Original Research Article

JOURNAL OF BASIC PHARMACOLOGY AND TOXICOLOGY

Phytochemistry, Antimicrobial and Toxicological Studies of *Greenwayodendron suaveolens* Seed Extracts

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Abstract

Greenwayodendron suaveolens is a tropical deciduous plant traditionally used to treat several aliments such as fever, rheumatism, pains, oedema and swollen glands. The antimicrobial properties, phytochemistry and toxicity of *G. suaveolens* aqueous and chloroform seed extracts were evaluated using referenced procedures as broth dilution, spread plate and frothing test. The test microbial isolates used were; *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans*. Alkaloids, saponins, cardiac glycosides, flavonoids and terpenoids were detected in the seed extracts. *S. aureus* was susceptible whilst *A. niger* showed the least zone of inhibition against chloroform seed extract. The maximal MIC values were exhibited by chloroform and aqueous seed extract against *S. aureus* and *E. coli*. *A. niger* and *C. albicans* had the least MIC values against the aqueous seed extract. All the test bacterial cultures showed multiple resistance against the control antibiotics. The administered aqueous seed extract was non-toxic to rats at the maximal dose, 400 mg/kg, reflecting the fact that the lethal dose (LD_{50}) of the aqueous seed extract was greater than 400 mg/kg. The observed difference in the mean body weights of the different groups of rats administered varying doses of *G. suaveolens* aqueous seed extract were statistically insignificant ($p > 0.05$).

Key words: *Greenwayodendron suaveolens*, Seed extract, Phytochemicals, Antimicrobial, Toxicity

1. Introduction

The nutritional and medicinal potentials of plants have been attributed to the phytochemicals and other chemical constituents contained in them [1]. Mbatchou *et al.* [2] stated that in spite of their importance, it has been reported that out of the 250,000 to 500,000 species of existing plants on earth, only about 300 species are utilized in the food, pharmaceutical, cosmetics and perfume industries. Goud *et al.* [3] reported that the screening of medicinal plants for antimicrobial agents has gained much importance in the development and utilization of medicinal plant resources in developing countries. This has conversely helped in the extension of health care to maximum number of population in these countries. Mohanta *et al.* [4] posited that the screening of compounds obtained from plants for their pharmacological assays has been a vast source of innumerable therapeutic agents represented by molecular diversity engineered by nature. Therefore, plants around us can be investigated for the purpose of identifying those that may be potent against infectious organisms and hence, useful in treating ailments caused by microorganisms [5].

Greenwayodendron suaveolens, (Annonaceae) is restricted to tropical Africa and is widespread from Southern Nigeria to Western Uganda, Northern Tanzania, Southern Democratic Republic of Congo and Cabinda (Angola) [6]. *G. suaveolens* is a deciduous, medium-sized to fairly large tree up to 35–45 m tall. The inner bark is fibrous and has a yellow to orange or pale brown color; it becomes brownish or blackish upon exposure, with a strong resinous smell [6]. Root, leaves and barks of this plant are used in traditional medicine for the treatment of fever, rheumatic pains, oedema, swollen glands, headache, stomach ache, constipation, hernia, facilitation of child birth, fertility and as ornamentals, anthelmintic and aphrodisiac [7, 8]. In Cameroon and Gabon, the bark is applied to scarifications for the treatment of malaria. In Democratic Republic of Congo, pounded bark is used in a mixture with other plants to make arrow poison while in Nigeria, the leaf has been recorded being taken internally for menorrhagia and the fruit is equally edible [9].

In view of the current ethnomedicinal usage of decoctions prepared from several parts of *G. suaveloens*, this research was aimed at evaluating the phytochemistry, antimicrobial properties and toxicity of *G. suaveolens* aqueous and chloroform seed extracts.

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2. Materials and Methods

2.1. Collection and extraction of sample

The seeds of *G. suaveolens* were collected at the premises of the Department of Plant Biology and Biotechnology, University of Benin, Benin City and identified by Dr. T. Odaro of the same department. The seeds were rinsed twice in clean tap water and dried in an oven at 40 °C for 24 hr and then blended to powder. About 100 g of the blended *G. suaveolens* seed was soaked in 100 ml of chloroform and distilled water separately for 24 hr. The extracts were filtered using Whitman No. 1 filter paper separately. The filtrates were concentrated in water bath at 40 °C seperately. After the complete evaporation, each extract was weighed and preserved aseptically at 4 °C.

2.2. Phytochemistry

Qualitative tests for alkaloids, tannins, phlobatannins, saponins, cardiac glycosides, flavonoids, steroids and terpenoids were conducted using standard methods such as Mayer's reagent test for determination of alkaloids and frothing test for saponin determination [10, 11].

2.3. Test microorganisms

The test microbial isolates were *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger and Candida albicans.* Pure cultures of the bacterial isolates and *Candida albicans* were obtained from the Department of Medical Microbiology, University of Benin Teaching Hospital, Benin City. Pure culture of *Aspergillus niger* was collected from the Microbiology laboratory, Edo Environmental Consults and Laboratory, Benin City, Nigeria.

2.4. Standardization of the microbial inoculum

All the test bacterial and fungal isolates were sub-cultured on freshly prepared nutrient agar plates and malt extract agar plates and incubated for 24 hr and 48 hr respectively. Portions of the streaked colonies were transferred into test tubes containing 5 mL of sterile nutrient broth and incubated for 3 hr at 37 °C. The growth of bacteria and fungi suspension obtained was compared to that of freshly prepared barium sulphate solution (0.5 mL of 1% barium in chloride to 99.5 mL of 1% H_2SO_4 (0.36 N) [12]. The turbidity was adjusted by adding more sterile nutrient broth to match 0.5 McFarland standard (10^6 cfu/ml).

2.5. Determination of antimicrobial activity of the seed extracts and control antibiotics

The agar-well diffusion procedure as described previously [13], was utilized to determine the inhibitory effects of the seed extract on the test cultures. The tests were carried out using a stock concentration of 200 mg/ mL prepared by dissolving 0.2 g of the respective chloroform and aqueous seed extracts into 10 mL of DMSO. Prepared and labeled Muller Hinton plates were seeded with 2 mL of standardized bacterial and fungal broth cultures respectively. Plating was done in triplicates for each microbial culture. The microbial lawn was done using a sterile glass rod. The seeded plates were allowed to dry. A 6 mm sterile cork borer was used to punch one equidistant hole in the middle of the labeled inoculated agar plates. The holes were filled with 0.2 mL of the differing concentrations of the seed extracts. The bored agar plates were left at room temperature for 10 min, allowing the diffusion of the extracts into the agar. The plates seeded with bacterial isolates and *C. albicans* were incubated at 35 °C for 24 hr. The plates with *A. niger* were kept at room temperature for 72 hr. At the end of the incubation period, the plates were observed and the antimicrobial activity of the seed extracts was assessed by an inhibition zone surrounding the well. The mean zones of inhibition was measured and expressed in millimeters. To another set of seeded Muller hinton agar plates, commercially available antibiotic discs were placed on the agar plates as positive control after which the plates were incubated overnight at 35 °C. At the end of the incubation period, the diameter of inhibition zone(s) was measured using a meter rule and recorded. Distances lesser than 14 mm were regarded as resistant (R), while distances which ranged from 14 mm to 17 mm were indicated as intermediate (I). Also, zones of inhibition greater than 17 mm were recorded as susceptible (S) for the respective isolates [14].

2.6. Minimum inhibitory concentration (MIC) determination

The MIC of both the chloroform and aqueous extracts of *G. suaveolens* seeds were ascertained using the broth dilution method [15]. The seed extracts were prepared to the maximal concentration of 100 mg/mL (stock concentration) dissolved in 10 mL of DMSO and serially diluted to give concentrations ranging from 50 mg/mL to 3.125 mg/ mL. Standardized microbial broth cultures (0.1 mL) were inoculated into the labeled tubes containing the diluted extracts. The tubes were incubated at room temperature for 24 hr for bacteria and at room temperature for 48r h for fungi [15]. The least concentration of the extract which inhibited the growth of the inocula was considered as the minimum inhibitory concentration.

2.7. Experimental animals

Twenty five wistar rats of both sexes weighing between 127 g – 155 g were kept and maintained in metal cages and fed. They were allowed to acclimatize for 21 days prior to the experiment after which they were thoroughly examined to establish their state of health and suitability for the experiment. The rats were randomly divided into 5 groups each with 5 rats. The rats were fasted for 16 hr prior to the administration of the extract (crude aqueous extract). Graded doses of extract (100, 200, 300 and 400 mg/kg) were administered to the respective corresponding groups of B, C, D and E. Rats in Group A (control) had distilled water

only. All the animals were observed closely and the body weight change was measured at a 7 day interval for 28 days.

2.8. Statistical analysis

The data are expressed as mean ± S.E.M. Analysis of variance (ANOVA) of the respective mean body weights obtained was conducted using SPSS 16.0. Duncan Multiple Range (DMR) test was conducted as the post hoc test. Significant differences in the mean body weights were determined at $p < 0.05$.

3. Results

The result of the phytochemical screening of *Greeenwayodendron suaveolens* seeds is shown in Table 1. The presence of alkaloids, saponins, cardiac glycosides, flavonoids and terpenoids were detected in the seed extract whilst tannins, phlobatannins and steroids were not detected in the analyzed seeds (Table 1). The results of the antimicrobial activity of *G. suaveolens* chloroform and aqueous seed extracts on test microbial isolates are presented on Table 2. *S. aureus* was the susceptible isolate exposed to the seed extract, displaying an inhibitory zone of 12.0 ± 0.82 mm whilst *A. niger* elaborated the least zone of inhibition (3.3 ± 0.47 mm) against the chloroform seed extract (Table 2). *A. niger* and *C. albicans* were resistant to the aqueous seed extract (Table 2).

Table 1. Qualitative phytochemistry of *G. suaveolens* aqueous seed extract

Phytochemical	Inference
Alkaloids	+
Tannins	-
Phlobatannins	-
Saponins	+
Cardiac glycosides	+
Flavonoids	+
Steroids	-
Terpenoids	+

Note: + indicates presence and - indicates absence

Table 2. Zone of inhibition (mm) of *G. suaveolens* chloroform and aqueous seed extracts on test cultures

Test isolates	Chloroform extract [a]	Aqueous extract [a]
Staphylococcus aureus	12.0 ± 0.82	5.7 ± 0.47
Escherichia coli	10.3 ± 0.47	7.3 ± 0.47
Bacillus subtilis	8.3 ± 0.47	4.7 ± 0.47
Pseudomonas aeruginosa	5.3 ± 0.47	3.7 ± 0.47
Aspergillus niger	3.3 ± 0.47	NMIZ
Candida albicans	7.0 ± 0.82	NMIZ

Note: a = Values are mean ± S.E.M. where number of replicates 'n'=3; NMZI=No Measurable Inhibitory zone.

The maximal MIC value of 25 mg/mL was exhibited by chloroform and aqueous seed extract against *S. aureus* and *E. coli* (Table 3). *A. niger* and *C. albicans* displayed the least values of MIC > 100 mg/mL against the aqueous seed extract (Table 3).

S. aureus and *B. subtilis* exhibited resistance against Zinacef, amoxicillin, Septrin Ampiclox and streptomycin and were susceptible against gentamicin and ciprofloxacin (Table 4). *S. aureus* displayed intermediate sensitivity to perfloxacin and erythromycin while *B. subtilis* showed sensitivity against perfloxacin and erythromycin (Table 4). *E. coli* and *P. aeruginosa* were resistant against chloramphenicol, sparfloxacin, amoxicillin, Augmentin and streptomycin and exhibited sensitivity against ofloxacin (Table 4). *E. coli* was susceptible towards gentamicin and ciprofloxacin whilst *P. aeruginosa* displayed intermediate sensitivity against ciprofloxacin (Table 4).

Table 3. Minimum inhibitory concentration (MIC) values of the chloroform and aqueous seed extracts of *G. suaveolens*.

Test isolates	Chloroform extract (mg/mL)	Aqueous extract (mg/mL)
Staphylococcus aureus	25	50
Escherichia coli	25	25
Bacillus subtilis	50	100
Pseudomonas aeruginosa	50	50
Aspergillus niger	100	>100
Candida albicans	100	>100

Grouped rats administered 400 mg/kg of aqueous seed extract had the minimal mean body weight at day 0 (130.90 ± 3.55 g) while rats administered with 300 mg/kg aqueous seed extract had the highest body weight (195.24 ± 1.36 g) at day 28 of the study (Table 5). The observed difference in the mean body weights were statistically insignificant ($p > 0.05$) as shown on Table 5.

4. Discussion

The seed extracts especially the chloroform extract exhibited marked antimicrobial activities against the test cultures (Table 2 and 3). This trend could be attributed to the presence of phytochemicals such as alkaloids, saponins, cardiac glycosides, flavonoids and terpenoids (Table 1). Aruoma [16] reported that mixtures of such chemicals have been known to exhibit a broad spectrum of biological effects and pharmacological properties. The presence of alkaloids in the seed extract was in agreement with an earlier report by Ajayi *et al.* [6] who detected alkaloids in defatted *G. suaveolens* seeds. The maximal antimicrobial attributes of the chloroform seed extract in comparison with the aqueous preparation (Table 2 and 3) might be the consequence of increased solubility of the bioactive phytochemicals in chloroform solvent. Subsequently, this might have resulted

Table 4. Antibiogram profiles of the test bacterial isolates exposed to several standard commercial antibiotic discs

Gram positive cultures	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
Staphylococcus aureus	I	S	R	R	R	R	S	R	R	I
Bacillus subtilis	S	S	R	R	R	R	S	R	R	S
Gram negative cultures	**SAX**	**CH**	**SP**	**CPX**	**AM**	**AU**	**CN**	**PEF**	**OFX**	**S**
Escherichia coli	R	R	R	S	R	R	S	I	S	R
Pseudomonas aeruginosa	R	R	R	I	R	R	R	R	S	R

KEY: S = Sensitive, I = Intermediate, R = Resistant, PEF = Perfloxacin, CN = Gentamicin, APX = Ampiclox, Z = Zinnacef, AM = Amoxacillin, R = Rocephin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, E = Erythromycin, CH = Chloramphenicol, AU = Augmentin, OFX = Ofloxacin, SP = Sparfloxacin.

Table 5. Body weight (mean ± S.E.M.) (g) of the grouped rats administered with different doses of G. *suaveolens* aqueous seed extract.

Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 28
Control	153.67±1.64	169.41±3.55	171.89±1.61	182.30±2.91	191.45±1.55
100 mg/kg	138.96±1.73	153.74±0.99	162.56±1.32	169.48±1.77	184.66±0.94
200 mg/kg	135.80±0.85	149.83±1.53	157.94±1.44	165.81±1.70	177.90±1.59
300 mg/kg	142.55±1.86	156.73±1.92	169.66±1.42	184.70±1.61	195.24±1.36
400 mg/kg	130.90±3.55	144.95±3.90	154.38±8.76	164.23±1.49	174.82±2.19

n=5; Means are not significantly different ($p > 0.05$) from each other using DMR.

Table 6. Effect of aqueous extract of *G. suaveolens* on organ weight (mean ± S.E.M.) (g) in albino rats

Organ	Control	100 mg/kg	200 mg/kg	300 mg/kg	400 mg/kg
Liver	4.53±1.21	4.25±1.21	4.31±0.12	4.70±0.16	4.50±1.20
Kidney	1.60±0.12	1.48±0.10	1.33±0.11	1.56±0.03	1.48±0.12
Lung	1.89±0.16	1.63±0.21	1.58±0.51	1.70±0.32	1.65±0.43
Heart	0.49±1.32	0.49±0.11	0.50±0.13	0.49±0.04	0.48±0.52
Intestine	1.64±0.24	1.35±0.13	1.43±1.21	1.60±1.52	1.39±0.12
Pancreas	0.37±0.22	0.31±0.23	0.26±0.23	0.33±0.12	0.32±0.33

Means are not significantly different ($p > 0.05$) from each other using DMR

Table 7. Effect of crude aqueous extract on serum biochemical parameters in albino rats.

Parameters	Control	100 mg/kg	200 mg/kg	300 mg/kg	400 mg/kg
Total bilirubin (mmol/L)	6.88±0.11	6.47±0.34	6.25±0.43	5.96±0.34	5.54±0.35
Total protein (g/L)	57.64±0.23	53.40±0.35	56.74±.011	52.93±0.52	53.17±0.82
Albumin (g/L)	33.28±0.32	33.20±0.43	36.41±0.12	30.86±0.63	32.74±0.15
Total cholesterol (mmol/L)	1.76±0.42	1.74±0.51	2.20±0.21	2.04±0.28	2.11±0.18
Creatinine (mmol/L)	67.90±0.32	72.65±0.12	78.91±0.13	82.49±0.92	80.86±0.14

Values are expressed as (mean ± S.E.M.), n=5; Means are not significantly different ($p > 0.05$) from each other using DMR.

in the increased bioavailability of these phytochemicals in both solid and liquid media leading to inhibition of microbial growth. Ahmad *et al.* [17] also reported that plant extracts in organic solvents exhibited more consistent antimicrobial activity compared to those extracted in water.

The antibiogram profiles of the test bacterial cultures revealed that all the bacterial isolates were resistant to five or greater than five of the total number of control antibiotics utilized in the study (Table 4). This trend was not surprising, given the fact that the bacterial isolates were of clinical origin and it also gives credence to an earlier opinion by Lawal *et al.* [18] who indicated the need to develop new antimicrobial agents and antibiotics as a result of the fact that microorganisms are developing resistance to many drugs and the tremendous increase in the mortality rate from infectious diseases.

In comparison with the control group, the administration of the crude aqueous extract on the animals had an increasing impact on mean body weight at the end of the study period (Table 5). However, the group fed with 300 mg/kg extract had maximal mean body weight at the end of the study period (Table 5). This might suggest that 300 mg/kg is the most effective dose which can have a positive influence on animals. Ajayi *et al.* [6] reported that deffated *G. suaveolens* seeds is a source of roughage and fibre and is also rich in magnesium and calcium which are essential for animal nutrition. The crude aqueous seed extract was non-toxic to the rats fed with different doses of the crude extracts as no mortality was observed in all the non-control grouped rats in this study. Vital organs such as liver, kidney, lung, heart, intestine and pancreas showed no significant difference in weight. This is an indication that long-term use of the plant does not have inflammatory effect on vital organs in the body. The biochemical parameters such as total bilirubin, total protein, albumin, total cholesterol and creatine showed no significant difference in values between control and treatment groups (Table 7). These are possible indications that *G. suaveolens* may not be toxic for long-term use. However, further study in this direction is encouraged to see the activity of the plant at the cellular level.

5. Conclusion

Aqueous and chloroform extracts of *G. suaveolens* exhibited broad spectrum antimicrobial activity against exposed multiple test antibiotic-resistant microbial isolates. Several medically significant phytochemicals such as alkaloids, saponins, cardiac glycosides, flavonoids and terpenoids were identified in the seed extract. The aqueous seed extract was non-toxic at the maximal dose at 400 mg/kg, which is suggestive of the fact that the lethal dose (LD_{50}) of the crude aqueous seed extract is greater than 400 mg/kg.

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