed, this end used to brush against the teeth, while the other end can be used as a toothpick. Most commonly plants that are used have a high content of tannins (astringent and antibacterial) or other compounds that benefit the health gums and teeth (Wolf, *et al*, 2009).

Teeth cleaning twigs can be obtained from a variety of tree species. Although many trees are used in the production of teeth cleaning twigs, some trees are better suited to clean and protect the teeth, due to the chemical composition of the plant parts. There are more than 180 plant species that can be used as a natural toothbrush. These species differ from each other on the basis of appearance, scent, texture and taste (Zafar, 2016).

Chewing stick or Toothpaste, Nigerian researchers have demonstrated that three local chewing sticks performed better than fluoride-based and conventional toothpastes in preventing tooth decay, the local chewing sticks are: *Fagara zanthoxyloides* (candlewood or Senegal prickly ash/orinata in Yoruba), *Vernonia amygdalina* (bitter leaf) and *Massularia accuminata* (pako ijebu in Yoruba/atu uhie in Igbo). Researchers have also shown that one of the chewing sticks; *Massularia accuminata*, increased testosterone and libido (The Guardian, 2018).

JUSTIFICATION OF THE STUDY

A good industrialized toothpaste, are expensive and has mostly inorganic substances that can harm the mouth generally. Toothpaste can also cause problems. Nearly twenty percent of the population suffers from small painful oral ulcers called canker sores or aphthous ulcers. Recent research suggests that a detergent found in 99 percent of all toothpastes called Sodium Lauryl Sulfate (SLS) may induce canker sores in twenty percent of the population (SYS Inc, 2010).

High alarming rate of tooth pain and decay, Adults 20 to 64 have an average of 3.28 decayed or missing permanent teeth and 13.65 decayed and missing permanent surfaces. Hispanic subgroups and those with lower incomes have more severe decay in permanent teeth. Black and Hispanic subgroups and those with lower incomes have more untreated permanent teeth (NIHRC, 2018).

This research work is of high importance to everybody more especially Africans, who are more under developed countries and localities in the world, and are still living within the poverty line. Hence, need to still explore the use of local herbs for the treatment of some diseases not for Lafia people alone as the topic is tagged.

In every community in Nigeria, there is one or so anti-microbial herbs present hence the need to identify them for better use. It is on this note that, the need to determine the anti-microbial and phytochemical activities on some local dental herbs; Guava, Neem, Lime used as chewing sticks in Lafia the Nasarawa State Capital was carried out.

MATERIALS AND METHODS;**MATERIALS;**

In this study, the equipment, reagents, chemicals and materials used include the following; **For Materials** we had the following; Test Tubes, Filter Papers, Oven, Water bath, Incubator, Microscope, Pestle, Mortar, Measuring cylinder, Petri-dishes, Glass rod, wire loop. While **the Reagents and Chemicals include**; Chloroform, Sulfuric acid, Acetic acid, Ethanol, Fecl₃, Fehling's solution, Nutrient broth, Potassium ferroxide, Hydrochloric acid, Nutrient agar, Dragendoff's reagents, Distilled water.

METHODS;

The plant materials (the bark) of the guava, the Neem and the Lime were collected from where they are prominently grown and found in abundance at Akurba, a suburb area in Lafia East Development Area of Nasarawa State. These plant materials were brought into the laboratory in the Polytechnic for identification and analysis.

LABORATORY PREPARATION OF SAMPLES AND ANALYSIS

The bark stem of the three plants; Neem, Guava and Lime brought to the laboratory was washed to remove sand. This was then oven dried for 2 days until they are properly dried. The dried sample was polarized using a manual blender (mortal) to obtain fine powder. This was also sieved (0.2mm) and stored in air-tight container until it's required for the study.

LABORATORY ANALYSIS (SCREENING).

Phytochemical screening of extract: The powdered sample of the three plant (Neem, Lime, and Guava) were subjected to phytochemical screening testing for the presence of alkaloids, tannins, flavoniod saponins, Hydroxymethyl Anthraquinone and reducing sugar.

20g of each of the dried stem extract were soaked in 100ml of ethanol in a separate extraction apparatus. This was then filtered out with dust filter paper and then heated to concentrated 50ml.

Again 20g of each of the powdered stem were soaked in distilled water for 18hours. It was then filtered and stored.

Screening procedure;***Test for Alkaloids***

2mls of the three different extract were strived with 5ml of 1% aqueous HCL in a water bath. 1ml of the filtrate of each sample were of each sample were tested with few drops of dragendoff's reagents and a second 1ml with mayer's reagents. Turbidity and precipitation with either of those reagents were taken as evidence of the presence of alkaloid (Trease and Evans, 1989). Also Wagner's test, a few drops of Wagner's reagent was added to 1ml of the sample, Orange colouration of precipitation indicates the presence of alkaloids.

Test for Tannins

2ml of the alcoholic plant extract was stirred with 10ml of distilled water in each of the three plants and was heated in the water bath. 1ml of 1% FeCl₃ was added to each of the filtrates, Blue-black, green presence of tannins.

Test for Saponins

2ml of alcoholic extract was diluted with 10ml of distilled water in each of the extract and was heated in a water bath. After heating, it was shaken vigorously, stable forms indicates the presence of Saponins.

Test for Reducing Sugar

2ml of plant extract was added in the test tubes and 5ml of Fehling's solution was added to it and it was heated in the water bath for 5mins. The formation of brick-red precipitation or colouration indicates the presence of reducing sugar compounds.

Test for Flavonoids

2ml of 2% NaoH solution was mixed with sample extract, intensive yellow colour was formed, but became colourless upon adding 2 drops of diluted acid solution, this result indicate the presence of flavonoids.

Test for Hydroxymethyl

2ml of alcohol extract was tested to a solution of 5ml of 5% ammonia. The formation of a red colour or precipitates indicates the presence of hydroxymethyl anthraquinones.

Antimicrobial Activity/Antimicrobial Screening

Microbial Strains-Clinical isolates of *Staphylococcus aureus*, *Streptococcus viridians*, *Escherichia coli* and *Candida albicans* were all obtained from Specialist Hospital Lafia.

Antimicrobial screening

Agar-well diffusion method was employed (NCCLS, et al, 1999).

Innocular of test organisms obtained from source; Specialist Hospital Lafia were carried out by growing each pure isolate in a nutrient broth for 18hrs at 37oc.

0.2ml was then used to seed a molten nutrient agar medium cooled at 45oc. This was poured into sterile Petri-dish and used for analysis.

Determination of Minimum Inhibitory Concentration (MIC)

The agar-well dilution methods were used to extract and were incorporated into molten nutrient agar at concentration of 2.5, 5.0, 7.5, 10, 15 and 20mgml⁻¹. A loopful of the test isolate diluted 10⁸CfUml⁻¹ was used to streak the plates and incubated. The minimum inhibitory concentration of the extract was regarded as the lowest concentration that did not permit growth of the test organisms.

RESULTS AND DISCUSSION

From the above analysis and screening of the various extracts from these three plants, it showed that the stem of *Azadirachta indica* (Neem), Orange and Guava was found to contain Alkaloids, Tannins, Flavonoids, Saponin and hydroxymethyl. Notable absent are in reducing sugar of Guava and Orange but it is present in Neem.

The stem of Neem, Guava and Orange were found to contain tannin, saponins, flavonoids and hydroxymethyl.

Table 1: Phytochemical Screening of Neem, Orange and Guava

S/N Constituent	OHE	NHE	GHE
1. Alkaloids	+	+	+
(b) Dragendoff reagent	+	+	+
(c) Mayer Solution	+	+	+
2. Tannins	+	+	+
3. Saponins	+	+	+
4. Reducing sugar	-	+	-
5. Flavoniod	+	+	+
6. Hydroxymethyl	+	+	+

KEY

OHE = Orange Hexane Extract

NHE = Neem Hexane Extract

GHE = Guava Hexane Extract

+ = Positive or Present

- = Absent or Negative

Antimicrobial Screening;**Neem Tree Extract;**

Ethanoic Neem extract showed inhabitation/growth for staphylococcus aureus. This therefore means that it poses antibacterial activities against staphylococcus aureus, which is gram positive and not gram negative.

Lime Orange Extract;

Ethanoic lime extract showed inhabitation on gram positive bacteria which is staphylococcus aureus rather than gram negative bacteria.

Guava Extract;

The cruel solvent prepared from stem of psidium (guava). Ethanol showed inhabitation activity against bacteria that are gram positive which are susceptible to ethanoic guava extract made, while neither or the gram negative bacterium showed any inhabitation that is in guava stem ethanoic

extract, but was only able to have inhabitation effect against staphylococcus and bacillus and no effect on salmonella.

The Phytochemical Screening of Neem, Guava and Orange extract showed that the n-hexane extract of the stem contains high or positive amount of flavonoid, alkaloids, reducing sugar, tannin, saponin and hydroxymethyl. There is absence of reducing sugar in guava and orange stem. The presence of these chemicals compound in Neem, Guava and Orange stems indicates that they are rich in carbohydrate and also has high proteins content.

The anti-microbial screening carried out on Neem, Guava and Lime orange extracts showed great growth in gram positive bacterial which are staphylococcus and bacillus cereus and no growth for gram negative bacterial were found. The findings therefore, showed that ethanoic Orange, Guava and Neem extracts are active against only gram positive bacterial, but also possess antimicrobial effect over gram positive control on staphylococcus aureus specifically. Chewing stick has revealed parallel and at times greater mechanical and chemical cleansing of oral tissues as compared to a toothbrush (Rathee, *et al.* 2009). Chewing sticks contain anti-microbial substance called tannin, which was the same as fluoride in toothpastes. The natural tannin in it helped to fight bacteria in the mouth, maintained the gums, made the teeth stronger and improved the oral hygiene more than toothbrushes (Segun, 2013). The tannin is in the bark of the chewing stick, so it must not be removed to enjoy the tannin.

RECOMMENDATION;

Having carried out the phytochemical and antimicrobial analysis/activities of these three local plants herbs namely: Neem, Guava and Orange, use as chewing sticks, the paper recommend as follows:

1. Due to its availability & affordability, it is advisable that all who cannot go for the conventional toothpaste should use either of the herbal plants as chewing stick.
2. For the purpose of keeping a healthy/hygienic condition of the mouth always, these herbal herbs are recommended for such.
3. Also for an odour free mouth & freshness, either of these chewing sticks should be used for that purpose.

4. Government should find ways of processing/standardizing and commercializing these products.

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