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Phytochemical Composition and Antimicrobial Activity of *Prosopis africana* Against Some Selected Oral Pathogens

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Abstract: Studies were carried out on phytochemical composition and antimicrobial activity of aqueous and ethanol extract of root and stem of *Prosopis africana* against clinical isolates of *Candida albicans, Streptococcus mutans* and *Staphylococcus saprophyticus*. Saponins, tannins and alkaloids were highly concentrated in the stem and root, with the former containing a significantly higher ($P<0.05$) quantity of these phytochemicals. Phenols and steroids were also present in the investigated plant parts. Results obtained revealed that both ethanol and aqueous extracts of the plant parts exhibited inhibitory effect on the growth of the tested microorganisms. For both aqueous and ethanol extracts, the inhibitory effect of the stem extract on *Candida albicans* was significantly higher ($P<0.05$) than that exhibited by the root extracts. In addition, ethanol extract exhibited a significant higher ($P<0.05$) inhibitory effect on *C. albicans* when compared to water extract.The inhibitory effects produced by the aqueous and ethanol extracts on *Streptococcus mutans* and *Staphylococcus saprophyticus* were not significantly different ($P>0.05$). In a similar trend, the effects produced by the stem and root extracts on *S.mutans* and *S. saprophyticus* were not significantly different ($P>0.05$). Data from the present study have implicated the stem and root of *P. africana* as a potential candidate plant parts in dentrifice production.

Key words: *Candida albicans* · Chewing stick · Dentifrice · *Prosopis africana* · *Staphylococcus saprophyticus* · *Streptococcus mutans*

INTRODUCTION

There is a long and venerable history of the use of plants to improve dental health and promote oral hygiene. In vast parts of the world where tooth brushing is uncommon, the practice of tooth cleaning by chewing sticks has been known since antiquity. The use of chewing stick persists today among many African and southern Asian communities as well as in isolated areas of tropical America and southern United States [1]. The plants used as chewing sticks are carefully selected for such properties as foaminess, hardness or bitterness. Kerry [2] reported that plants have also been incorporated into dentifrices and there are several modern examples of this practice. He further stated that plants are also used to provide natural chewing gums for oral hygiene, to treat toothache, gingivitis and periodontal disease.

Akande and Hayashi [3] reported that in Nigeria, some of the chewing sticks being used are obtained from the following plants: *Garcinia manni, Masularia accuminata, terminalia glaucescens, Anogeissus leiocarpus, Pseudocedrela kotschyi, Xanthoxyllum gilletti and Azadiracta indica.* In a related development, Agboola [4] stated that *Prosopis africana* is used as chewing stick by Yorubas in south western Nigeria.

P. africana is a perennial leguminous tree of the subfamily Mimosidae [5] and is mostly found growing in the savanna regions of Western Africa. In many areas, its fermented seeds are used as a food condiment [4] and its young leaves and shoots are fodder that is highly sought after towards the end of the dry season. Almost all parts of the tree are used in medicine. In Mali the leaves, bark, twigs and roots are used to treat and relieve bronchitis, dermatitis, tooth decay, dysentery, malaria and stomach cramps. In Ghana, boiled roots serve as poultice for sore throat, root decoction for toothache and bark as a dressing or lotion for wounds or cuts [6].

The need to process and package indigenous medicinal plants that are of oral importance into herbal toothpaste has been proposed [7]. Interestingly, barely two years after this proposition, a particular Nigeria-based company released a plant based dentifrice into Nigerian

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market. However, this present trend requires that the bioactivity of many of these medicinal plants against common oral pathogens be scientifically established. In this regard, the present work focused on providing scientific information on the phytochemical composition and antimicrobial activity of aqueous and ethanol extracts of stem and root of *P. africana on* oral pathogens such as *Candida albicans, Streptococcus mutans* and *Staphylococcus saprophyticus*.

MATERIALS AND METHODS

Plant Collection and Pre-extraction Preparation: Different plant parts such as leaves, stem, root and fruit of *P. africana* were collected from Oke-Ogun axis of south Western Nigeria (a woody Savannah vegetation). The plant was identified by a plant Taxonomist at the Forestry Research Institute of Nigeria, Ibadan. Nigeria. The stem and root of the plant was sun-dried for seven days, pounded using pestle and wooden mortar.

Extraction Procedure: The ethanol extract preparation was done as previously described by Ogundiya *et al*. [7]. However, for water extraction, the procedure was basically the same except that soaking was done for 48 h and the filterate was evaporated to dryness. The crude extracts were reconstituted into aqueous solution using sterile distilled water to obtain extract concentrations of 0.4 and 0.2 g ml^{-1}.

Microorganisms: Pure cultures of *Candida albicans, Streptococcus mutans and Staphylococcus saprophyticus* isolated from patients with dental diseases were obtained from the Medical Microbiology Department of the University College Hospital (UCH) Ibadan. Nigeria. Bacterial cultures were maintained on Nutrient agar slant and the fungus on Potato dextrose agar slant, both at 6-8°C.

Phytochemical Studies: Both qualitative and quantitative analyses of the phytochemicals present were carried out using methods described by Fadeyi *et al*. [8] and Harbone [9].

Antimicrobial Assay: The antimicrobial activity of different concentrations of both ethanol and aqueous extracts was determined by modified agar-well diffusion method of Perez *et al*. [10] as described by Popoola *et al*. [11]. The bacterial plates were incubated at 37°C (fungal plates at 28°C) and the zone of inhibition measured in mm after 24 h, 48 h and 72 h of growth. A control experiment was set up by using an equal amount of sterile distilled water in place of different extract concentrations.

Statistical Analysis of Data: Data were expressed as mean±standard deviation. The data obtained were subjected to ANOVA test to determine whether there was significant difference between extract used and also between the length of incubation.

RESULTS

Table 1 shows the results of phytochemical analysis of the stem and root of *P. africana*. Saponnins, alkaloids and tannins are highly concentrated in the stem and root of this tested plant, with those in the stem being significantly higher ($P<0.05$). To a lesser extent, phenolic and steroidal compounds are also present in the investigated plant parts. Cyanoglycoside was present in the root while it was not detected in the stem.

The results of the antimicrobial assay of the root and stem extract of *P. africana* is presented in Table 2-4.

Table 1: Results of the quantitative estimation of the phytochemicals (mg/100g) present in the ethanol extracts of *Prosopis africana*

	Alkaloid	Steroid	Phenol	Tannin	Cyanoglycoside	Saponnin
Stem	102.4	8.8	10.2	85.9	ND	110.0
Root	73.2	4.6	4.1	31.0	1.5	87.7

Values are mean of triplicate determinations. ND implies not detected

Table 2: Inhibition of *Candida albicans* by aqueous and ethanol extract of *Prosopis africana*

		Aqueous extract		Ethanol extract	
		Concentration (g ml^{-1})			
Plant part	Incubation period (h)	0.4	0.2	0.4	0.2
Root	24	19.5±0.5	17.5±2.0	31.5±0.5	31.0± 4.0
	48	28.5±0.5	26.5±1.5	30.5±3.5	30.0±6.0
	72	10.5±0.5	10.5±0.5	30.5±3.5	31.5±4.5
Stem	24	40.0±3.0	35.5±1.5	40.0±0.0	38.0±0.0
	48	27.0±1.0	27.5±0.5	35.5±0.5	38.0±2.0
	72	23.0±1.2	24.0±2.5	33.5±3.0	32.5±1.5

Values are mean±standard deviation (n = 3)

Table 3: Inhibition of *Streptococcus mutans* by aqueous and ethanol extract of *Prosopis africana*

		Aqueous extract		Ethanol extract	
		Concentration (g ml^{-1})			
Plant part	Incubation period (h)	0.4	0.2	0.4	0.2
Root	24	28.0±1.0	27.5±2.5	26.5±2.5	24.5± 1.5
	48	29.0±2.0	28.0±1.0	35.5±4.5	34.5±5.5
	72	28.0±1.6	27.0±1.2	35.5±4.5	34.5±5.5
Stem	24	26.5±0.5	26.5±3.5	36.5±2.0	34.0±2.0
	48	28.0±2.0	22.0±3.0	35.0±5.0	33.0±7.0
	72	29.5±0.5	16.5±4.5	28.5±3.5	27.5±2.5

Values are mean±standard deviation (n=3)

Table 4: Inhibition of *Staphylococcus saprophyticus* by aqueous and ethanol extract of *Prosopis africana*

		Aqueous extract		Ethanol extract	
		Concentration (g ml^{-1})			
Plant part	Incubation period (h)	0.4	0.2	0.4	0.2
Root	24	22.0±1.0	17.0±1.0	30.0±2.0	28.5± 2.0
	48	35.5±2.5	34.5±1.5	29.5±1.5	27.0±0.0
	72	21.5±0.3	16.5±2.5	30.5±2.0	28.0±1.0
Stem	24	32.5±4.0	24.5±3.5	39.0±0.0	34.0±2.0
	48	30.0±2.0	33.5±0.5	34.5±3.5	31.5±0.5
	72	10.5±0.5	14.0±2.0	34.0±2.0	33.5±4.5

Values are mean±standard deviation (n=3)

Results obtained revealed that both ethanol and aqueous extracts of the plant parts exhibited inhibitory effect on the growth of the tested microorganisms. For both aqueous and ethanol extraction, the inhibitory effect of the stem extract on *Candida albicans* was significantly higher ($P<0.05$) than that exhibited by the root extracts. In addition, ethanol extract exhibited a significant higher ($P<0.05$) inhibitory effect on *C. albicans* when compared to water extract.

The inhibitory effects produced by the aqueous and ethanol extracts on *Streptococcus mutans* were not significantly different ($P>0.05$). In a similar trend, the effects produced by the stem and root extracts on *S.mutans* were not significantly different ($P>0.05$). ANOVA test of the data obtained from the antimicrobial assay of the different extracts on *Staphylococcus saprophyticus* revealed a trend similar to that observed on *Streptococcus mutans.*

DISCUSSION

Various chemicals such as alkaloids, tannins, saponnins, cyanoglycosides, terpenoids, oleic and stearic acids which are naturally present in plants have been implicated in the confernment of antimicrobial activities on the plant containing them [12,13,14]. The presence of some of these plant secondary metabolites in a significant amount in the investigated parts of *P. africana* may have conferred antimicrobial activity on both stem and root extracts of this plant. In this regard, the higher concentration of these phytochemicals in the stem extract may have been responsible for a significantly higher ($P<0.05$) inhibition exhibited by the stem extract on *C. albicans*, when compared to root extracts.

It has been reported that the root decoction of *P. africana* is used to treat toothache in Ghana and the bark and root used to treat and relieve tooth decay in Mali [6]. Results from the present study have given scientific basis for such traditional medicine practices. In addition, the relative efficiency of each part of *P. africana* to inhibit the growth of oral pathogenes has been presented in the present report.

Higher plants, as sources of medicinal compounds continue to play dominant role in maintenance of human health since antiquities. Over 50% of all modern clinical drugs are of natural product origin [15] and natural products play an important role in drug development programs of the pharmaceutical industry [16,17]. In this regard, the use of plants in the production of dentifrice and natural chewing gums for oral hygiene and to treat toothache, gingivitis and periodontal disease has been reported by Kerry [2]. Data from the present study have implicated the stem and root of *P. africana* as a potential candidate plant parts in such application. And it is hoped that this discovery would be utilized to better the oral health of Africans whose landscape is replete with this plant.

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