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Phytochemical Screening and Antibacterial Activity of Extracts of *Garcinia mannii* and *Terminalia avicennoides* on Some Oral Bacterial Pathogens

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Abstract

Garcinia mannii and Terminalia avicennoides twigs are commonly used as chewing sticks for dental hygiene among many tribal groups in Nigeria. The antibacterial activity of methanol and aqueous extracts of G. mannii and T. avicennoides were tested on some isolates of oral bacterial pathogens (Micrococcus luteus, Streptococcus mutans, Pseudomonas. aeruginosa, Klebsiella pneumoniae, Streptococcus pneumoniae and Streptococcus pyogenes). The percentage yield of the extract revealed 10.64% and 10.46% for methanol and aqueous extract of G. mannii and 22.24% and 22.36% for methanol and aqueous extract of T. avicennoides. Preliminary phytochemical screening revealed the presence of alkaloids, phenols, tannins, flavonoids, cardiac glycosides, anthraquinones, steroids, phlobatannins, terpenes and saponins. Quantitative phytochemical analysis (mg/100g) showed total phenols ranged from 706.95±147.58-1017.01±18.85, flavonoids; 198.35±2.13-366.750±14.53, alkaloids; 120.31±3.15-25.54±21.96, tannins; 138.50±6.03- 224.65 ± 1.94 and saponins; $4.81\pm0.00-8.28\pm0.28$ in aqueous and methanol extracts of G. mannii and T. avicennoides. The antibacterial test based on the diameter of clear zones of inhibition on agar plates ranged from $6.33\pm0.67 - 25.00\pm0.57$ mm at concentrations of 20, 30 and 40mg/mL in aqueous and methanol extracts of G. mannii and T. avicennoides. These were comparable to the 5mg/mL of both standard antibiotics used (Amoxicillin and Ampiclox) with zones of inhibition ranging from 15.67±0.33 to 28.00±1.00 on all the test organisms. Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the two active extracts ranged from 0.625-20mg/mL and 1.25-40mg/mL respectively. These results confirmed the efficacy of G. mannii and T. avicennoides in traditional dental care. The extract could contain bioactive compounds with potential in the formulation of new dental care medicaments.

1. Introduction

According to the World Health Organization (WHO), a medicinal plant is any plant in which one or more of its organs or parts, contains chemical substances that can be used for therapeutic purposes, or which are precursors for synthesis of other bioactive metabolite. Such a plant will have all of its parts employed in the control or treatment of

a disease condition and therefore contains chemical components that are medically active. These bioactive components are often referred to as phytochemicals or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pests [1], [2], [3].

Medicinal plants contain biologically active components which have been employed in traditional medical practice for the treatment of human infections [4]. According to some studies carried out on some medicinal plants in Nigeria it is reported that Garcinia mannii and Terminalia avicennoides are used in treatment of pains and oral infections. Investigations have shown that these chewing sticks possesses antimicrobial activity against oral microbial flora [5]. The species of trees and shrubs from which chewing sticks are made is numerous, but in Southern Ghana the most popular varieties belong to the Garcinia species, including G. mannii, G. kola, and G. epunctata [6]. Notwithstanding the relative abundance of literature on the benefits of chewing sticks, information on their chemical and pharmacological components is generally scanty [6], and probably nonexistent in the specific case of Garcinia mannii.

In Africa, chewing sticks commonly used for maintaining oral hygiene. The roots, stem and twigs of numerous plants are employed for this purpose. Chewing sticks are recommended for oral hygiene by the World Health Organization [7] and some of them, or their extracts, are also used in the ethno medical treatment of oral infections [8].

The use of chewing sticks have been documented since ancient times. And have been used by the Babylonians some 7000 years ago [9]. The cleansing efficacy of chewing sticks is attributed to the mechanical effects of its fibers, or release of beneficial chemicals or a combination of both [7]. Some Africa chewing sticks are reported to contain fluoride ions, silicon, tannic acid and other natural plaque inhibiting substances that can reduce bacterial colonization and plaque formation [10]. A significant percentage of the Nigeria population uses chewing sticks, mainly because they are readily available and cheap [11].

Recent interest in chewing sticks and their extracts have focused on their effects on organisms that are involved in oral infections. The stick is usually chewed or tapered at one end until it becomes frayed into a brush. Soaking it in water for few hours softens the natural fibers, helping them to separate. [12]. The stick is held by one hand in a pen-like grip and the brush end is used with an up and down or rolling motion. A two finger or and a five finger grip technique maybe used. When the brushy edge is shred after being frequently used, the stick gets ineffective and it is then cut and further chewed to form a fresh edge. In this way, it can be used for few more weeks [13].

There are known 173 different types of trees, which can be used as chewing sticks, belonging to the families *Rubiaceae*, *Rutaceae*, *Combretaceae*, *Euphorbiaceae*, *Meliaceae*, *Mytaceae* and *Asteraceae* [14]. The most popular chewing stick or fibrous sponges include *Salvadorapersica* and *Azadirachta indica* [8].

The human oral cavity is maintained at a relatively comfort temperature (35°C-36°C), thus providing suitable conditions for a larger number of microorganisms [12]. The ecological conditions in the mouth are never stable for long periods of time being affected by intermittent feeding and age. With change in the natural ecosystems such as the use of antibiotics, contributes to the variations in microbial communities [15].

Oral infections are disease conditions found in the mouth. The mouth includes not only the teeth and the gums but their supporting connective tissues, ligaments, bones, soft and hard palates. These conditions are generally grouped into three main categories, as diseases of the teeth, diseases of the gums and oral cancers. This study is aimed at authenticating and scientifically verify the claims of antibacterial properties by local marketers and users of some selected chewing sticks in some parts of Nigeria.

2. Materials and Methods

2.1. Materials

2.1.1. Collection and Identification of Plant Materials

Fresh stem of *Garcinia mannii* and *Terminalia avicennoides* were collected from Okija in Anambra state and Maikunkele in Minna, Niger State both in Nigeria in February, 2016. The plants were identified at National Institute of Pharmaceutical Research and Development, Idu-Abuja, Nigeria and assigned a voucher number NIPRD/H/6786 and NIPRD/H/6797 for *G. mannii* and *T. avicennoides* respectively.

 Table 1. Local names, ethno medicinal uses and locations of collection of the plants used.

Scientific name	Local names	Ethnomedicinal uses	Part used	Location
Garcinia mannii	Namijin goro (H), Aku-ilu (I)	Antimalarial, antibacterial, used as preservatives, dye e.t.c	stem	Okija, Anambra State
Terminalia avicennoides	Wawan kurmi (H), erin mado (Y), okwe (I)	Use to treat wound and skin infections, dysentery, amoebiasis and diarrhea	Stem	Maikunkele, Niger State

Keys: I - Igbo, H - Yoruba, H - Hausa

2.1.2. Sources and Characterization of Bacteria Pathogens

Samples were collected from oro-dental patients attending General Hospital Minna, using sterile swab stick soaked with sterile normal saline. The swab stick sample was inoculated into prepared nutrient broth and incubated for 6 h, so as to activate the bacteria. After activation in the nutrient broth, the nutrient broth culture was subcultured on nutrient Agar, Blood Agar, MacConkey Agar and Manitol Salt Agar so as to isolate the bacteria. This was characterized after isolation and compared with known existing taxa [16]. The bacteria pathogens identified include: Micrococcus luteus, Streptococcus mutans, Pseudomonas pneumoniae. Klebsiella aeruginosa, Streptococcus pneumoniae and Streptococcus pyogenes.

2.1.3. Ethical Consideration

Ethical clearance to conduct this research was sought from the research ethics and publication committee of the hospital. Informed consent was obtained for each respondent before physical examination. Subjects less than 18 years had their consent sought from their relatives or guidance.

2.2. Methods

2.2.1. Plant Processing and Extraction

The stem *G. mannii* and *T. avicennoides* were collected washed and air-dried at room temperature at the Centre for Genetic Engineering and Biotechnology, Federal University of technology, Minna, Niger State. The dried stem were then blended using blending machine to obtain a fine powder [16]. Fifty grams (50 g) of the plant powder was extracted with 400mL of methanol and distilled water using reflux method at a temperature of 45°C for 2 hours and the extract was filtered using muslin cloth followed by further filtration using whattman No 1 filter paper with pore size of 0.7µm to obtain a fine filtrate. The filtrate was then concentrated using RE-6000 rotary evaporator at 50°C and further concentrated using the water bath at 45°C to ensure the extract is totally free from the solvent used in the extraction.

The resultant concentrates was kept in the refrigerator for further use.

2.2.2. Qualitative Phytochemical Screening of Extracts

Preliminary qualitative phytochemical screening which involved performing simple chemical tests to detect the presence of secondary metabolites such as tannins, flavonoids, phenols, phenolic compounds, saponins, and glycosides, was carried out according to Trease and Evans and Sofowora [10], [18].

2.3. Quantitative Determination of Phytochemicals

Quantitative estimation of phytochemicals such as alkaloids and saponins was carried out according to [21], total phenolic content [23] and flavonoids using Aluminum Chloride colorimetric method [26].

2.4. Determination of Antibacterial Activity of the Extract

2.4.1. Assay for Antibacterial Activity

Agar well diffusion method was used to evaluate the antibacterial activity of the Crude extracts [37]. Eighteenhour culture of bacteria adjusted to 0.5 McFarland standard was used as inoculum on sterile Mueller Hinton agar. The plate was kept on flat bench for 30 minutes to solidify. Five wells (4mm) deep were made in the agar using a sterile 6mm diameter cork borer. Then 0.5 mL of the reconstituted extract at a concentration of 20, 30 and 40 mg/mL was pipetted into the wells using micro pipette. Zero point five milliliter (0.5mL) each of 5mg/mL of Ampiclox and Amoxiline solution were used as positive controls and 0.5mL of Di-Methyl Sulphoxide (DMSO) as a negative control. The plates were allowed to stand on a flat bench for 30 min to allow diffusion of the extract into the agar before incubation at 37°C for 24 h. Each test was carried out in triplicates and mean zone diameter of inhibition was recorded.

2.4.2. Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was determined using the double fold dilution. An aliquot of 1mL of the reconstituted extract with 50% DMSO at a concentration of 40 mg/mL was diluted serially to give concentrations of 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/mL in eight test tubes. One milliliter (1mL) of 18h culture of bacteria previously adjusted to 0.5 McFarland standard (1.0x10⁶cfu/mL) was added to each of the test tubes and the content was mixed thoroughly. The tubes were incubated at 37°C for 4 h. The 9th test tube contained 1mL of 50% DMSO with no extract served as negative control. The 10th and 11th test tubes containing a solution of 5 mg/mL of Ampiclox and Amoxiline served as positive control. The procedure was repeated for the test and the control. The test tube with the lowest concentration of the extract without visible turbidity of growth was taken as the MIC [19].

2.4.3. Determination of the Minimum Bactericidal Concentration (MBC)

From each of the test tubes without any visible growth, a loopfull of the broth was aseptically inoculated on a sterile Mueller Hinton agar. The inoculated plates were incubated for 24hr at a temperature of 37°C. After incubation, the MBC was determined as the lowest concentration with no visible growth on the plate [20].

2.5. Statistical Analysis

Data obtained in this study were analysed using the IBM Statistical Package for Social Science (SPSS) 20.0, 2011 version (SPSS Inc., Chicago, Illinois, USA). Numerical data were presented as mean±standard error of mean (SEM) of the triplicate.

3. Results

Plants sample	Solvent used	Weight of extract (g)	% yield
C. mamuii	Methanol	5.32	10.64
G. mannu	Distilled water	5.23	10.46
T	Methanol	11.12	22.24
1. avicennoiaes	Distilled water	12.18	24.36

Table 2. Percentage yield of the extracts of G. mannii and T. avicennoides.

Table 3. Results of Phytochemical contents in aqueous and methanol extracts of G. mannii and T. avicennoides.

Plants	Extract	Alkaloids	Phenols	Tannins	Saponins	Cardiac glycosides
G. mannii	Methanol	+	+	+	+	+
	Aqueous	+	+	+	+	+
T. avicennoides	Methanol	+	+	+	+	+
	Aqueous	+	+	+	+	+

Table 3. Continued.								
Plants	Extract	Phlobatannins	Anthraquinones	Steroids	Terpenes	flavonoids		
C. mannii	Methanol	+	+	+	+	+		
G. mannii	Aqueous	+	+	+	+	+		
T. avicennoides	Methanol	+	+	+	+	+		
	Aqueous	+	+	+	+	+		

Keys: +: Present, -: Absent

Table 4. Results of Quantitative phytochemical contents in methanol and aqueous extract of G. mannii and T. avicennoides (mg/100g).

Plants extract	Total phenols	Total flavonoids	Alkaloids	Tannins	Saponins
TAA	706.945±147.58	255.333±17.61	151.606±14.07	169.550±2.91	4.807±0.02
TAM	788.364±1.80	233.111±12.27	125.482±1.90	155.283±5.67	7.012±0.90
GMA	900.518±8.90	198.352±2.13	120.312±3.15	138.500±6.03	8.280±0.28
GMM	1017.011±18.85	366.750±14.53	247.538±21.96	224.625±1.94	4.882±0.11

Values are expressed in mean±standard error of mean of duplicate determination.

Keys: TAA: Aqueous extract of *T. avicennoides*, TAM: Methanol extract of *T. avicennoides*, GMA: Aqueous extract of *G. mannii*, GMM: Methanol extract of *G. mannii*.

Table 5. Mean zones of inhibition (mm) of methanol and aqueous extracts of Garcinia mannii against teste organisms.

Test organisms	GMA 20mg/mL	GMA 30mg/mL	GMA 40mg/mL	GMM 20mg/mL	GMM 30mg/mL	GMM 40mg/mL	Amoxicilin* 5mg/mL	Ampiclox* 5mg/mL
M. luteus	13.67±0.33°	18.33±0.67 ^d	21.00±1.53 ^e	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	9.33±0.67 ^b	26.33±0.33 ^f	22.33±0.33 ^{ef}
P. aeruginosa	$11.00{\pm}1.00^{a}$	14.00 ± 0.00^{bc}	16.67±0.67 ^d	11.33±0.67 ^a	14.00±0.58 ^{bc}	15.67±0.33°	13.67±0.33 ^b	20.67±0.33e
S. mutans	12.33±0.33ª	14.00 ± 0.00^{b}	22.33±0.33 ^{de}	12.33±0.33ª	21.33±0.67 ^d	13.670.33°	25.67±0.33e	28.00 ± 1.00^{f}
K. pneumoniae	$0.00{\pm}0.00^{a}$	$0.00{\pm}0.00^{a}$	$0.00{\pm}0.00^{a}$	19.67±0.67 ^c	21.33±0.88cd	16.67±0.67 ^b	19.00±1.00 ^c	24.33±0.33 ^d
S. pneumoniae	15.33±0.67 ^b	16.33±0.33 ^{bc}	11.33±2.03 ^a	15.67±1.20 ^b	21.00±0.58°	21.33±0.33°	15.670.33 ^b	24.33±0.67 ^d
S. pyogenes	$15.33{\pm}0.88^{a}$	17.00±1.15 ^b	16.00 ± 1.15^{ab}	17.33±0.88 ^b	19.67±0.88°	19.67±0.67°	$26.00{\pm}0.58^{d}$	27.67±0.33 ^{de}

Values are expressed in mean±standard error of mean, values with the same superscript on the same row have no significant difference (p>0.05), n=3 GMA: *Garcinia mannii* aqueous extract, GMM: *Garcinia mannii* methanol extract

* Specification for Amoxicillin and Ampiclox are: ≤19mm (resistance) and ≥20mm (susceptible) (CLSI, 2012).

Table 6. Mean zones of inhibition (mm) of Methanol and Aqueous Extract of Terminalia avicennoides against test organisms.

Test organism	TAA 20mg/mL	TAA 30mg/mL	TAA 40mg/mL	TAM 20mg/mL	TAM 30mg/mL	TAM 40mg/mL	Amoxicilin* 5mg/mL	Ampiclox* 5mg/mL
M. luteus	$10.00{\pm}0.58^{a}$	15.33±0.67 ^{bc}	16.67±0.33°	14.00±0.58 ^b	17.67±0.88 ^d	22.33±1.67 ^e	26.33 ± 0.33^{f}	22.33±0.33e
P. aeruginosa	$9.00{\pm}1.00^{a}$	14.67±0.67°	19.00±0.58 ^d	20.67±1.20 ^{de}	21.33±0.67 ^e	19.00±0.58°	13.67±0.33 ^b	20.67±0.33 ^{de}
S. mutans	$14.00{\pm}0.58^{a}$	16.00±0.58 ^b	16.00±0.58 ^b	22.00±1.15 ^d	18.00±0.58°	19.33±0.33 ^{cd}	25.67±0.33 ^e	28.00 ± 1.00^{f}
K. pneumoniae	6.33±0.67 ^a	12.67±0.33 ^b	14.33±0.33°	23.67±0.33 ^{ef}	24.33 ± 0.33^{f}	25.00±1.00g	19.00 ± 1.00^{d}	24.33 ± 0.33^{f}
S. pneumoniae	$14.00{\pm}0.58^{a}$	18.33±0.33 ^b	18.00±1.15 ^b	21.33±0.67°	22.67±1.33 ^{cd}	24.33±0.33 ^d	15.670.33 ^{ab}	24.33±0.67 ^d
S. pyogenes	18.33 ± 0.33^{a}	21.33±0.88 ^b	22.67±0.88 ^{bc}	18.33±0.33 ^a	21.33±0.88 ^b	22.67±0.88 ^{bc}	26.00±0.58°	27.67±0.33 ^{cd}

Values are expressed in mean±standard error of mean, values with the same superscript on the same row have no significant difference (p>0.05), n=3

TAA: Terminalia avicennoides aqueous extract, TAM: Terminalia avicennoides methanol extract

* Specification for Amoxicillin and Ampiclox are: ≤19mm (resistance) and ≥20mm (susceptible) (CLSI, 2012).



Figure 1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Methanol extract of G. mannii (mg/mL).



Figure 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of aqueous extract of G. mannii (mg/mL).



Figure 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of methanol extract of T. avicennoides (mg/mL).



Figure 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of aqueous extract of T. avicennoides (mg/mL).

4. Discussions

The efficacy of a medicinal plant against any target disease solely depends on the presence and concentration of the phytochemicals that are resident in that plant [21]. Preliminary phytochemical screening of the extract reveals the presence of alkaloids, phenols, tannins, cardiac glycosides, phlobatannins, anthraquinones, steroids, terpenes and flavonoids and saponins in all the extracts. (Table 3). The phytochemicals listed above except alkaloids have also been reported in methanol extract of G. mannii [24]. Quantitative phytochemical analysis of the extracts shows high amount of total phenols and flavonoids, alkaloids tannins and saponins; 1017.01 ± 18.85 , 366.75 ± 14.53 , 247.54 ± 21.96 , 224.63 ± 1.94 and 4.88±0.11mg/100g in methanol extract of G. mannii followed by aqueous extract of G. mannii, methanol and aqueous extract of T. avicennoides with phenolic content of 900.52±8.90, 788.36±1.80 and 706.95±147.58mg/100g respectively, alkaloids contents ranged from 120.31±3.15-247.54±21.96mg/100g, tannins; 138.50±6.03-224.63±1.94 in four extract of the two plants while saponins have the lowest concentration in all the extract ranging from 4.80±0.11-8.28±0.28mg/100g.

Phytoconstituents secreted by plants to protect them against pathogenic insects, bacteria, fungi or protozoa have found applications in human medicine [25]. Some phytochemicals such as phenolic acids act essentially by inhibiting adherence of organisms to the cells lining the bladder, and the teeth, which ultimately lowers the incidence of urinary-tract infections (UTI) and the usual dental caries. It is worthy of note that antimicrobial activity results of the same plant part tested most of the time varied from researcher to researcher. This is possible because concentration of plant constituents of the same plant organ can vary from one geographical location to another and from one part of the plant to another depending on the age of the plant, differences in topographical factors, the nutrient concentrations of the soil, extraction method as well as method used for antimicrobial study [27].

Different mechanisms of action of phytochemicals have been suggested. They may inhibit microorganisms, interfere with some metabolic processes or may modulate gene expression and signal transduction pathways [28]; [29]; and [30]. Phytochemicals may either be used as chemotherapeutic or chemo preventive agents with chemoprevention referring to the use of agents to inhibit, reverse, or retard tumorigenesis. Plant extracts and essential oils may exact different modes of action against bacterial strains, such as interference with the phospholipids bilayer of the cell membrane which has as a consequence may increase cell permeability and exudation of cellular constituents, damage or changes in the enzymes involved in the production of cellular energy and synthesis of structural components, and destruction or inactivation of genetic material. In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents [31].

The antibacterial potentials of these plants were judged based on the zones of inhibition of bacterial growth of the test organisms. The mean zones of inhibition of the isolates are a function of the relative antibacterial activities of the extracts. The zones of inhibition is the area on the agar plate that remains free of bacterial growth. The size of the zones of inhibition is usually related to the level of antibacterial activity of the sample or extract; a larger zone of inhibition usually means the antibacterial potency of the extract is more [32].

The zones of inhibition of the extract ranged from 6.33±0.67 - 25.00±0.57 mm at a concentration of 20, 30 and 40mg/ml in methanol and aqueous extract of G. mannii and T. avicennoides (Table 5 and 6) which is an indication of the antibacterial potency of these extracts. The activity of these extract maybe attributed to the presence of the phytochemicals at a relatively high concentration [34]. The antimicrobial activity of alkaloids which is also present in this extract have also been reported [35]. Mechanism of action of tannins involve the precipitation of protein to water soluble compounds and as a result, bacterial are inactivated by the direct damage done to their cell membrane [36]. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) which is the minimum concentration required to inhibit the growth of the microorganism or completely kill the microorganism respectively was also recorded (Figure 1-4). The MIC and MBC of the methanol and aqueous extract of the plant ranged from 0.625-20mg/ml and 1.25-40mg/ml respectively. The lower the MIC and MBC the higher the potency of the extract. This MIC and MBC values also indicate that the extract of this plant contains bioactive compounds which can serve as a precursor in the synthesis of new drugs [24]. Therefore, the presence of these phytochemicals in good quantity is an indication of its medicinal potency and why it is used by traditional herbalist in treating many ailments [19].

4.1. Conclusions

From the results of this study, aqueous and methanol extracts of *T. avicennoides* and *G. mannii* possesses active phytochemical constituents and antibacterial activities against the selected oral bacterial pathogens, it can therefore be concluded that methanol and aqueous extracts of these plants may be used as chewing sticks since it contains bioactive components that can also inhibit the growth and activities of the microorganisms in the oral cavity. Also, the plants can also be used in the manufacture of herbal paste and drugs which can be use in the treatment of ailments caused by these microorganisms.

4.2. Conflict of Interest

The author has declared there is no conflict of interest.

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