

## STUDIES ON ANTIBACTERIAL ACTIVITIES OF SOME SELECTED CHEWING STICKS USED IN NORTHEASTERN NIGERIA

J. Muazu\*, S. G. Zakama, B. Timi, G. T. Mohammed and S. J. Madu

Department of Pharmaceutics and Pharmaceutical Microbiology University of Maiduguri,  
Nigeria.

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**\*Correspondence for  
Author**

**J. Muazu**

Department of  
Pharmaceutics and  
Pharmaceutical  
Microbiology University  
of Maiduguri, Nigeria.

### ABSTRACT

**Objective:** This study is aimed at assessing the antibacterial activity of the aqueous extract of some selected chewing sticks (*Azadirachta indica*, *Citrus sinensis*, *Vernonia amygdalina*, *Eucalyptus obliqua* and *Salvadora persica*) commonly used in Maiduguri Metropolitan Council and its environs in order to ratify their use in ethno medical treatment of oral infections. **Methods:** The antibacterial activity of the aqueous extract of some selected chewing sticks was assayed against some clinical isolates (*Staphylococcus aureus* and *Escherichia coli*) using the disc diffusion method. **Results:** The findings emanating from this study indicates a concentration dependent growth inhibition against *Staphylococcus aureus* ranging from 0.00 to 12.00 mm.

However only *Eucalyptus obliqua* showed graded dose activity against *Escherichia coli* (8.00-9.00 mm) with the other samples showing no activity. **Conclusion:** Conclusively, the study shows that apart from mechanical removal of debris, cleaning of gums and teeth, most of the chewing sticks have some antibacterial activities against some bacteria's that are not residents of the oral cavity, hence ratifying their use in ethno medical treatment of oral infections.

**KEYWORDS:** Chewing stick, Oral cavity, Antibacterial activity, Disc diffusion method and Northeastern Nigeria.

### INTRODUCTION

It is generally accepted that maintenance of oral hygiene through regular removal of dental plaque and food deposits is an essential factor in the prevention of dental caries and periodontal disease.<sup>[1]</sup> Methods for oral hygiene vary from country to country and from

culture to culture. Despite the widespread use of toothbrushes and toothpastes, natural methods of tooth cleaning using chewing sticks prepared from selected twigs, stems or roots from a variety of plant species have been practiced for thousands of years in Asia, Africa, the Middle East and the America.<sup>[1]</sup>

Chewing sticks are important Non Timber Forest Productions (NTFP) widely used for dental cleaning in the tropical West Africa.<sup>[2]</sup> Almost the entire rural population of Nigeria uses chewing sticks for orodental hygiene.<sup>[3]</sup> The efficacy of chewing sticks is attributed to mechanical effects of its fibers, release of beneficial chemicals or a combination of both <sup>[4]</sup> Chewing sticks are recommended for oral hygiene by the World Health Organization, and some of them, or their extracts, are also used in the ethno medical treatment of oral infections.<sup>[3]</sup>

Some of the plants used as chewing sticks in West Africa includes; *Terminalia arabica*, *Acacia arabica*, *Serindei wernecker*, *Masularia acuminita* and *Vernonia amygdalina*.<sup>[5]</sup> *Citrus aurantafolia*, *Citrus sinensis*, the roots of *Cassia sieberianba* were used in Sierra Leone and *Azadirachta indica*.<sup>[6]</sup> *Garcinia kola*, *Fagara xanthoxaloides* and *Bridelia ferruginea* are also used.<sup>[7]</sup>

The development of virile herbal toothpaste is consequent upon the bioactivity of the constituent chewing stick against a wide range of oral pathogens.<sup>[8]</sup> Thus, these studies aims at assessing the antibacterial activity of the aqueous extract of some chewing sticks used locally in some parts of North Eastern Nigeria and ratify their use in ethno medical treatment of oral infections.

## MATERIALS AND METHODS

**Source of plant material, collection and identification:** Five different samples of chewing sticks (*Azadirachta indica*, *Citrus sinensis*, *Vernonia amygdalina*, *Eucalyptus obliqua* and *Salvadora persica*) commonly utilized in Maiduguri Metropolitan Council (MMC) and its environs were collected April 2013, from MMC. The samples were subsequently identified by Professor S. S. Sanusi, a Taxonomist in the Department of Botany, University of Maiduguri.

**Preparation of Extracts:** The method used by Osho and Adeyemi<sup>[9]</sup> was adopted with some modifications. Fresh samples of chewing sticks (*Azadirachta indica*, *Citrus sinensis*,

*Vernonia amygdalina*, *Eucalyptus obliqua* and *Salvadora persica*) were air dried to a constant weight and subsequently chopped into bits prior to pulverization in a mill (TYPE YC100L-4, China) and stored in an air tight container for further use. A 10g weight of each of the pulverized chewing stick was put into a screw-capped bottle to which 100ml of sterile distilled water was added. The mixture was soaked for 48 hours with occasional mechanical shaking and subsequently centrifuged (Micro Centrifuge 4214, Italy) at 2000 rpm for 10 minutes. The supernatants were passed through a 0.45 mm membrane filter and the extract stored in a refrigerator.

**Source of the Microorganisms:** Clinical isolates of the test organisms (*Staphylococcus aureus* and *Escherichia coli*) were obtained from the Department of Medical Microbiology, University of Maiduguri Teaching Hospital (UMTH) and confirmed for identity using biochemical test with 24 hour broth culture.<sup>[10]</sup>

#### **Antibacterial activities of the selected samples**

##### **Sterilization of the equipment's and disinfection**

All work surfaces were mopped with a moist hand towel and was disinfected with Dettol® (Chlorhexidine and Cetrimide) so as to reduce microbial load on working surface.<sup>[11]</sup>

**Dry heat sterilization:** A hot air oven (Venticell, Germany) was used to sterilize the conical flasks, forceps, wire loop, pipettes, and beakers at 160 °C for 2 hr.<sup>[11]</sup>

**Moist heat sterilization:** Moisture insensitive equipment's and materials used for microbiological processes were sterilized in an autoclave (YX-280B, China) at 121°C for 15 minutes.<sup>[11]</sup>

**Preparation of Media:** The medium (Nutrient Agar and Nutrient broth) were prepared according to manufacturer's instruction and sterilized in an autoclave at 121 °C for 15 min. The agar was then poured into 90 mm diameter of sterile disposable plastic petri dish to a depth of 4mm. The plates were then dried upside down in an incubator (Surgifriend, England) at 37 °C with their lids opened and inverted so that moisture would not condense back into the Nutrient agar.

**Preparation of test organisms:** About 1ml each of the 24 hr pure broth culture of all the bacteria was added to 9 ml of sterile Sodium Chloride (NaCl) solution (8.5 mg/ml) and

sterilized in an autoclave at 121 °C for 15 min. 1 ml of these was then drained and added to another 9 ml of the NaCl solution to give a final dilution which was used for the study.

### **Preparation of discs containing graded concentration of the various test samples and positive control**

Whatman filter paper number one was punched into circular discs (6 mm in diameters), with the aid of an office punch. The discs were then placed into a glass petri dish and sterilized in a hot air oven at 60 °C for 30 min. 1 ml each of the different concentration (0.1, 1, 10 and 100 mg/ml) of the extract were pipetted into sterile glass plates and ten (10) sterile disc were then put into each glass plate of different concentrations using a sterile forceps (discs were checked to ensure that they don't stick to each other) to soak the extract and subsequently allowed to dry.<sup>[12]</sup>

The positive control was prepared by dissolving 100 mg of amoxicillin capsule (500 mg) powder in 1 ml of distilled water (100 mg/ml). Similar procedure for the treatment discs of the various extracts as above was then employed. This concentration was prepared because the pilot study revealed that the commercially available amoxicillin disc (20 mg) does not demonstrate antibacterial effect on the clinical isolates employed.

### **Antibacterial study**

Similar to Ndukwe *et al.*<sup>[13]</sup>, the disc diffusion method was adopted. 1 ml each of the  $c \times 10^3$  test organisms were introduced into the different plates and the excess discarded after sufficiently going round the surface of the entire medium. With the aid of a sterile forceps, impregnated paper discs containing the graded concentrations of extracts/control were arranged radially and pressed firmly onto the inoculated agar surface to ensure even contact. Each disc was sufficiently spaced out and kept at least 15 mm from the edge of the plate to prevent overlapping of zones. This was done in triplicate for each of the concentrations used. The plates were incubated at 37 °C for 24 hr. Diameters of zones of inhibition were measured and recorded using a transparent millimeter rule.<sup>[14]</sup>

### **Statistical Analysis**

Paired-Samples T-Test was used in the analysis to determine the level of significance of the various bacterial zones of inhibition observed. P-value < 0.05 was considered significant.

## RESULTS

**Table 1: Susceptibility test for *Staphylococcus aureus* showing zones of inhibition (mm) (n = 3)**

Plant extract	Concentration (mg/ml)			
	100	10	1	0.1
Positive control	14.00 ± 0.29	NT	NT	NT
<i>Eucalyptus obliqua</i>	11.00 ± 0.50	10.00 ± 1.50	8.00 ± 0.47	8.00 ± 0.47
<i>Azadirachta indica</i>	10.00 ± 0.99	9.00 ± 0.48	9.00 ± 0.91	9.00 ± 1.41
<i>Vernonia amygdalina</i>	12.00 ± 0.77	10.00 ± 0.55	8.00 ± 0.39	7.00 ± 0.91
<i>Salvadora persica</i>	10.00 ± 0.20	11.00 ± 1.45	12.00 ± 0.50	10.00 ± 0.19
<i>Citrus sinensis</i>	9.00 ± 1.62	8.00 ± 0.31	8.00 ± 1.30	0.00 ± 0.00

\*Indicates significant difference with positive control at p-value < 0.05. (T-Test), NT = Not tested, Positive control = Amoxicillin.

**Table 2: Susceptibility test for *Escherichia coli* showing zones of inhibition (mm) (n = 3)**

Plant extract	Concentration (mg/ml)			
	100	10	1	0.1
Positive control	10.00 ± 0.50	NT	NT	NT
<i>Eucalyptus obliqua</i>	9.00 ± 1.25	8.00 ± 1.50	8.00 ± 0.50	8.00 ± 0.86
<i>Azadirachta indica</i>	0.00 ± 0.00*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>Vernonia amygdalina</i>	0.00 ± 0.00*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>Salvadora persica</i>	0.00 ± 0.00*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>Citrus sinensis</i>	0.00 ± 0.00*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

\*Indicates significant difference with positive control at p-value < 0.05 (T-Test), NT = Not tested, Positive control = Amoxicillin.

**Susceptibility test for *Staphylococcus aureus***

As shown in Table 1, the result acquired designates a concentration dependent inhibition of the clinical isolate (*Staphylococcus aureus*) for the various aqueous extracts of the chewing sticks employed with the exception of *Salvadora persica* which shows its highest zone of inhibition at the concentration of 1 mg/ml. All the zones of inhibition for the aqueous extracts at 100mg/ml were compared with the positive control using Paired-Samples T-Test. No significant difference exists at p-value < 0.05.

**Susceptibility test for *Escherichia coli***

The aqueous extract of *Azadirachta indica*, *Vernonia amygdalina*, *Salvadora persica* and *Citrus sinensis* showed no activity against *Escherichia coli* at all the concentrations utilized. On the other hand, *Eucalyptus obliqua* demonstrated a concentration dependent response.

Paired-Samples T-Test was carried out for zones of inhibition at 100mg/ml and the result showed significant difference with all the extracts except for *Eucalyptus obliqua* (Table 2).

## DISCUSSION

Although, to the best of our knowledge, no study around our locality have been reported as to the antimicrobial effect of *Eucalyptus obliqua* extract as chewing stick, our findings is comparable to a study<sup>[15]</sup> **reported on antibacterial effect** of roots, stem-bark and leaves extracts of *Eucalyptus camaldulensis*. However, contrary findings<sup>[6]</sup> showed no inhibition against *Staphylococcus aureus* observed with aqueous stem extract of *Azadirachta indica*, while our study showed a similar result<sup>[16]</sup> to the reported a dose dependent effect with ethanol stem bark extract of *Azadirachta indica* against *Staphylococcus aureus*. Our findings are in concordance to results reported<sup>[5]</sup> and<sup>[17]</sup> which showed dose dependent effect with *Vernonia amygdalina* and *Salvadora persica* respectively. *Citrus sinensis* fruit peel aqueous extract also demonstrated growth inhibition against *staphylococcus aureus*<sup>[18]</sup> and thus supporting our findings. The positive control (amoxicillin 100 mg/ml) showed a slightly higher zone of inhibition compared with the aqueous extracts at same concentration (100 mg/ml). However, this difference is not significant at p-value < 0.05. The slight difference may be attributed to the fact that the positive control is a pure compound compared with the extracts utilized.<sup>[19]</sup>

The findings of these study also indicates that the aqueous extract of *Azadirachta indica*, *Salvadora persica* and *Citrus sinensis* as shown in Table 2 does not have any activity against *Escherichia coli*. These findings are contrary to findings<sup>[16], [17]</sup> and<sup>[18]</sup> respectively who all reported growth inhibition against *Escherichia coli* which could be attributed to difference in environmental condition which is believed to affect the chemical composition of plants.<sup>[20]</sup> However, similar to our findings, the aqueous extract of *Vernonia amygdalina* chewing stick had no effect on *Escherichia coli* at a concentration of 125 mg/ml.<sup>[5]</sup> *Eucalyptus obliqua* however shows a comparable activity with the positive control (100 mg/ml) but no significant difference at P-value < 0.05. These findings simulate that of reported results<sup>[15]</sup> that the water extract of the stem bark, leaf and roots of *Eucalyptus camaldulensis* demonstrated growth inhibition against *Escherichia coli*. The activity of the positive control is slightly higher due to similar reason stated above.

## CONCLUSION

This study established that apart from mechanical removal of debris, cleaning of gums and teeth, the aqueous extract of the five samples used as chewing sticks assayed have a dose dependent antibacterial activity against the clinical isolates employed, which according to reports are not residents of the oral cavity. It should however, be noted that as opposed to the concentration dependent effects of all the samples against *Staphylococcus aureus*, only *Eucaleptus obliqua* aqueous stem extract had effect on *Escherichia coli*.

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