Antimicrobial Compounds from *Spondias purpurea*

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The dichloromethane, ethyl acetate, ethanol and water extracts of the freeze-dried bark of Spondias purpurea were tested for antimicrobial activities against seven microorganisms. Results of the study indicated that all the extracts at 30 μg have low antimicrobial activities against E. Coli, P. aeruginosa and T. mentagrophytes. The water and ethanol extracts showed low activity against C. Albicans, while the ethyl acetate extract gave low activity against A. niger. All the extracts were inactive against S. Aureus and B. subtilis. The dichloromethane extract was fractionated by silica gel chromatography. It afforded 1, 2 and a mixture of hydrocarbons. Compounds 1 and 2 were identified by comparison of their ^1H NMR spectral data with those of lutein and sistosterol, respectively. Sitosterol and the mixture of hydrocarbons were tested against the same microorganisms. Result of the study indicated that both compounds had low activity against E. Coli and P. aeruginosa at 30 μg and inactive against S. Aureus, B. subtilis, T. mentagrophytes and A. niger. Sitosterol gave low activity against C. albicans.

INTRODUCTION

Spondias purpurea Linn. commonly known as sineguelas, is a medicinal plant which has antimicrobial properties and is also used for the treatment of tonsilitis and stomatitis for children[1]. Previous studies reported on the isolation of polysaccharides[2] and fatty acids, monoterpenes and sesquiterpenes[3] from the leaf oil of *S. purpurea*.

We now report the antimicrobial test results of the dichloromethane, ethyl acetate, ethanol and water extracts from the freeze-dried bark of *S. purpurea*. The antimicrobial properties of sitosterol and the mixture of hydrocarbons isolated from the dichloromethane extract are likewise reported. Another compound isolated from this extract is lutein which was reported to have antimicrobial and antimutagenicity potentials[4].

RESULTS AND DISCUSSION

The dichloromethane, ethyl acetate, ethanol and water extracts of the freeze-dried bark of *S. purpurea* were tested for potential antimicrobial activities against seven microorganisms, namely, *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, *T. mentagrophytes*, *C. albicans* and *A. niger*. Results of the study (Table 1) indicated low antimicrobial activities of all the extracts tested at 30 μg against *E. coli*, *P. aeruginosa* and *T. mentagrophytes*. The water and ethanol extracts showed low activity against *C. albicans*, while the ethyl acetate extract was active against *A. niger*. All the extracts were active against *S. aureus* and *B. subtilis*. Thus, the extracts have low activities against the tested microorganisms. This may be due to the very low concentration of the tested extracts (30 μg) which is the same as the concentration of the stantard antibiotics (30 μg).

For this study, only the least polar dichloromethane extract was fractionated to identify its constituents and to determine the effect of purification on the antimicrobial activity of the constituents of the extract. Fractionation by silica gel cromatography afforded **1** and **2** and a mixture of hydrocarbons. The ^{1}H NMR spectral data of **1** were compared with those found in the literature for lutein[4], while the ^{1}H NMR spectral data of **2** were compared with those found in the literature for sitosterol[5]. The spectra matched in all essential respects.

Lutein was not tested for antimicrobial activity since a previous study indicated that it has high activity against a number of pathogenic bacteria and fungi. It was also found to have high antimutagenic activity[4]. Thus, only sitosterol and the mixture of hydrocarbons were tested for antimicrobial activity against the seven microorganisms used in the antimicrobial tests of the crude extracts. Results of the study (Table 2) indicated that sitosterol has low antimicrobial activity against *E. coli*, *P. aeruginosa* and *C. albicans*. It was found inactive against *S. aureus* and *A. niger*. The mixture of hydrocarbons indicated low activity against *E. coli* and *P. aeruginosa*. It was found inactive against the other microorganisms tested.

EXPERIMENTAL

Instrumentation and General Procedure. The NMR spectra were recorded in $CDCl_3$ with the use of a JEOL Lambda Fourier Transform 400 MHz NMR. Silica gel type 60 (Merck) was used for column chromatography and plastic backed plates coated with silica gel F_{254} (Merck) for thin layer chromatography. Fractions were monitored by TLC and spots were visualized by spraying with vanillin-sulfuric acid, then warming. All reagents used were analytical grade reagents.

Sample collection

The bark of *Spondias purpurea* was collected from San Miguel, Bulacan in December 1999. It was identified as *Spondias purpurea* Linn. at the Philippine National Museum.

Extraction and isolation

The ground bark (346 g) was freezed-dried and extracted exhaustively with dichloromethane (1 L). The dichloromethane extract was concentrated under vacuum to afford a crude extract (1 g) which was subject to gravity column chromatography using increasing proportions of ethyl acetate in petroleum ether (10% increments) as eluents. The fractions eluted with petroleum ether was rechromatographed (3x) in the same solvent to afford the mixture of hydrocarbons (5.4 mg). The 20% ethyl acetate in petroleum ether fraction was rechromatographed (2x) in 20% ethyl acetate in petroleum ether to afford sitosterol (20.5). The 30% ethyl acetate in petroleum ether fraction was rechromatographed (3x) in 30% ethyl acetate in petroleum ether to afford lutein (2 mg).

The freeze-dried bark after extraction with dichloromethane was again extracted with ethyl acetate (1L), then ethanol (1L) and finally water (1L).

Antimicrobial Test

The microorganisms used in these tests are *Staphylococcus aureus* UPCC 143, Bacillus subtilis UPCC 1, *Escherichia coli* UPCC 195, *Pseudomonas aeruginosa* UPCC 244, *Candida albicans* UPCC 2168, Aspergillus niger UPCC 4063 and *Trichophyton mentagrophytes* UPCC 4193.

A microbial suspension containing approximately 107 cells/mL was prepared for each test organism for 24-hour agar culture using 0.1% peptone water. One-tenth mL of the bacterial suspension was transferred into prepoured 30 mL deep nutrient agar plate, the yeast suspension into glucose yeast peptone agar plate and the fungal suspension on potato dextrose agar plate. About 5 mL of the corresponding melted agar cooled to about 45 ^{0}C was immediately poured into the plate. The plate was swirled to distribute the microbial cells evenly on the plate. After the overlay agar has solidified, three 1-cm diameter holes were bored from equidistant points using a sterile cork borer.

Two-tenths mL portions of samples were placed in duplicate holes per organism. A similar volume of the solvent ethanol and of the corresponding antibiotic for each test organism was placed in the remaining two wells on the plate. Plates were incubated at room temperature to prevent condensation of liquid on the petri lid that may cause interference in distribution of organisms on the surface. Bacterial and yeast plates were read after 24 hours, while the mold plate was read after three days. The diameter of the holes and the clearing zones were measured in millimeter (mm). The average for the compounds was taken and the antimicrobial activity index (AI) was computed as the mm clearing zone minus mm hole divided by mm hole.

CONCLUSION

The dichloromethane, ethyl acetate, ethanol and water extracts of the freeze-dried bark of *S. purpurea* indicated low antimicrobial activities against several microorganisms at 30 μg. The low activity may be attributed to the low concentration of the extracts tested which is the same as the concentration of the standard antibiotics.

Fractionation of the dichloromethane extract afforded lutein, sitosterol and a mixture of hydrocarbons. Antimicrobial tests on sitosterol and the mixture of hydrocarbons showed low antimicrobial activities at 30 μg against a few of the microorganisms tested. Lutein, a carotenoid which is known to have high antimicrobial and antimutagenic properties[1] also contributed to the antimicrobial properties of the bark of *S. purpurea*.

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Table 1. Antimicrobial test results of Crude Extracts.

Test Organisms	Sample[a]	Clearing Zone, mm[b]	AI
P. aeruginosa	A	12	0.2
	B	12	0.2
	C	12	0.2
	D	12	0.2
	Tetracycline	30	2.0
B. subtilis	A	-	0
	B	-	0
	C	-	0
	D	-	0
	Chloramphenicol	40	3.0
E. coli	A	12	0.2
	B	12	0.2
	C	12	0.2
	D	12	0.2
	Tetracycline	30	2.0
S. aureus	A	-	0
	B	-	0
	C	-	0
	D	-	0
	Chloramphenocol	30	2.0
C. albicans	A	12	0.2
	B	12	0.2
	C	-	0
	D	-	0
	Chlotrimazole	25	1.5
T. mentagrophytes	A	12	0.2
	B	12	0.2
	C	12	0.2
	D	12	0.2
	Chlotrimazole	38	2.8
A. niger	A	-	0
	B	-	0
	C	12	0.2
	D	-	0
	Cycloheximide	16	0.6

A = water, B = ethanol, C = ethyl acetate, D = dichloromethane
[a]30 ml of 1 µg/ml solution; [b]Average of 3 trials

Table 2. Antimicrobial Test Results of Sitosterol (A) and the Mixture of Hydrocarbons (B).

Test Organisms	Sample[a]	Clearing Zone, mm[b]	AI
P. aeruginosa	A	12	0.2
	B	12	0.2
	Tetracycline	20	1.0
B. subtilis	A	-	0
	B	-	0
	Chloramphenicol	40	3.0
E. coli	A	13	0.3
	B	12	0.2
	Tetracycline	30	2.0
S. aureus	A	-	0
	B	-	0
	Chloramphenicol	30	2.0
C. albicans	A	13	0.3
	B	-	0
	Chlotrimazole	25	1.5
T. mentagrophytes	A	-	0
	B	-	0
	Chlotrimazole	38	2.8
A. niger	A	-	0
	B	-	0
	Cycloheximide	16	0.6

[a]30 ml of 1 µg/ml solution; [b]Average of 3 trials