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FULL LENGTH RESEARCH PAPER

In-Vitro Activity of the Extracts of Some Nigerian Plants used as Chewing Sticks on Human Odontopathogens

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ABSTRACT

The effects of water and chloroform extracts of five Nigerian chewing stick plants namely Fagara zanthoxyloides (fasa kwari) root Prosopis africana (kiryaa) stem/root, Guiera senegalensis (sabaaraa) stem, Khaya senegalensis (Madachi) stem/root and Azadirachta indica (Dogonyaro) stem/root on odontopathogens; Eikenella corrodens and Staphyllococcus aureus, which causes tooth decay in humans, was investigated. The isolates obtained from Dental Laboratory stock cultures of Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, Nigeria were treated with water and chloroform extracts of these plants for antibacterial activity using invitro well diffusion method. Residues of the extracts were made into suspensions using sterile distilled water at concentrations of (10mg/ml, 30mg/ml, 60mg/ml and 80mg/ml). K. senegalensis was the most effective followed by A. indica. The effectiveness of various extracts against E. corrodens and S. aureus, were in the decreasing order of Khaya senegalensis, A. indica, F. zanthoxyloides, P. africana and G. senegalensis. Extracts of G. senegalensis showed poor antibacterial activities. The leaves extracts of these plants at concentrations below 60mg/ml distorted the growth pattern of E. corrodens while Streptococcus sp. showed appreciable sensitivity at extract concentration of 80mg/ml. The chloroform extract was observed to show a greater distortion in growth pattern than the water extract. Distortions in the growth patterns of these odontopathogens and subsequent inhibition in growth at higher concentrations suggests that these plants may be valuable in the management of human oral infections.

Keywords: *oral infections, odontopathogens, plant chewing sticks stock cultures*

INTRODUCTION

Good oral hygiene is necessary for healthy teeth, gum and fresh breath. A number of methods are used in oral hygiene to prevent and cure oral diseases. It is of pertinent importance to look at the role plants play in oral hygiene as a number of them have medicinal properties. When compared to toothpaste, mouthwashes and denitrifies, plants used for oral hygiene stand out. In many African homes, teeth are cleaned in the morning by chewing the root or slim stem of certain plants until they acquire brush-like ends. The Babylonians recorded the use of chewing sticks in 7000 BC and its use ultimately spread throughout the Greek and Roman Empires, it is also used by Egyptians, Jews and in the Islamic empires. It is believed that the counterpart of the modern day toothbrush was unknown in Europe until about 300 years ago. Presently, chewing sticks are being used in Africa, South America, the Middle East and Asia. Oral hygiene is the practice of keeping the mouth clean and healthy by brushing and flossing to prevent tooth decay and gum diseases. The chewing stick has different names depending on different societies for instance, Miswak, Siwak or Arak is used in Middle East, Miswaki in Tanzania, Datan in India and Pakistan. It is used deeply in many cultures (Almas, 1995). A number of plants are used as chewing sticks in West Africa, the lime tree (*Citrus aurantifolia*) and the orange tree citrus (*Citrus sinensis*) sometimes provides chewing sticks. The roots of senna (*Cassia vinnea*) were used by American negroes and those of African Laburnum (*Cassia sieberiana*) were used in Sierra Leone. Neem (*Azadirachta indica*) is widely used to provide chewing sticks in the Indian sub-continent, these

are just to mention a few of the plants used as chewing sticks.
Chewing sticks impact varying taste sensations, a tingling, peppery taste and numbness is provided by *Zanthoxylum zanthoxyloides* waterman *(Fagara zanthoxyloides* lam) root, a strong bitter taste and frothing from *Masularia accuminata* (G. Don) Bullox ex Hoyle stem and initial bitterness becoming sweet later from *Vernonia amygdalina* (Deli root). The root of *Terminalia glaucesens* planch, produces a discolouration of the mouth. The most popular chewing sticks are ones having good flavour and texture and a recognised effect on teeth and supporting tissues. Freshly cut specimens are always desirable because they are more easily chewed into a brush. Some of them possess such tough fibers that penetrate the gum during use thus causing some discomfort (Lewis, 1980).

Some parts of other plants are also used for oral health, such parts include leaves of Eucalyptus, which is been chewed to cure bad breath, preparation of onion and lime juices can also be gargled to relieve gingivitis or toothache, Nutmeg can also be used for sweet breath and masking bad breath when chewed and held in the mouth. Barks of some plants are also used in oral health such as sour sop. Seeds of some plants can also be useful in oral hygiene; examples are seed of cloves and others. Modern toothpaste, mouthwashes, dentrifices, etc. are available in different forms, types, sizes, colours, and in different packs. Most toothpastes are clearly marked out with fluoride which is an element that is expected to prevent tooth decay. Some other chemical components of toothpastes are Aqua, hydrated silica, sorbitol, glycerin, sodium lauryl sulphate, flavour, titanium dioxide, xanthan gum, sodium saccharin, sodium fluoride and a number of others.

For modern cleaning to take place in our daily lives, a toothbrush is required which comes in different shapes, texture (hard or soft), colours etc. It is important to know which type of toothbrush is best for us; some tooth brushing cannot take away the remnants of food at the corners of the mouth, in between the teeth and even the inner sides of the cheeks (Akinremisi, 1997).

The oral cavity contains the teeth used for mastication, the gum surrounded by periodontium and alviolar bone, the root of the mouth is known as the hard plate and posterior to it is the soft plate. These and other inner tissues of the cheeks are lined with oral mucous. The tongue is a mobile muscular organ contained in the mouth; it is concerned with speech, mastication, swallowing and taste because taste buds are present on it. The lips preventing contents in the mouth from falling out of the mouth. Since the oral cavity plays these roles with the various parts inside it, it becomes of paramount importance to take the best care of it using the effective methods, materials and making the best use of the resources that are available for oral hygiene (Cawson, 1984).

Plants used in oral hygiene

A number of plants are used in oral hygiene; some of them are herbs, shrubs and trees. The following plants and their various parts are used in oral hygiene for healthy teeth gum and fresh breath.

Azadirachta Indica, *Melia azadirachta*
Others names: Neem, Nimb, Nimba.
Local names: Dogonyaro (Hausa), Eke-Oyinbo (Yoruba)
Azadirachta indica belongs to the family Meliaceae.

Originally, native to India but it is seen in Indonesia, Malaysia, Australia, Sri Lanka, Burma, Pakistan and Africa. In Nigeria it is found commonly on farmlands, homes and streets. It is one of the fastest growing trees in semi-arid and arid regions. *Azadirachta indica* has been used for centuries as the country store of developing nations. The Neem posses different compounds example is azadirachta-A to azadirachtin-G (Hardie, 1995).

Other forms of Oral Hygiene Using Plants
Dentrifices
Abrasive substances in most commercially available toothpastes are now largely of inorganic origin, but in place of this, natural abrasives are widely used. Plants are used as abrasives; examples of such plants are *Acorus calamus* (sweet flag) and *Rumex crispus* (yellow dock) whose powdered roots are used in Europe and North America.

Similarly, the powdered bark of *Cinchona officinalis* (Peruvian bark) is used in South East Asia, Europe and North America. Ashes of burned branches of *Vitis vinifera* (European grape) are used as dentrifice in England. Also the powdered stem of toothbrush tree is used in Central America. Users also attribute gum strengthening and tooth whitening properties to these natural products (Hardie, 1995).

Chewing Gums
Gums from *Silphium spp*. (Rosinweed) were used by North American Indians and early settlers, to clean their teeth and keep them white. Also gum from *Myroxylan balsamum* is chewed continuously by Indians in South America to keep their teeth clean, to tighten and sooth them as well. Gum exudates from the trunk of *Croton*

xalapensis is likewise used in Mexico for cleaning teeth. In addition to the chewing gums from plants, latex is also chewed from plants such as *Ficus platyphylla*. These gums and latex are quite bitter, unlike the commercially available gums that are flavoured with mint and sugar to improve taste and flavour. Sugary substances encourage the incidence of caries. Therefore, use of plants (gums and latex has an advantage over commercial chewing gums that promote caries.

Chewing Sponges

These are popular means of cleaning teeth in Ghana. They are prepared from certain plants such as *Acacia pennata, Hibiscus rostellatus* and *Lasiant hera africana*, which grows in cape coast region. The stems or vines are collected from the forest, bark removed and beaten on rocks until they become fibrous. This is washed and made into sponges that are about five inches in diameter. To clean teeth, a small portion of the sponge is placed in the mouth and vigorously chewed for few minutes (20-30 minutes). This activity produces foam and stimulates saliva flow. After chewing the sponge is then taken in the fingers and nabbed over the teeth and gums. Water is then used to rinse the mouth (Hardie, 1995). The present work was aimed at providing baseline knowledge on the efficacy of our indigenous chewing stick plant extract on human odontopathogens

MATERIALS AND METHODS

Fresh and dried plant samples were obtained from Herb sellers and identified at the herbarium of the Botany Unit Usmanu Danfodiyo University, Sokoto, Nigeria. Fresh chewing stick of the selected plants were collected and dried in the oven at a temperature of 60^{o}C. The dried stem/root was ground to a powder.

Extract preparation

Separately 400g each of the powder was suspended in 500ml of distilled water in a litre flask for 24 hrs at room temperature. The mixture was then sieved through a fine muslin cloth followed by filtration using filter paper to trap the finer particles that went through the cloth. The filtrate was then mixed with the chloroform in a separating funnel, the mixture shaken until separation was observed in form of two layers; the water top and the chloroform extract lower layer. The different layers were run out into separate beakers and placed in an oven to dry at 50^{o} C. Residues of the extracts were made into suspensions using sterile distilled water at concentrations of 10mg/ml, 30mg/ml, 60mg/ml and 80mg/ml (Cheesbrough, 2000).

Culture of the test organisms

The Bacterial cultures used in this study were obtained from the Dental Laboratory stock cultures of Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria (UDUTH). The stock cultures, odontopathogens *Eikenella corrodens* and *Staphyllococcus aureus* were, isolated from human decayed tooth.

Standardization of the inoculum

The test organisms were suspended in prepared broth and incubated at 37°C for 24 hours.

They were then inoculated into prepared molten agar medium by surface plating method. The paper discs prepared earlier were placed at 45° opposite to each other on the plate containing the bacterial culture, and all the plates were labelled and incubated at 37°C for 24 hours. Clear zones of inhibition were observed after the incubation period (Cheesbrough, 2000).

Assay Procedure

The *E. corrodens* and *S. aureus* were grown on nutrient agar and maintained on slants. The agar well diffusion method was employed. Four 9mm ditches (wells) were made in each plate of solidified nutrient agar. Five (5) mls of each concentration of the extracts (10mg/ml, 30mg/ml, 60mg/ml and 80mg/ml) was measured out and mixed with 0.3g of agar. The well was carefully filled with this mixture and left to solidify. Each of the plates were seeded with each of the different odontopathogens and incubated in the incubator for 24 hrs. The zone of inhibition diameter was measured using a meter ruler to the nearest whole number of a millimetre (Cheesbrough, 2000).

RESULTS AND DISCUSSION

The activity of *khaya senegalensis Azadirachta indica, Fagara zanthoxyloides Prosopis africana* and *Guiera senegalensis* stem/root extracts on the growth of odontopathogens; *Eikenella corrodens* and *Staphyllococcus aureus,* are presented in Tables 1 to 5 respectively.

Table 1 and 2 shows the activity of chloroform and water extracts of the root/stem of *K. senegalensis* and *A. indica* tested against *E. corrodens* and *S. aureus.* The concentrations below 60mg/ml showed growth inhibition zones at various degrees. While Tables 3, 4 and 5 shows that the performance of chloroform and water extracts of the stem/root of F. *zanthoxyloides P. africana* and *G. senegalensis* against *E. corrodens* and *S. aureus* respectively was appreciable. In all the trials chloroform extracts were observed to show a greater distortion in growth pattern than the water extracts.

Table 1: Activity of chloroform and water extracts of the root/stem of *Khaya senegalensis* on the growth of *E. corrodens* and *S. aureus*

Test Isolate	Extract	Concentration mg/ml	Diameter of inhibition zones (mm)
E. corrodens	Water	10	6
		30	6
		60	8
		80	9
	Chloroform	10	10
		30	10
		60	21
		80	21
S. aureus	Water	10	6
		30	6
		60	7
		80	8
	Chloroform	10	9
		30	9
		60	20
		80	20

Table 2: Activity of chloroform and water extracts of the root/stem of *Azadirachta indica* on the growth of *Eikenella corrodens* and *Staphyllococcus aureus*

Test Isolate	Extract	Concentration mg/ml	Diameter of inhibition zones (mm)
E. corrodens			
	Water	10	5
		30	5
		60	6
		80	8
	Chloroform	10	6
		20	7
		30	12
		80	18
S. aureus	Water	10	5
		30	5
		60	6
		80	8
	Chloroform	10	6
		20	8
		30	9
		80	16

Table 3: Activity of chloroform and water extracts of the root/stem of *Fagara zanthoxyloides* on the growth of *Eikenella corrodens* and *Staphyllococcus aureus*

Test Isolate	**Extract**	**Concentration mg/ml**	**Diameter of inhibition zones (mm)**
E. corrodens	Water	10	0
		30	4
		60	11
		80	16
	Chloroform	10	9
		30	14
		60	16
		80	18
S. aureus	Water	10	0
		30	2
		60	9
		80	15
	Chloroform	10	15
		30	17
		60	18
		80	18

Table 4: Activity of chloroform and water extracts of the root/stem of *Prosopis africana* on the growth of *Eikenella corrodens and Staphyllococcus aureuss*

Test Isolate	Extract	Concentration **mg/ml**	Diameter of inhibition zones **(mm)**
E. corrodens	Water	10	0
		30	0
		60	5
		80	6
	Chloroform	10	2
		30	4
		60	8
		80	12
S. aureus	Water	10	0
		30	0
		60	5
		80	6
	Chloroform	10	0
		30	0
		60	6
		80	12

Table 5: Activity of chloroform and water extracts of the stem of *Guiera senegalensis* on the growth of *Eikenella corrodens and Staphyllococcus aureus*

Test Isolate	Extract	Concentration **mg/ml**	Diameter of inhibition zones **(mm)**
E. corrodens	Water	10	0
		30	0
		60	0
		80	6
	Chloroform	10	4
		30	4
		60	8
		80	18
S. aureus	Water	10	0
		30	0
		60	2
		80	2
	Chloroform	10	4
		30	6
		60	9
		80	16

According to Vlicktnick *et al*. (1995) antibacterial activity is recorded when the zone of inhibition is greater or equal to 9mm. The implication here being that for *E. corrodens* and *S. aureus* antibacterial activity were reported for all concentrations in both the water and chloroform extracts. For the extracts of *G. senegalensis* only mild antibacterial activity was recorded at the highest concentration in both the water and chloroform extracts. For the extracts of *F. zanthoxyloides* and *P. africana* there were no activities at the lowest concentration for water extracts but as the concentration increased antibacterial activities were recorded.

Similar results were obtained by Ahmad *et al*. (2000), Bonjar (2004), Muhammad and Muhammad (2005). The use of chewing sticks, infusion from plants as Astringents, chewing gums and sponges from plants remains effective means of preventing, controlling and curing oral diseases in all users irrespective of age. The usage of plants has been practiced from time immemorial and their effects on oral hygiene have been encouraging. The continual usage is very important so as to prevent and cure oral diseases that affect individuals. If constantly used, the incidence of oral diseases will reduce to the lowest level, because of the natural healing substances that are present in them.

Tooth brushing with brushes and fluoride based toothpastes have been found to be accompanied by some disadvantages such as the usage of hard toothbrushes with horizontal sawing action which causes abrasion in adults, tooth brushing is also suspected to be responsible for erosion of tooth. A number of disadvantages are also associated with fluoride based toothpastes; these plants have been seen to have an upper hand in curing oral diseases rather than incurring infections or diseases to the users (Sofowora, 1993).

The present study showed that F. *zanthoxyloides, P. africana, G. senegalensis*, *K. senegalensis A. indica* root/stem extracts were capable of inhibiting the growth of the two odontopathogens studied. These findings open the possibility of extracting bactericidal chemicals from these plants.

The possibility of the active compounds of the extracts of these chewing sticks being effective is shown by their inhibition on growth, this study exposed the possibility of these compounds being responsible for observed antibacterial activities of their extracts. This opens a way for more research into this area to bring out conclusively the actual active compounds which may be the basis for large-scale production of new generation non synthetic toothpaste.

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