International Scholars Journals

International Journal of Medicinal Plants Research ISSN 2169-303X Vol. 8 (12), pp. 001-004, December, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Phytochemical composition and *in vitro* antimicrobial activity of *Anogeissus leiocarpus* on some common oral pathogens

Adejumobi, J.A., Ogundiya, M. O.[1,2], Kolapo, A.L[2] and Okunade, M. B[1]

[1]Department of Chemistry, The Polytechnic, Ibadan. Nigeria.
[2]Department of Biology, The Polytechnic, Ibadan. Nigeria

Accepted 19 October, 2019

An assessment of phytochemical composition and antimicrobial activity of aqueous and ethanolic extract of root and stem of *A. leiocarpus* against clinical isolates of *Candida albicans, Streptococcus mutans* and *Staphylococcus saprophyticus* was carried out. Saponins, tannins and alkaloids were highly concentrated in the stem and root, with the later containing a significantly higher (P<0.05) quantity of these phytochemicals. The results of investigation showed that all the extracts had inhibitory effect on the growth of all the isolates. For both aqueous and ethanol extracts, a two way ANOVA test revealed that extract concentrations did not have significant effect (P>0.05) on the inhibition of *C. albicans* while the length of incubation period had a significant effect (P<0.05) on its inhibition. The inhibitory effect produced by the ethanol extract of the root and stem on *S. saprophyticus* and *S. mutans* was significantly higher (P<0.05) than the effect produced by aqueous extract. On a general note, root extracts exhibited significant inhibition (P<0.05) compared to stem extracts. Results from this study strongly indicates that *A. leiocarpus* is a potential candidate plant whose extract could be incorporated into dentifrice.

Key words: *Anogeissus leiocarpus Candida albicans*, chewing stick, dentifrice, oral pathogen, *Staphylococcus saprophyticus*, *Streptococcus mutans*.

INTRODUCTION

Anogeissus leiocarpus is a graceful tree of the Sahel to forest zones, straight tapering boles branching from low down, often gregarious and effectively killing out grasses (Dalziel, 1937) . The leaves serve as fodder to livestock (Burkill, 1985). It is also used in traditional medicine as a remedy for many ailments of livestock and man, which include helminthosis, schistomiasis, leprosy, diarrhea and psoriasis (Burkill, 1985; Onyeyili 2000). In addition to these applications, Hollist (2004) reported that *A. leiocarpus* is one of the major plants commonly used as chewing stick in Nigeria. Its use in the treatment of oral disease such as thrush and black tongue was also reported by this same author.

Herbal remedies have long history of use for gum and tooth problems. In many traditional cultures, there are no plastic-bristle brushes, rather, the use of herbal chewing sticks for relieving dental problems is common (Bo, 2008).

*Corresponding author. E-mail: adelodunkolapo@yahoo.com

Many studies have demonstrated the antimicrobial, anti-carries, anti-periopathic and antifungal properties of both aqueous and ethanolic extracts of various chewing sticks (Buada and Boak-Yiadom, 1973; Rotimi et al., 1988; Akande and Hayashi, 1998; Ugoji et al., 2000).

There are documented reports on the antimicrobial activity of *A. leiocarpus* on oral microflora. Ndukwe et al. (2005) reported the antimicrobial effect of its root extract on *Staphylococcus aureus* and *Pseudomononas aeroginosa*. Rotimi et al. (1988) documented the antibacterial activity of its bark extract on *Bacteriodes gingivalis* and *Bacteriodes malaninogenicus*. In an earlier report, we reported a significantly higher antibacterial activity of ethanolic extract of *A. leiocarpus* root against *Staphylococcus aureus* and *Streptococcus pyogenes* (Ogundiya et al., 2006).

The need to process and package indigenous medicinal plants that are of oral importance into toothpaste has been proposed (Ogundiya et al., 2006). However, this requires that the bioactivity of these medi-

Table 1. Results of the quantitative estimation of the phytochemicals (mg/100g) present in the ethanol extracts of *Anoeissus leiocarpus*.

	Alkaloid	Steroid	Phenol	Tannin	Cyanoglycoside	Saponnin
Stem	29.5	13.5	3.3	32.5	ND	73.0
Root	47.0	5.5	9.5	71.9	0.9	89.5

Values are mean of triplicate determinations. ND implies not detected.

medicinal plants against common oral pathogens be scientifically established. In this regard, the present study was aimed at providing information on the phytochemical composition and antimicrobial activity of aqueous and ethanol extracts of stem and root of *A. leiocarpus* 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 *A. leiocarpus* 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 were 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. (2006). However, for water extraction, the procedure was basically the same except that soaking was done for 48 h and the filtrate 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.

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^{O}C.

Phytochemical studies

Both qualitative and quantitative analyses of the phytochemicals present were carried out using methods described by Fadeyi et al. (1987) and Harbone (1998).

Antimicrobial assay

The antimicrobial activity of different concentrations of both ethanolic and aqueous extracts was determined by modified agar-well diffusion method of Perez et al. (1990) as described by Poppola et al. (2007). The bacterial plates were incubated at 37^{O}C (fungal plates at 28^{O}C) and the zone of inhibition measured in mm after 24, 48 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

The results of phytochemical analysis of the stem and root extract of *A. leiocarpus* is shown in Table 1. Saponnins, tannins and alkaloids were highly concentrated in the stem and root of the tested plant, with those contained in the root being significantly higher ($P<0.05$). Steroidal compounds in the stem extract were higher while the root contained higher quantity of phenol than the stem. Cyanoglycosides was not detected in the stem extract while the root contained a small quantity of this phytochemical.

The results of the antimicrobial assay of the root and stem extract of *A. leiocarpus* are presented in Tables 2 - 4. From the present data, it is evident that both ethanol and aqueous extracts of the plant parts exhibited inhibitory activity on the growth of the three tested microbes. For both aqueous and ethanol extracts, a two way ANOVA test revealed that extract concentrations did not have significant effect ($P>0.05$) on the inhibition of *C. albicans*, however, the length of incubation had signify-cant effect ($P<0.05$) on the inhibition of *C. albicans*.

The inhibitory effect produced by the ethanol extract of the root and stem on *S. mutans* was significantly higher than the effect produced by the aqueous extract. In a similar trend, root extracts exhibited a significant ($P<0.05$) inhibition of *S. mutans* compared to the effect produced by the stem extracts. However, the length of incubation had no significant effect ($P>0.05$) on the level of inhibition observed for this organism. ANOVA test of the data obtained from the antimicrobial assay of the different extracts on *S. saprophytticus* revealed a trend similar to that observed on *S. mutans.*

DISCUSSION

A large number of constitutive plant compounds have been reported to have antimicrobial activity. Well known examples include phenols, unsaturated lactones, sapon-

Table 2. Inhibition of *Candida albicans* by aqueous and ethanol extract of *Anogeissus leiocarpus*.

Plant part		Aqueous	Extract	Ethanol	Extract
	Time of incubation (h)	Extract (g/ml)	Concentration	Extract (g/ml)	Concentration
		0.4	0.2	0.4	0.2
Root	24	33.5 ± 1.5	26.0 ± 6.0	29.0 ± 3.0	25.5 ± 2.5
	48	26.0 ± 1.0	25.0 ± 1.0	28.0 ± 3.0	26.0 ± 0.5
	72	21.0 ± 0.5	19.1 ± 0.6	23.5 ± 6.5	22.5 ± 2.5
Stem	24	30.0 ± 1.0	25.5 ± 1.5	38.5 ± 0.5	39.0 ± 2.0
	48	28.0 ± 2.0	26.5 ± 0.5	37.5 ± 1.5	31.5 ± 2.5
	72	19.5 ± 0.5	19.5 ± 0.5	35.5 ± 0.5	26.0 ± 2.0

Values are mean ± standard deviation (n = 3).

Table 3. Inhibition of *Streptococcus mutans* by aqueous and ethanol extract of *Anogeissus leiocarpus.*

Plant part		Aqueous	Extract	Ethanol	Extract
	Time of incubation (h)	Extract (g/ml)	Concentration	Extract (g/ml)	Concentration
		0.4	0.2	0.4	0.2
Root	24	29.5 ± 1.0	22.0 ± 0.0	40.0 ± 0.0	37.0 ± 1.0
	48	31.0 ± 1.0	26.0 ± 0.0	38.0 ± 2.0	30.5 ± 0.5
	72	18.0 ± 0.0	20.0 ± 0.0	34.5 ± 0.5	22.5 ± 2.0
Stem	24	25.0 ± 0.0	24.5 ± 2.5	29.0 ± 2.0	27.0 ± 3.0
	48	25.5 ± 0.5	28.5 ± 0.5	31.5 ± 1.5	26.0 ± 0.0
	72	17.5 ± 2.5	18.5 ± 1.5	31.5 ± 0.5	26.0 ± 0.0

Values are mean ± standard deviation (n = 3).

Table 4. Inhibition of *Staphylococcus saprophyticus* by aqueous and ethanol extract of *Anogeissus leiocarpus.*

Plant part		Aqueous	Extract	Ethanol	Extract
	Time of incubation (h)	Extract(g/ml)	Concentration	Extract (g/ml)	Concentration
		0.4	0.2	0.4	0.2
Root	24	29.5 ± 3.5	21.0 ± 1.0	36.0 ± 4.0	33.3 ± 2.0
	48	30.0 ± 4.0	26.5 ± 0.5	33.5 ± 2.5	33.5 ± 2.5
	72	33.5 ± 2.5	18.5 ± 0.5	30.0 ± 1.0	30.0 ± 4.0
Stem	24	16.0 ± 1.0	16.0 ± 0.0	25.0 ± 0.0	26.5 ± 1.5
	48	10.0 ± 0.5	10.0 ± 1.5	29.0 ± 2.0	26.5 ± 1.5
	72	8.0 ± 0.6	7.5 ± 0.3	13.0 ± 1.0	12.0 ± 0.0

Values are mean ± standard deviation (n = 3).

nins, cyanogenic glycosides and glucosinolates (Ingham, 1973; Osbourn, 1996). The presence of these phytochemicals in the investigated plant parts of *A. leiocarpus* would be responsible for the demonstrated antimicrobial activity of the extracts. In this regard, the higher concentration of these phytochemicals in the root extract may have been responsible for a relatively higher antimicrobial activity demonstrated by the root extract on the tested oral pathogens.

Previous reports have indicated that the root of *A. leiocarpus* is often used as chewing stick (Akande and Hayashi, 1998; Ndukwe et al., 2005). Result from the present study is possibly giving insight on the reason why the root rather than the stem of *A. leiocarpus* has been utilized as chewing stick from a dateless past. Paradoxically, this study also showed that the stem also contained active agents against the tested oral pathogens, and thus could be used in the absence of the root of this plant.

Earlier reports from our lab and elsewhere (Ogundiya et al., 2006; Adekunle and Odukoya, 2006; Okunade et al., 2007) indicated no activity of *A. leiocarpus* extract on

C. albicans. Result from the present study has clearly demonstrated that at higher concentrations such as in the present study, *C. albicans* is sensitive to both aqueous and ethanol extract of *A. leiocarpus*. Also, the inhibition of *S. saprophyticus* and *S. mutans* observed in this study has confirmed that the antimicrobial principles in *A. leiocarpus* which inhibited microorganisms like *B. gingivalis, B. melaninogenicus* (Rotimi et al., 1988), *S. aureus, P. aeroginosa* (Ndukwe et al., 2005) are equally active against the tested microorganisms in the present study.

Kerry (2008) stated that plants have been incorporated into dentrifices and there are several modern examples of this practice. In an earlier report, Ndukwe et al. (2005) have opined that the increasing tendencies of oral pathogens to develop resistance to synthetic antimicrobials is a strong indication for a renewed interest in the usage of chewing sticks for good oral hygiene. Results from the previous and present studies have established that *A. leiocarpus* is a potential candidate plant which could be incorporated into orodentrifice. Furthermore, this study has shown that the stem in addition or absence of the root of this plant could be used for good oral hygiene.

REFERENCES

Adekunle AA, Odukoya KA (2006). Antifungal Activities of Ethanol and Aqueous Crude Extracts of Four Nigerian Chewing Sticks. EthnoBotanical Leaflet http://www.siu/~ebl/leaflets

Akande JA, Hayashi Y (1998) Potency of extract contents from selected tropical chewing sticks against *Staphylococcus aureus* and *Staphylococcu auricularis*. World J. Microbiol. and Biotechnol. 14: 235-238

Bo A (2008) Importance of Herbs for dental Health. Worldental.org

Buada CV, Boakye-Yiadom K (1973) The antibacterial activity of some Ghanian chewing sticks. Ghana pharma. J 1:150-151

Burkill HM (1985) The useful plants of West Africa. Vol I. Families A-D. Royal Botanical Garden. Kew. p 417.

Dalziel JM (1937) The useful plants of West tropical Africa. Crown agents for overseas Governments. Milbank, London Uk pp 201-204

Fadeyi MO, Adeoye AO, Olowokudejo ID (1987). Epidermal and phytochemical Studies in the genus *Bohervia* (Nyctaginaceae) in Nigeria. Int. J. Crude drug research 27: 178-184

Harbone JB (1998) Phytochemical methods: Aguide to modern techniques of plant analysis. 3rd Edition. Chapman and Hill, London. p..279

Hollist NO (2004) A collection of traditional Yoruba Oral and dental medicaments.Book Builders Publishers Ltd. Ibadan. pp 83-87

Ingham JL (1973) Disease resistance in higher plants. The concept of pre-infectional and post-infectional resistance. Phytopathol. Z. 78: 314-335

Kerry B (2008). Phytotherapy for Periodontal disease and improved oral hygiene (Phytotherapy Review and Commentary) http://www.mediherb.com

Ndukwe KC, Okeke W, Lamikanra A, Adesina SK, Aboderin O (2005) Antibacterial activity of aqueous extracts of selected chewing sticks. J. Contemp. Dent. Pract 6(3): 086-094

Ogundiya MO, Okunade MB, Kolapo AL (2006). Antimicrobial activities of some Nigerian Chewing sticks http://www.siu/~ebl/leaflets

Okunade MB, Adejumobi JA, Ogundiya MO, Kolapo AL (2007). Chemical, Phytochemical compositions and antimicrobial activities of some local chewing sticks used in South Western Nigeria. Journal Phytopharmacotherapy and Natural products 1(1): 49-52

Onyeyili PA (2000). Antihelminthic efficacy of some plants used in ethno veterinary practices in the Arid zone of North Eastern Nigeria. RGA No28 Project Report

Osbourn AE (1996). Preformed Antimicrobial Compounds and Plant Defense against Fungal Attack. The Plant cell 8:1821-1831

Perez C, Pauli A, Bazerque P (1990). An antibiotic assay by agar-well diffusion method. Acta Biol. Med. Exp. 15: 113-115

Popoola TOS, Yangomodu OD, Akintokun AK (2007). Antimicrobial Activity of Cassava Seed Oil on Skin Pathogenic Microorganisms. Res. J. Medicinal Plant 1(2): 60-64

Rotimi VO, Laughon BE, Bartlett JG, Mosadomi HA (1988). Activities of Nigerian chewing sticks extracts against *Bacteriodes-gingivalis* and *Bacteriodes- melaninogenicus.* Antimicrobiological Agents and Chemotherapy 32(4): 598-600

Ugoji E, Egwari LO, Obisesan B (2000). Antibacterial activities of aqueous extracts of ten African chewing sticks on oral pathogens. Nig. J. Internal medicine 3(1): 7-11