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Phytochemistry and Bioassay Studies of Fijian *Palaquium stehlinii* Christoph (Sapotaceae)

Subramanium Sotheeswaran*[1], Mirja Sharif[1], Sadaquat Ali[1], and Noel W. Davies[2]

[1]Department of Chemistry, Univesity of the South Pacific, Suva, Fiji
[2]Central Science Laboratory, University of Tasmania, Australia

Abstract – Water extract of the bark of *Palaquium stehlinii* has been bioassayed for antimicrobial activity and was found to inhibit the growth of *Escherichia coli* and *Penicillium chrysogens*. Phytochemical study revealed the presence of amyrin esters and 3,4',5,7-tetrahydroxyflavanone in the extract.

Keywords – *Palaquium stehlinii*, Sapotaceae, bark, dihydrokaempferol, amyrin esters, bioassay.

Introduction

Palaquium stehlinii belonging to the family Sapotaceae is a medicinal plant well known among the indigenous people of Fiji. It is also a common timber tree growing in the islands of Viti Levu, Vanua Levu and Kadavu in Fiji[1].

Water extract of the bark of *P. stehlinii* inhibited the growth of two microorganisms namely, *Escherichia coli* and *Penicillium chrysogens*. This paper reports the phytochemistry and bioassay results of the water extract from the bark of *P. stehlinii*.

Experimental

Bark of *Palaquium stehlinii* was provided by Mr Saula Vodonaivalu of the South Pacific Herbarium. Bark (100 g) was soxhlet extracted using hexane, chloroform and methanol. The hexane and chloroform extracts gave an oil representing a yield of 7.8% (with respect to the bark) which on thin layer chromatography (TLC) gave essentially one major spot. Neither vacuum liquid chromatography (VLC) nor radial chromotography yielded any pure compounds.

GCMS analysis showed that the oil consisted principally of acetates and cinnamates of α- and β-amyrin (See Table 1 and the Results and Discussion section). On-column injections onto a short-thin film column were necessary to ensure the absence of other high boiling components. The GC-MS determinations were carried out on a HP-MSD, using a 7 m × 0.32 mm HP-1 column (0.17 μm film), with an SGE on-column injector, and temp. programming from 30-240℃ at 30° min^{-1} then to 300℃ at 10° min^{-1} using He as carrier gas. The ^{1}H and ^{13}C NMR spectra were recorded at ambient temp. at 250 and 300 MHz, respectively, using TMS as internal reference. The mass spectral data of the triterpenoid esters are given below:

β-Amyrin acetate: m/z 468(10%), 218(100), 189(40), 95(30), 43(47).

α-Amyrin acetate: m/z 468(8), 218(100), 189(20), 43(30).

Unknown triterpene acetate(1): m/z

*Author for correspondence.

468(18), 289(25), 229(28), 189(100), 95(31), 43(63).

Unknown triterpene acetate(2): m/z 453(95), 393(51), 301(100), 241(66), 163(22), 95(31), 43(72).

11-Keto-α-amyrin acetate: m/z 466(18), 407(5), 273(100), 232(60), 135(48), 43(55).

β-Amyrin dihydrocinnamate: m/z 393(4), 218(100), 133(6), 105(8), 69(12).

α-Amyrin dihydrocinnamate: m/z 558(7%), 408, 339(7), 270(4), 218(100), 133(27), 105(36).

Unknown triterpene cinnamate: m/z 554 (5%), 393(7), 257(3), 218(100), 131(40), 44(50).

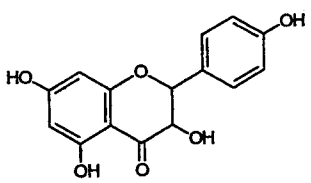
β-Amyrin cinnamate: m/z 556(4), 393(7), 270(3), 218(100), 131(60), 44(35).

α-Amyrin cinnamate: m/z 556(3), 393(7), 270(4), 218(100), 131(73), 69(28).

11-Keto-α-amyrin cinnamate: m/z 422(17%), 273(100), 217(7), 175(25), 135(35), 67(14).

Methanol extract (1.86%) was evaporated and the residue re-extracted with n-butanol and the extract was separated by preparative thin layer chromatography (PTLC) using 10% MeOH-$CHCl_3$ to yield the flavanone I (45 mg, 0.05%), m.p. 151-3℃; IR (ν_{max} cm^{-1} 3350 (OH), 1720 (C=O), 1615 (aromatic C=C), 1082 (C-O-C); EIMS m/z (% rel. intensity) 288 (M^+, 43), 259 (52%), 153 ($C_7H_5O_4$, 100); ^1H nmr ($CDCl_3$/MeOD) See Results and Discussion. ^{13}C NMR ($CDCl_3$/MeOD) δ_c (ppm) : 181.8s, 132s, 128s, 118s, 115d, 112d, 111d, 110d, 109d, 108d, 106d.

The flavanone (I) was acetylated (pyridine/acetic anhydride) to yield an acetate: m.p.



I

83-6℃; IR (ν_{max} cm^{-1}) no OH absorption, 1738 (C=O), 1716 (C=O), 1618 (aromatic C=C), 1076 (C-O-C); EIMS m/z (% rel. int.) 414 (M^+-42, 34%), 372 (14), 354 (18), 312 (28), 270 (41), 153 (62), 136 (61), 107 (46), 43 (100); ^1H nmr $CDCl_3$ (δ ppm) 7.46 (2H, d, J=8.32 Hz), 7.12 (2H, d, J=8.52 Hz), 6.28 (1H, s), 6.24 (1H, s), 5.66 (1H, d, J=11.98), 5.36 (1H, d, J=12.34), 2.32 (3H, s), 2.30 (6H, s) and 2.08 (3H, s).

Water extract for bioassay was obtained by extracting the plant material at room temperature.This extract was tested for activity with the following microorganisms: *Escherichia coli, Staphylococcus aureus, Penicillium chrysogens and Aspergillus niger.* For the bacteria, nutrient agar medium and for the fungi, potato dextrose agar medium were used. The preparation of microbial plates was as follows:

Bacteria strains – Nutrient agar (28 g) was suspended in distilled water (1.0 l). This was boiled to dissolve the agar completely. The plate was sterilized by autoclaving at 121℃ for 15 min at 15 psi. The agar solution was poured onto the plates after autoclaving and cooled to 55℃.

Fungal strains – Potato dextrose agar (39 g) was suspended in 1.0 litre of distilled water and boiled to completely dissolve the agar. This solution was sterilised by autoclaving at 121℃ for 15 min at 15 psi. It was then mixed well, cooled to 55℃ and poured onto plates to settle and form the gel.

Streaking procedure employed was as follows: A wire loop was heated over the bunsen flame until red hot to sterilize the loop. The loop was plunged into a bottle of bacteria or fungi. The loop became filled with the microbe. Using this loop, the agar was streaked very lightly all over the plate.

Three small wells were dug in each microbial plate and samples of the test extract and garlic extract as 10% aqueous solutions were taken in two wells. The third well had distilled water as the reference.

Results and Discussion

The combined n-hexane and chloroform extracts gave an oil which was found to be a

Table 1. Oil Constitutents of *Palaquium stehlinii*.

Name of Compound		M^+, m/z	Base peak, m/z
β-Amyrin acetate	(2.5)	468	218
α-Amyrin acetate		468	218
unknown triterpene acetate (1)	(0.5)	468	189
unknown triterpene acetate (2)	(0.5)	468	301
11-Keto-α-Amyrin acetate	(0.5)	482	273
β-Amyrin dihydrocinnamate	(1.0)	not recorded	218
α-Amyrin dihydrocinnamate		558	218
unknown triterpene cinnamate	(0.1)	554	218
β-Amyrin cinnamate	(0.5)	556	218
α-Amyrin cinnamate		556	218
11-Keto-α-Amyrin cinnamate	(0.01)	not recorded	273

*Percentage occurrence with respect to the dry weight of bark is given in parenthesis.

mixture of at least twelve compounds by GC. MS fragmentation studies showed the presence of several triterpene esters in the oil (Table 1). Six of the esters were identified as amyrin esters from the mass spectral data. All of them had the *retro-diels alder* fragment ions at m/z 218 as their base peaks. Five of the esters were acetates and had characteristic fragments at m/z 43 $[CH_3CO]^+$. The cinnamates (Table 1) had mass fragment ions at m/z 131 $[C_6H_5CH{=}CH\text{-}CO]^+$, whereas the dihydrocinnamates had ions at m/z 133 $[C_6H_5CH_2CH_2CO]^+$.

The n-butanol extract of the residue of the methanol extract on investigation yielded a flavanone which was identified as 3,4',5,7-tetrahydroxy flavanone as follows:

The HREIMS data gave the molecular formula as $C_{15}H_{12}O_6$ (M^+, m/z found 288.0619, calc. 288.0631). The CIMS data ($M+NH_4^+$, m/z 306) confirmed the parent molecular ion which was recorded in the EIMS. The IR spectrum of the flavanone showed a strong OH absorption (3350 cm^{-1}) and indicated the presence of a C=O (1720 cm^{-1}). The ^{13}C NMR spectrum indicated the presence of a carbonyl group (δ_c 181.8s). No peaks were observed in the range δ_c 55-65 indicating the absence of any OMe groups and confirmed the absence of any O-methylations in the natural product.

The acetylated product had a formula weight of 456 (EIMS) indicating the flavanone to be tetrahydroxylated.

The ^1H nmr spectrum of the flavanone showed a two proton doublets at δ_H 7.37 (J= 8.54 Hz) and 6.87 (J=8.56 Hz) for the *ortho*-coupled protons in the B ring and an overlapped two proton signal at δ_H 5.90 for the free A ring aromatic protons[2]. The presence of one proton doublets at δ_H 4.99 and 4.57 suggested a substitution pattern typical of a flavanone skeleton, i.e. an aromatic group at C-2 and a hydroxyl substituent at C-3. The ^1H nmr of tetraacetate of I (See Experimental) confirmed the structure of the flavanone as 3,4',5,7-tetrahydroxyflavanone (dihydrokaempferol)(I). The EIMS data matched with those of the reference spectrum of dihydrokaempferol.

The water extract of the bark was obtained for bioassay. It was tested for antimicrobial activity using the microbes *Escherichia coli, Staphylococcus aureus, Penicillium chrysogens* and *Asperigillus niger* and this activity was compared with the water extract of garlic. The results are presented in Table 2. Even though the water extract of

Table 2. Antibiotic Activity of the Water Extract of *Palaquium Stehlinii*

Microbe	Distilled water	Garlic extract	Bark extract of *P. stehlinii*
Escherichia coli	-	++	+
Staphylococcus aureus	-	++	-
Penicillium chrysogen	-	++	+
Asperigillus niger	-	++	-

+, ++ means the test substance inhibited the growth of the microbe.

the bark was not as active as that of garlic extract, it did show marginal activity against *E. coli* and *P. chrysogens*.

Neither the flavonane nor the amyrin esters showed the antibiotic activity that was recorded for the water extract of the bark (see Experimental).

References

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