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Antifungal Compounds from *Anacardium occidentale*

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The nutshell oil of *Anacardium occidentale*, commonly known as kasoy afforded mixtures of anacardic acids and cardols in varying degrees of unsaturation. Their structures were elucidated by extensive 1D and 2D NMR and high resolution mass spectrometry. The anacardic acids were identified as 1a (50%), 1b (17%) and 1c (33%), while the cardols were identified as 2a (80%) and 2b (20%). Small amounts (<1%) of mono-, di- and triunsaturated seventeen-carbon side-chain analogues of sample 1, and bilobol, the monounsaturated analog of sample 2, were also observed by mass spectrometry. The mixture of 1a, 1b, and 1c indicated slight activity against *E. coli* and *P. aeruginosa* and moderate activity against *S. aureus*, *B. subtilis*, *C. albicans*, *T. mentagrophytes* and *A. niger*. The mixture of 2a, and 2b indicated slight activity against *P. aeruginosa* and *C. albicans*, moderate activity against *B. subtilis* and *T. mentagrophytes*, and high activity against *A. niger*.

Keywords: *Anacardium occidentale*, Anacardaceae, cardols, anacardic acids, antimicrobial

Anacardium occidentale, commonly known as kasoy, is a small tree found throughout the Philippines. It is cultivated for its fruits and kernel of the nuts which are sold commercially. A decoction of the bark and leaves could be used as a herbal astringent to treat toothaches, sore throats and sore gums. The bark decoction when orally taken could treat extreme diarrhea (Quisumbing 1978).

Chemical studies on the nut shell oil of the plant reported the isolation of anacardic acids, cardanols and cardols by HPLC (Shobha et al. 1992, Nagabhushana & Ravindranath 1995) and phase separations (Tyman et al. 1992). Anacardic acids and their analogs were reported to have antitumor activities (Kubo et al. 1993). Cashew nut shell oil was reported to be mutagenic with and without metabolic activation using the *Salmonella typhimurium*/microsome system (Polasa & Rukmini 1987). Topical anti-acne preparations containing cashew nut shell oils for topical treatment of *Acne vulgaris* have also been reported (Sato et al. 1992, Shimomuta & Koizumu 1992). The antimicrobial activity of phenolic compounds from the nut shell oil were tested against *B. subtilis*, *E. coli*, *S. cereviceae* and *P. chrysogenum*. They have potential antimicrobial activity only against gram-positive bacteria (Himejima & Kubo 1991).

We now report the fractionation, structure elucidation and antimicrobial test results of mixtures of anacardic acids and cardols from the ethyl acetate extract of the nutshell of *A. occidentale*. This is the first report on the percentage composition of anacardic

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acids and cardols from the nutshell oil of *A. occidentale* and their antifungal properties.

Methods

General Experimental Procedure

NMR spectra were recorded in $CDCl_3$ on a Bruker AMX at 300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR. The high resolution EIMS were recorded on a Micromass Autospec mass spectrometer. Column chromatography was performed with silica gel 60 (70-230 mesh), while TLC was performed with plastic-backed plates coated with silica gel F_{254}. The plates were visualized by spraying with vanillin-H_2SO_4 and warming.

Sample Collection:

The nut shell of *Anacardium occidentale* Linn. was collected from a cashew processing factory in Antipolo City in June 1999.

Extraction and Isolation:

The dried nut shell of *Anacardium occidentale* Linn (800 g) were mashed and pulverized, then extracted with ethyl acetate at room temperature for two days to afford the crude extract (340 g). Ten mL of the crude extract was chromatographed (silica gel 60, 70-230 mesh) using hexane, 30% ethyl acetate in hexane, 60% ethyl acetate in hexane, ethyl acetate and acetone as eluents. The 60% ethyl acetate in hexane and ethyl acetate fractions were combined and rechromatographed (3x) in 10% hexane in ethyl acetate to afford sample 1 (0.8 g) and sample **2** (1.1 g).

Antimicrobial Tests

The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These are *Pseudomonas aeruginosa* UPCC 1244, *Bacillus subtilis* UPCC 1149, *Escherichia coli* UPCC 1195, *Staphylococcus aureus* UPCC 1143, *Candida albicans* UPCC 2168, *Trichophyton mentagrophytes* UPCC 4193 and *Aspergillus niger* UPCC 3701.

Microbial suspension containing approximately 6×10^8 cells/mL was prepared from each test organism for 24-hour culture of *S. aureus, E. coli, P. aeruginosa, B. subtilis* and *C. albicans* and from a 5 day old *A. niger* and *T. mentagrophytes*. The suspending medium used for each microbial suspension was 0.1% peptone water. One-tenth (0.1) mL of the bacteria, yeast and molds were transferred into pre-poured nutrient agar (NA, DISCO Laboratories, Detroit, Michigan), glucose yeast peptone agar (GYP) [14] and potato dextrose agar (PDA, DISCO Laboratories, Detroit, Michigan), respectively. About 5 mL of corresponding medium, autoclaved and cooled to about 45°C was poured into the 90 mm petri dish. The plate was swirled to distribute the microbial cells evenly on the plate and the agar overlay was allowed to solidify. Three 10 mm wells were cut from equidistant points of the seeded agar plates using sterile cork borer. Thirty (30) µg of samples dissolved in 95% EtOH were transferred in each well. For the standard agent, 30 µg were used.

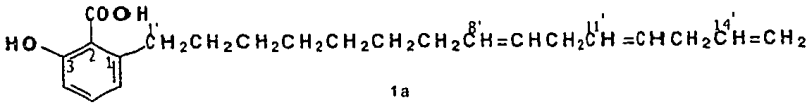
The NA, GYP and PDA-based cultures were incubated at 30 ± 1°C for 24, 48 and 72 hours, respectively. Antimicrobial effects were determined by measuring the zone of the growth inhibition represented by a clear zone in mm. The average diameter of the clear zones was used to calculate an antimicrobial index (Guevara & Recio 1985).

Results and Discussion

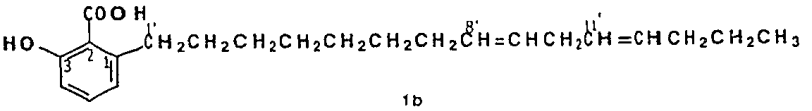
Anacardium occidentale nut shells were extracted with ethyl acetate, and the extract separated by silica gel chromatography into fractions containing mixtures of anacardic acids and cardols, in varying degrees of unsaturation. Structures were elucidated by extensive 1D and 2D NMR and mass spectrometry, as follows.

The high resolution mass spectrum of sample **1** indicated a mixture of three compounds varying in degrees of unsaturation. The mass spectrum gave molecular ion peaks (M^+) at 342.2195, 344.2347 and 346.2509 corresponding to molecular formulas of $C_{22}H_{30}O_3$ (**1a**), $C_{22}H_{32}O_3$ (**1b**) and $C_{22}H_{34}O_3$ (**1c**), respectively.

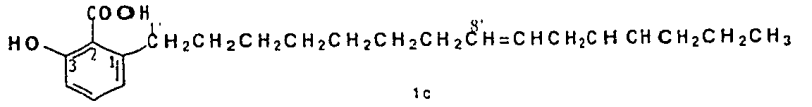
The ^1H NMR spectral data of sample **1** (Table 1) gave resonances at δ 6.80, 7.37 and 6.89. The proton at δ 6.80 is ortho coupled (J = 7.5 Hz) to the hydrogen at δ 7.37 which is also ortho coupled (J = 8.2 Hz) to the proton at d 6.89. The protons at δ 6.80 and 6.89 Hz are meta coupled (J = 1.2 Hz) to each other. This indicates a 1,2,3-trisubstituted aromatic system. The COSY spectrum indicated additional coupling as follows. The benzylic protons at δ 3.01 are coupled to the methylene hydrogens at δ 1.65, which are in turn coupled to the overlapping methylene protons at δ 1.40. The protons at δ 1.40 are also coupled to the allylic hydrogens at δ 2.05, which are in turn coupled to the olefinic protons at δ 5.40 and 5.38. Doubly-allylic hydrogens at δ 2.81 are coupled to the previous olefinic protons, and also to another set of olefinic protons at δ 5.44 and 5.42, which are in turn coupled to the other doubly-allylic hydrogens at δ 2.84, which are finally coupled to terminal olefinic protons at δ 5.82 and 5.06. The olefinic proton at δ 4.99 is geminally coupled to



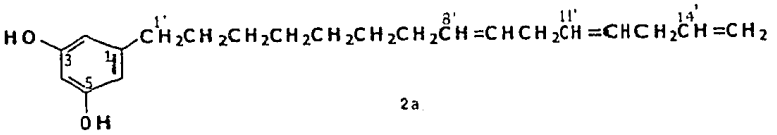
1a



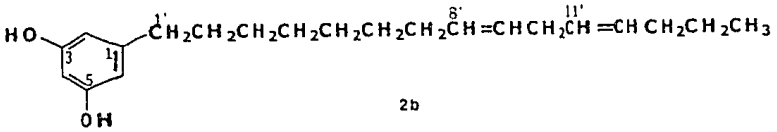
1b



1c



2a



2b

Figure 1. Compounds from *Anacardium occidentale*.

Table 1. 300 MHz ^{1}H NMR data of **1a**, **1b** and **1c** in $CDCl_3$.

Carbon No.	^{1}H, δ (**1a**)	^{1}H, δ (**1b**)	^{1}H, δ (**1c**)
1	---	---	---
2	---	---	---
3	---	---	---
4	6.89 (1H, dd, J = 1.2, 8.3 Hz)	6.89 (1H, dd, J = 1.2, 8.3 Hz)	6.89 (1H, dd, J = 1.2, 8.3 Hz)
5	7.37 (1H, dd, J = 7.6, 8.2 Hz)	7.37 (1H, dd, J = 7.6, 8.2 Hz)	7.37 (1H, dd, J = 7.6, 8.2 Hz)
6	6.80 (1H, dd, J = 1.3, 7.5 Hz)	6.80 (1H, dd, J = 1.3, 7.5 Hz)	6.80 (1H, dd, J = 1.3, 7.5 Hz)
1'	3.01 (2H, t, J = 7.1 Hz)	3.01 (2H, t, J = 7.1 Hz)	3.01 (2H, t, J = 7.1 Hz)
2'	1.65 (2H, m)	1.65 (2H, m)	1.65 (2H, m)
3'	1.4 (2H, m)	1.4 (2H, m)	1.4 (2H, m)
4'	1.4 (2H, m)	1.4 (2H, m)	1.4 (2H, m)
5'	1.4 (2H, m)	1.4 (2H, m)	1.4 (2H, m)
6'	1.4 (2H, m)	1.4 (2H, m)	1.4 (2H, m)
7'	2.05 (2H, m)	2.05 (2H, m)	2.05 (2H, m)
8'	5.40 (1H, m)	5.40 (1H, m)	5.40 (1H, m)
9'	5.38 (1H, m)	5.38 (1H, m)	5.38 (1H, m)
10'	2.05 (2H, m)	2.05 (2H, m)	1.3 (2H, m)
11'	5.44 (1H, m)	5.44 (1H, m)	1.3 (2H, m)
12'	5.42 (1H, m)	5.42 (1H, m)	1.3 (2H, m)
13'	2.84 (2H, m)	2.05 (2H, m)	1.3 (2H, m)
14'	5.82 (1H, ddt, J = 6.2, 10.1, 17.1 Hz)	1.40 (2H, m)	1.40 (2H, m)
15'	5.06 (1H, dq, 1.8, 17.1 Hz), 4.99 (1H, dq, 1.8, 10.1 Hz)	0.91 (3H, t, J = 7.0 Hz)	0.93 (3H, t, J = 7.3 Hz)

the hydrogen at δ 5.06 by 1.8 Hz and vicinally coupled to the hydrogen at δ 5.82 by 10.1 Hz, indicating cis coupling. The proton at δ 5.06 is coupled to the hydrogen at δ 5.82 by 17.1 Hz, indicating trans coupling. The presence of **1b** and **1c** in the mixture is indicated by two almost overlapping triplets for methyl hydrogens at δ 0.93 and 0.91, which couple to the δ 1.40 methylene protons.

The ^{13}C NMR spectral data of sample **1** (Table 2) indicated twenty-two major carbon resonances as follows. A carbonyl carbon of a carboxylic acid at δ 176.1; an oxygenated aromatic carbon at δ 163.5; two non-protonated olefinic carbons at δ 110.5 and 107.7; nine protonated olefinic carbons at δ 122.7, 135.4, 115.9, 130.3, 127.6, 129.3, 126.8, 136.8 and 114.5; nine methylene carbons at δ 25.5-36.4 for **1a**. Many of the carbon resonances for **1a** overlap with those for **1b** and **1c**, except in regions where their structures differ. Signals for **1a**, **1b** and **1c** have been tentatively assigned from the relative intensity of signals (see below). Additional methyl and methylene carbon resonances for **1b** and **1c** were found at δ 14.0 and 13.9, d 22.7 and 22.6, and δ 29.6 and 29.3, 31.5 and 31.7. Olefinic carbons for **1b** and **1c** were observed at δ 130.1, 129.90, 129.86, 129.79, 128.8 and 128.0.

Table 2. 75 MHz ^{13}C NMR data of **1a**, **1b** and **1c** in $CDCl_3$.

Carbon No.	^{13}C, δ (1a)	^{13}C, δ (1b)	^{13}C, δ (1c)
1	147.7	147.7	147.7
2	110.5	110.5	110.5
3	163.5	163.5	163.5
4	115.9	115.9	115.9
5	135.4	135.4	135.4
6	122.7	122.7	122.7
1'	36.4	36.4	36.4
2'	31.5	31.5	31.5
3'	29.7	29.7	29.7
4'	29.3	29.3	29.3
5'	29.2	29.2	29.2
6'	29.7	29.7	29.7
7'	27.2	27.2	27.2
8'	130.3	130.1	129.90
9'	127.6	128.0	128.8
10'	25.5	28.9	29.6*
11'	129.3	129.86*	29.3*
12'	126.8	129.79*	29.3*
13'	31.9	31.5	31.7
14'	136.8	22.6	22.7
15'	114.6	14.0	13.9
COOH	176.1	176.1	176.1

*maybe be interchanged

The ^{1}H and ^{13}C assignments for sample **1** (Tables 1 & 2) were verified by the heteronuclear 2D experiment HMQC and connectivity was verified by the inverse long-range heteronuclear experiment HMBC (Table 3) optimized for J = 10 Hz. The hydroxyl group was attached to C-3 (δ 163.5) due to its deshielded resonance and the long-range correlation of this carbon to H-4 and H-5. The alkyl group was attached to C-1 since this carbon is long-range correlated to H-5, H-6 and H-1'. The carboxylic acid was bonded to C-2 due to long-range correlation of the carbonyl carbon to H-6. The following long-range correlations were also observed: C-1' to H-6, H-2' and H-3'; C-2' to H-1' and H-3'; C-3' and C-6' to H-1', H-2' and H-7'; C-7' to H-6', H-8' and H-9'; C-13' to H-12', H-14' and H-15'. All long-range correlations observed are consistent with the structure of **1a**. Additional long-range correlations were observed for **1b** as follows: C-12' to H-10', H-11' and H-13'; C-14' to H-15'; and C-15' to H-13' and H-14'. For **1c**, the following long-range correlations were also observed: C-14' to H-15' and C-15' to H-13' and H-14'.

Literature search revealed that sample **1** is a mixture of anacardic acids in varying degrees of unsaturation. Compound **1a** has been previously reported from *Ginkgo biloba* leaf extracts (Furukawa, 1935) and cashew nut shell oil (Izzo & Dawson 1949, Shobha et al. 1992, Tyman et al. 1992, Nagabhushana & Ravindranath 1995).

The composition of the anacardic acid mixture was determined by NMR integration and mass spectrometry. In the NMR analysis, integrals were compared for the terminal olefin hydrogens (**1a** only), methyl hydrogens (**1b** and **1c**), and singly- and doubly-allylic hydrogens (**1a** and **1b**), with those for the benzylic hydrogens (common to all three), and the ratio of **1a:1b:1c** which best fitted all integration data determined. A 53:19:28 ratio was estimated. This is in close agreement with the HRMS molecular ion ratio of 50:17:33 for **1a:1b:1c** respectively. Cashew nut shell extract has been previously reported (Izzo & Dawson 1949) to contain at least 25% of **1c** in the mixture.

The HRMS of sample **1** also indicated small amounts of anacardic acid analogs with seventeen carbon side-chains. Molecular ions were observed at 370.2517 ($C_{24}H_{34}O_3$), 327.2659 ($C_{24}H_{36}O_3$) and 374.2819 ($C_{24}H_{38}O_3$). These acids have also been previously reported (Furukawa 1935, Izzo & Dawson 1949, Shobha et al. 1992, Tyman et al. 1992, Nagabhushana & Ravindranath 1995) as constituents of *Ginkgo biloba* and cashew nut shells.

The high resolution mass spectrum of sample **2** indicated a mixture of two compounds. The mass spectrum gave molecular ion peaks (M^+) at 314.2252, and 316.2406 corresponding to the molecular formula of $C_{21}H_{30}O_2$ (**2a**, 79%) and $C_{21}H_{32}O_2$ (**2b**, 21%), respectively.

The ^{1}H NMR spectral data of sample **2** (Table 4) indicated resonances for meta coupled aromatic

Table 3. 300 MHz HMBC correlations of **1a**, **1b** and **1c** in $CDCl_3$.

Carbon No.	HMBC Correlations of **1a**	HMBC Correlations of **1b**	HMBC Correlations of **1c**
1	H-4, H-5, H-1'	H-4, H-5, H-1'	H-5, H-6, H-1'
2	H-4, H-6, H-1'	H-4, H-6, H-1'	H-4, H-6, H-1'
3	H-5, H-6	H-5, H-6	H-4, H-5
4	H-4	H-4	H-6
5	H-6	H-6	H-6
6	H-1, H-6	H-1, H-6	H-1', H-4
1'	H-2', H-6, H-5	H-2', H-6, H-5	H-6, H-2', H-5
2'	H-1', H-3'	H-1', H-3'	H-1', H-3'
3'	H-1', H-2', H-4'	H-1', H-2', H-4'	H-1', H-2', H-4'
4'	H-2', H-4', H-5'	H-2', H-4', H-5'	H-2', H-5'
5'	H-3', H-5'	H-3', H-5'	H-3', H-5'
6'	H-7'	H-7'	H-7'
7'	H-6, H-8', H-9'	H-6, H-8', H-9'	H-6, H-8', H-9'
8'	H-6', H-7'	H-6', H-7'	H-6', H-7'
9'	H-7'	H-7'	H-7', H-10'
10'			H-11', H-12'
11'	H-13'	H-13'	H-13'
12'	H-13'	H-13'	H10', H-11',H-13'
13'	H-12', H-14', H-15'	H-12', H-14', H-15'	H-12', H-14', H-15'
14'		H-15'	H-15'
15'		H-13', H-14'	H-13', H-14'
COOH	H-6	H-6	H-6

Table 4. 300 MHz ^1H NMR data of **2a** and **2b** in $CDCl_3$.

Carbon No.	^1H, δ (**2a**)	^1H, δ (**2b**)
1	...	---
2	6.25 (1H, d, J = 2.0 Hz)	6.25 (1H, d, J = 2.0 Hz)
3	...	---
4	6.25 (1H, d, J =2.0 Hz)	6.25 (1H, d, J =2.0 Hz)
5	...	---
6	6.25 (1H, d, J = 2.0 Hz)	6.25 (1H, d, J = 2.0 Hz)
1'	2.48 (2H, t, J = 7.4 Hz)	2.48 (2H, t, J = 7.4 Hz)
2'	1.56 (2H, m)	1.56 (2H, m)
3'	1.31 (2H, m)	1.31 (2H, m)
4'	1.31 (2H, m)	1.31 (2H, m)
5'	1.31 (2H, m)	1.31 (2H, m)
6'	1.31 (2H, m)	1.31 (2H, m)
7'	2.05 (2H, q, J =6.5 Hz)	2.05 (2H, q, J = 6.5 Hz)
8'	5.40 (1H, m)	5.40 (1H, m)
9'	5.38 (1H, m)	5.38 (1H, m)
10'	2.80 (2H, m)	2.80 (2H, m)
11'	5.44 (1H, m)	5.44 (1H, m)
12'	5.42 (1H, m)	5.42 (1H, m)
13'	2.85 (2H, m)	2.05 (2H, q, J = 6.5 Hz)
14'	5.82 (1H, ddt, J = 6.2, 10.1, 17.1 Hz)	1.31 (2H, m)
15'	5.01 (1H, dd, J = 1.8, 10.1 Hz), 5.06 (1H, dd, J = 1.8, 17.1 Hz)	0.92 (3H, t, J = 7.6 Hz)
3-OH, 5-OH	4.86 (2H, br)	4.86 (2H, br)

protons at δ 6.25 (2H, d, J = 2.0 Hz) and 6.19 (1H, d, J = 1.9 Hz), indicating 1,3,5 trisubstitution. The olefinic protons at δ 5.01 and 5.06 are geminally coupled to each other by 1.8 Hz. The proton at δ 5.01 is coupled cis to the hydrogen at δ 5.82 by 10.1 Hz, while the proton at δ 5.06 is coupled trans to the hydrogen at δ 5.82 by 17.1 Hz. Additional olefinic protons were found at δ 5.37-5.43 (4H, m). Allylic protons were

attributed to the resonances at δ 2.48 (2H), 2.05 (2H), with doubly-allylic protons at δ 2.80 (2H) and 2.82 (2H). A broad singlet at δ 1.31 is assigned to methylene protons of almost similar environment. A hydroxyl resonance was found at δ 4.86 (2H, s, br). The presence of **2b** in the mixture is indicated by the resonance for a methyl group at δ 0.89. The **2a**:**2b** ratio was again calculated from the ^{1}H NMR integrals, and found to be 80:20 respectively, in good agreement with the HRMS molecular ion intensity ratio.

The ^{13}C NMR spectral data of sample **2** (Table 5) indicated resonances for twenty-one carbons with the following functionalities: three non-protonated [δ 156.5 (2C) and 146.1] and three protonated [δ 108.1 (2C) and 100.2] aromatic carbons; one methylene (δ 114.7) and five methine (δ 130.4, 127.8, 129.3, 126.9, and 136.8) olefinic carbons; and nine methylene carbons [δ 31.8, 31.5, 31.0, 29 (4C), 27.2 and 25.6]. Additional carbon resonances were found at δ 14.0, 22.5, 25.6, 31.0, 127.7 and 129.9 for **2b**.

Table 5. 75 MHz ^{13}C NMR data of **2a** and **2b** in $CDCl_3$.

Carbon No.	^{13}C, δ (2a)	^{13}C, δ (2b)
1	146.1	146.1
2	108.1	108.1
3	156.5	156.5
4	108.1	108.1
5	156.5	156.5
6	108.1	108.1
1'	35.8	35.8
2'	31.0	31.0
3'	29	29
4'	29	29
5'	29	29
6'	29	29
7'	27.2	27.2
8'	130.4	130.4
9'	127.8	127.8
10'	25.6	25.6
11'	129.3	129.9
12'	126.9	127.7
13'	31.5	31.0
14'	136.8	22.5
15'	114.7	14.0

The ^{1}H and ^{13}C assignments for sample **2** (Tables 4 & 5) were verified by the heteronuclear 2D experiment HMQC and connectivity was verified by the inverse long-range heteronuclear experiment HMBC (Table 6) optimized for J = 10 Hz. The hydroxyl groups were attached to C-3 (δ 156.5) and C-5 (δ 156.5) due to their deshielded nature and the long-range correlation of these carbons to H-2, H-4 and H-6. The alkyl group was attached to C-1 (δ 146.1) since this carbon is long-range correlated to H-1'. All long-range correlations observed are consistent with the structures of **2a** and **2b**.

Table 6. 300 MHz HMBC correlations of **2a** and **2b** in $CDCl_3$.

Carbon No.	HMBC	HMBC
1	H-1'	H-1'
2	H-2, H-4, H-1'	H-2, H-4, H-1'
3	H-2, H-6	H-2, H-6
4	H-2, H-6, H-1'	H-2, H-6, H-1'
5	H-2, H-4	H-2, H-4
6	H-2, H-6, H-1'	H-2, H-6, H-1'
1'	H-4, H-6	H-4, H-6
2'	H-1', H-3'	H-1', H-3'
3'	H-2', H-4'	H-2', H-4'
4'	H-3'	H-3'
5'	H-3', H-4', H-6'	H-3', H-4', H-6'
6'	H-4', H-5', H-7'	H-4', H-5', H-7'
7'	H-5', H-6', H-8,	H-5', H-6', H-8,
8'	H-7'	H-7'
9'	H-7', H-10'	H-7', H-10'
10'	H-9', H-11', H-12'	H-9', H-11', H-12'
11'	H-13'	---
12'	H-10'	H-10', H-13'
13'	H-12', H-15'	H-12', H-14', H-15'
14'	H-13'	H-13', H-15'
15'	---	---

Literature search revealed that **2a** and **2b** are cardol I and cardol II, respectively. They were earlier reported as constituents of the nut shell oil of *A. occidentale* (Izzo & Dawson 1949, Shobha et al. 1992, Tyman et al. 1992, Nagabhushana & Ravindranath 1995).

The HRMS of sample **2** also indicated a small amount (molecular ion at 318.2562) of the monounsaturated analog, bilobol (Furukawa 1935, Gellerman & Schlenk 1968), reported from *Gingko biloba*.

Earlier studies reported that phenolic compounds from the nut shell oil of *A. occidentale* have potential antimicrobial properties against gram-positive bacteria only (Himejima & Kubo 1991). The antimicrobial properties of a mixture of **1a**, **1b** and **1c** and a mixture of **2a** and **2b** from the nut shell oil of a Philippine collection of *A. occidentale* were tested against the gram-positive bacteria: *E. coli* and *P. aeruginosa*; gram-negative bacteria: *S. aureus* and *B. subtilis*; and the fungi: *C. albicans*, *T. mentagrophytes* and *A. niger*.

Results of the study (Table 7) indicated that sample **1** showed slight activity against *E. coli* and *P. aeruginosa* with activity index (AI) of 0.2 and moderate activity against *S. aureus*, *B. subtilis*, *C. albicans*, *T. mentagrophytes* and *A. niger* with AI of 0.8, 1.0, 0.8, 0.5 and 0.4, respectively. However, sample **1** is less active than the standard antibiotics which gave higher activity index. Sample **2** indicated slight activity against *P. aeruginosa* and *C. albicans* with AI of 0.2, moderate activity against *B. subtilis*

Table 7. Antimicrobial test results on sample 1 and sample 2.

Sample	Concn. (µg)	*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Bacillus subtilis*		*Candida albicans*		*Aspergillus niger*		*Trichophyton mentagrophytes*	
		C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.	C.Z. (mm)	A.I.
1	30	18	0.8	12	0.2	12	0.2	20	1.0	18	0.8	14	0.4	18	0.8
2	30	–	0	–	0	12	0.2	17	0.7	12	0.2	16	0.6	25	0.5
Standard Antibiotic	30	30 Chloramphenicol	2.0	30 Tetracycline	2.0	20 Tetracycline	1.0	40 Chloramphenicol	3.0	25 Chlortrimazole	1.5	16 Cycloheximide	0.6	38 Chlortrimazole	2.8

CZ - clear zone, * - average of three trials, AI - activity index

and *T. mentagrophytes* with AI of 0.7 and 0.5, respectively and high activity against *A. niger* with AI of 0.6. Sample 2 has the same activity as the standard antibiotic, cycloheximide against *A. niger*. *E. coli* infects the urinary tract, biliary tract and other abdominal tracts which cause diarrhea, sepsis, meningitis and urinary tract infection, while *B. subtilis* causes some forms of food poisoning and a causative agent of the disease anthrax (Brooks et al. 1997). The fungus, *C. albicans* is found in the mucous membrane, gastrointestinal and genital tracts causing thrombophlebitis and endocartitis (Brooks et al. 1997). *T. mentagrophytes* is a parasitic fungus in the skin, hair and nails which causes infection in the outermost layer of the skin (Jawwetz 1991), while *A. niger* is a fungus pathogen causing aspergillosis which affects the lungs (Brooks et al. 1997). Results of the study imply that the mixture of anacardic acids (**1a**, **1b**, **1c**) and cardols (**2a**, **2b**) could be used to prevent the proliferation of the tissues of any infections caused by these microorganisms.

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