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Production and evaluation of chewable tablets obtained from extracts of *Zanthoxylum tessmannii*

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ABSTRACT

Following claims of the use of the root bark of *Zanthoxylum tessmannii* in remedies for mouth and throat infections, particularly pharyngitis and oral thrush, it became necessary to examine the possibility of formulating the extract into chewable tablets. The root bark was extracted using different solvents, namely methanol, chloroform, n-hexane and ethylacetate. The phytochemical composition of the methanolic extract was established and the activity of the extracts was tested against clinical strains of *Staphylococcus aureus, Streptococcus spp., Klebsiella spp., Candida albicans, and Pseudomonas aeruginosa*, as well as laboratory strains of *E. coli* and *Staphylococcus aureus* using standard methods. The extract was formulated into chewable tablets using wet granulation with compression in a tableting machine. The compressed tablets were evaluated for friability, hardness and uniformity of weight. The phytochemical tests revealed the presence of alkaloids, flavonoids, anthraquinones, carbohydrate/sugars and trace amounts of tannins, resins, glycosides and oils. The extract showed significant activity against *Candida albicans* and Streptococcus spp. The chewable tablets passed the test for uniformity of weight but fell short on hardness and friability. We conclude that the root and bark extract of *Zanthoxylum tessmannii* can be formulated into chewable tablets for oral thrush and other conditions suitable for paediatric and geriatric patients, with an optimized formula.

KEYWORDS- *Zanthoxylum tessmannii*; Chewable tablet; Antifungal; Antimicrobial agent

INTRODUCTION

Zanthoxylum tessmannii grows in the rain forests of southern Nigeria and the roots, bark and leaves have been used in traditional societies as remedies for toothache, coughs, leprous ulcerations, rheumatism and lumbago (1). Other uses include sore throat and oral thrush, and tonsillitis, these indications necessitating formulation of the root and back extracts into lozenges in previous studies (1). Even though it is also believed to have analgesic properties, especially in such conditions as urns, rheumatism, headache, stomach-ache, toothache and post-partum states, by far, its most popular use is as a chewing stick which characteristically produces a warm pungent benumbing effect on the palate.

Of the 120 or so active compounds currently isolated from higher plants and widely used in modern medicine today in different countries, 80 % show a positive correlation between their modern therapeutic use and the traditional use of the plants from which

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they are derived (2, 3). These reasons informed the further production of the extract into chewable tablets. Chewable tablets are ideal for local treatment of oropharyngeal conditions and also for systemic ailments. They are a preferred choice for administration of drugs to paediatric and geriatric patients who may have difficulties swallowing whole tablets. They are normally pleasantly flavoured and do not need intake of water, which, which makes them very convenient. They are ideal for delivering large doses of drugs (e.g. antacids). This helps to reduce the number of tablets to be swallowed by the patient. They are also used to provide prompt action, due to circumvention of disintegration time, e.g. in glyceryl trinitrate in angina. The replacement of disintegration with mastication helps to promote absorption by the increased surface area of the small particles produced. Other advantages include acceptability to elderly and geriatric patients in order to improve compliance and direct release of medicament in the mouth, which can be highly beneficial in cough and other conditions.

In this project, the extract from the root and back of the plant was formulated into chewable tablets flavoured with mannitol and menthol. Mannitol is sweeter than sucrose and has smooth mouth feel. It is non-hygroscopic and thus suitable for moisture sensitive drugs (4), such as herbal extracts whose diverse composition may provide a substrate form microorganisms in the presence of moisture. The ideas was to provide a herbal remedy ideal for treating both local and systemic conditions with prompt onset of action, especially in paediatric and geriatric patients.

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MATERIALS & METHODS

Materials

The roots and bark of *Zanthoxylum tessmannii* were harvested locally and identified by Mr. Alfred Ozioko, a taxonomist with the International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu State. Methanol, chloroform, n-hexane, ethyl acetate, and dimethylsulphoxide (DMSO) were all from Sigma-Aldrich (Germany). Nutrient agar, Mueller Hinton agar (MHA), nutrient broth, Mueller Hinton broth (MHB), MacConkey agar were all prepared according to manufacturer's specifications.

Clinical strains of *Staphylococcus aureus, Streptococcus spp., Klebsiella spp., Candida albicans* and *Pseudomonas aeruginosa* were obtained from the Sputum Laboratory in the University of Port Harcourt Teaching Hospital (UPTH). *Eschericia coli* and *Staphylococcus aureus* (both from Automated Type Culture Collection, ATCC) were obtained from the Department of Pharmaceutical Microbiology in our Institution. All other materials were of laboratory grades and were used as such without modifications.

Methods

Extraction of the plant

The roots and bark of the plant were washed adequately and air dried. The dried plant parts were then milled to fine powder. The powder (1 kg) was weighed on a weighing balance (Ohaus Adventurer) soaked in 2.5 L of methanol for four days in a macerating jar. On the fourth day, the extract was filtered with the aid of Muslin cloth. The methanolic solution was then filtered using filter paper (5 mm) and the resulting filtrate dried under atmospheric conditions with the aid of porcelain crucibles, depositing the brownish-black resinous extract. The residue was further washed with another 2.5 L of methanol and the resinous residues were pooled together. About 15 g of the crude extract was suspended in 50 mL of distilled water and partitioned successively with five times the volume of n-hexane, chloroform and ethyl acetate. The partition in each case was collected and air dried to obtain the various extract.

Phytochemical analysis

These were carried out on the methanolic extract. The various tests followed standard published methods (5-7).

Hospital strain isolation and susceptibility test

The following organisms were after streaking on Mueller Hinton agar (MHA) slants followed by incubatation for 24 hours at 37 °C: *Candida albicans, Streptococcus spp., Klesiella spp., Pseudomonas aerugenosa and Staphylococcus aureus*. Plates of MHA were prepared and each seeded with the hospital isolates as well as the laboratory strains of E. coli and *S. aureus* at 0.5 McFarlands each. A sterile cork-borer was used to bore 6 mm holes in the solidified medium and about 50 µl of 100 mg/ml of extract (in DMSO) was incorporated. Incubation was done for 24 h at 37 °C. This was done for each extract.

Antifungal test

A sterile petri dish was seeded with *Candida albicans* at 0.5 McFarlands and molten MHA was poured into the plate. The dispersion was swirled gently to ensure even dispersion. After solidification, a sterile cork-borer was used to bore holes in the medium and about 50 µl of the extract (concentration 100 mg/ml) was incorporated. Incubation was done at 37 oC for 24 hours as before. This was done for each extract.

Preparation of chewable tablets using wet granulation

The formula used here was a modification of the formula used in preparing lozenges of *Z. tessmannii* in a previous work (Okorie et al. 2009). Mannitol (120 g) was weighed out into a porcelain mortar. This was triturated with the extract and then blended with acacia (as mucilage) to give a damp mass. This mass was forced through a sieve (4 mm) and the resulting wet granules were dried in hot air oven at 55 oC. The granules were further sized by passing through a sieve (2 mm). They were then mixed slightly with menthol and peppermint oil, and after the mass dried, compression was done with the aid of a single punch tableting machine (Cadmach, India). A total of 50 chewable tablets were compressed.

Evaluation of chewable tablets

Friability test

Twenty dust-free chewable tablets were weighed together on the analytical balance. They were then subjected to motion in an Erweka friabilator (Germany) set at 25 revolutions per minute for 4 minutes. At the end, they were de-dusted and weighed together. The difference in the total weights before and after the test was obtained. The percentage difference is the friability of the tablet batch.

Hardness test

Ten tablets were selected at random and a Monsanto-type hardness tester (Thermonik, India) was used to measure the force required to break each.

Weight uniformity test

Twenty tablets of the chewable tablets were randomly selected and weighed together and the total weight and average determined. They were then weighed individually and the percentage deviation from the average value was calculated for each (8)

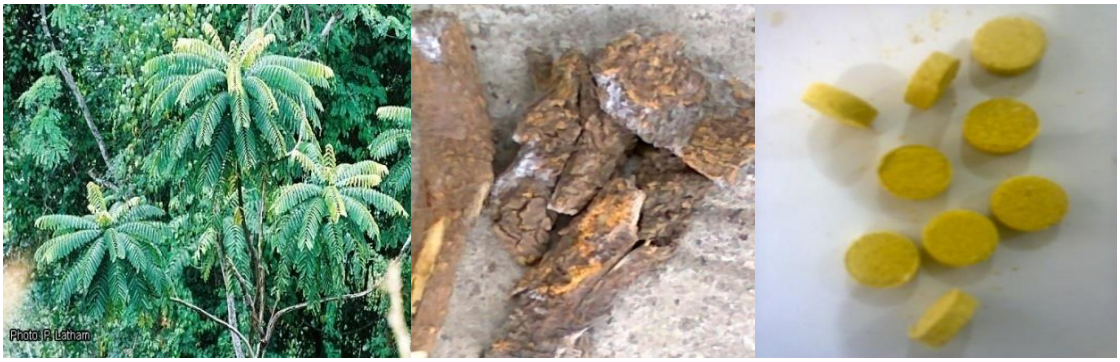
RESULTS

Extraction, phytochemistry and antimicrobial screening

The extract was gummy and dark-brown in colour, with a characteristic odour, bitter taste and a warm feel in the mouth. It was insoluble in water but soluble in organic solvents including DMSO, n-hexane, and methanol etc. The percentage yield obtained using one kilogram of the powdered drug was 4.18 %. Ethyl acetate gave less than 1 %. The methanolic extract was found to contain very high concentrations of alkaloids and flavonoids, with moderate concentrations of oils, tannins, anthraquinones and resins. It also contained reducing sugars, glycosides and deoxy sugars, but not proteins and saponins. The antimicrobial profiles of the extracts are depicted in Table 1. The inhibition zones produced against the test organisms are presented in Table 2.

Tablet properties

The formula used in the tablet production is presented in Table 3. Brown rounded tablets (Figure 1) were produced. They were sweet tasting and with a minty flavor and characteristic warm feel. The friability was 5.3 %. The average hardness was 4.21 Kgf (± 0.27). The batch had an acceptable weight variation, with none exceeding 10 % of 600 mg (the mean weight). All ranged between 590 - 620 mg.



A B C

Figure 1: Photograph of A) *Zanthoxylum tessmannii ;* B) Stem and root bark; C) chewable tablets obtained from the stem and root back

Table 1: Microbial susceptibility test

Organism	Extract	Sensitivity
S. aureus (ATCC)	Methanol	**+++**
	Chloroform	**+++**
	N-Hexane	**---**
	Ethyl acetate	**+++**
Candida albicans	Methanol	**++++**
	Chloroform	**++++**
	N-Hexane	**---**
	Ethyl acetate	**---**
Streptococcus spp.	Methanol	**++++**
	Chloroform	**++**
	N-Hexane	**---**
	Ethyl acetate	**+**
Klebsiella spp.	Methanol	**---**
	Chloroform	**++**
	N-Hexane	**++**
	Ethyl acetate	**++**
P. aeruginosa	Methanol	**+++**
	Chloroform	**+**
	N-Hexane	**---**
	Ethyl acetate	**---**
Staphylococcus aureus	Methanol	**++**
	Chloroform	**---**
	N-Hexane	**---**
	Ethyl acetate	**++**
E. coli (ATCC)	Methanol	**---**
	Chloroform	**+**
	N-Hexane	**+**
	Ethyl acetate	**---**

Key:

++++ = Highly effective
+++ = Very effective
++ = Effective
+ = Less active
--- = No effect

Table 2: Extracts and inhibition zones

Organism	Extract	Inhibition zones (mm)
S. aureus (ATCC)	Methanol	14
	Chloroform	14
	N-Hexane	---
	Ethylacetate	14
Candida albicans	Methanol	19
	Chloroform	16
	N-Hexane	---
	Ethylacetate	---
Streptococcus spp.	Methanol	19
	Chloroform	10
	N-Hexane	---
	Ethylacetate	---
Klebsiella spp.	Methanol	---
	Chloroform	12
	N-Hexane	9
	Ethylacetate	12
P. aeruginosa	Methanol	16
	Chloroform	10
	N-Hexane	---
	Ethylacetate	---
Staphylococcus aureus	Methanol	12
	Chloroform	---
	N-Hexane	---
	Ethylacetate	10
E. coli (ATCC)	Methanol	---
	Chloroform	8
	N-Hexane	10
	Ethylacetate	---

DISCUSSION

Zanthoxylum has a diverse chemistry, consistent with its use in many conditions. The presence of significant to moderate concentration of alkaloids, anthraquinones, tannins, sugars, cardiac glycosides, flavonoids, some of the having been associated with antibacterial activities (9), and may therefore be a basis for the activity of the methanolic extract against the organisms. Though the chloroform extract had a broader spectrum, it produced less intense activity against susceptible organisms than the methanolic extract. The intensity of activity demonstrated against *C. albicans, P. aeruginosa, and Streptococcus spp* point at a potential for use in oropharyngeal conditions. Streptococcus is commonly implicated in sore throat and commonly colonizes the pharynx (10), while C. albicans is associated with oral thrush (11).

The inclusion of methol in the formula will beneficial in both conditions, due to local anaesthetic effects (12). The treatment of oral thrush currently consists in the use of nystatin (13) which is preferred to systemic agents due to its very poor absorption (11). Due to a lack of antibacterial activity and also contradindication in congenital candidiasis and renal insufficiency (14), the chewable tablet of *Z. tessmannii* may be a useful alternative, particularly in renally-challenged elderly patients. The analgesic properties of the extract would

be a welcome relief in inflammation. As far as the antibacterial and antifungal properties are concerned, the activity profiles established here provide a scientific basis for the continued use of the plant in traditional medicine.

High friability could affect the integrity of the tablets in the course of storage in containers, transportation and handling. Even though this is not an official test, a friability value of 1 % is normally considered an upper limit (15). A value of 3 Kgf is considered acceptable for chewable tablets (16). Since both friability and hardness measure different aspects of overall tablet hardness (by attrition and fracture respectively), the implication of these results is that formula or compaction pressure would need further optimization.

CONCLUSION

This study details the extraction of antimicrobial principles from the roots and bark of *Zanthoxylum tessmannii* and the formulation of the extract into chewable tablets ideal for treatment of oropharyngeal conditions such as thrush and sore throat, particularly in children and also elderly patients. The extracts, especially the methanolic extract demonstrate very high activities against both clinical and laboratory strains of test microorganisms. Flavoured chewable tablets hold a great potential to prompt rapid onset of both local and systemic action and the preliminary preparation and characterization of the tablet produced with mannitol indicate that this presentation is suitable, pending further optimization.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare

Table 3: Formula for production of chewable tablets

Ingredient	Amount per tablet	Amount per 50 tablets
Extract	400 mg	20 g
Acacia (mucilage)	72 mg	3.6 g
Peppermint oil	0.8 ml	40 ml
Menthol	4.8 ml	240 ml
Mannitol to	2.4 g	120 g
Magnesium stearate (0.9 %)	21.6 mg	1.08 g

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