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Antimicrobial Activity of the Alkaloidal Constituents of the Root Bark of *Eupomatia laurina*

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Abstract

From the root bark of *Eupomatia laurina* were isolated four anti-microbial alkaloids; sampangine (I), eupomatine-1 (II), liriodenine and lanuginosine. The isolated alkaloids, the fraction from which I + II were isolated (EI), the dichloromethane fraction of the root bark (RD) and the ethyl acetate fraction of the stem bark (SE) were screened against 13 $Gram^+$ and 12 $Gram^-$ bacteria, a protozoan and nine fungi. All extracts were found to be active against all the bacteria and protozoan. Antifungal activity was demonstrated by EI, RD and SE fractions only. Compound II and fraction EI were found to be far superior to the standard used, both in broadness and level of activity. MIC was performed on EI and was found to be in most cases 0.5 µg while in some case it was 0.1 µg. Broad-spectrum antifungal activity was exhibited by fraction EI (10 µg disc), RD (4 mg disc) and SE (4 mg disc). MIC was done on EI and was 10 µg. The activity of EI, RD and SE was found to be far superior to the standard ketoconozole (20 mg disc) and griseofulvin (25 mg disc).

Keywords: *Eupomatia laurina* R. Br., alkaloids, anti-microbial activity.

Introduction

Herbal medicine is part and parcel of the much needed health care in most of the developing countries including Papua New Guinea. As part of our investigation on medicinal plants (Khan, 1998, 1999, 2001; Khan & Omoloso, 1998; Khan et al., 1998, 2000, 2001a–e) we recently reported the antimicrobial activity of *Eupomatia laurina* (Khan et al., 2001e). The dichloromethane fraction of the root bark and the ethyl acetate fraction of the stem bark exhibited the highest level of activity against both the bacteria and the fungi.

Eupomatia laurina R. Br belongs to a monogeneric family, Eupomatiaceae, with only two species and localised to Australisia; a number of alkaloids and lignans having anti-tumour (against Sarcoma 180) and pharmacological activities (Collins et al., 1990) have been reported from these species (Taylor, 1985; Read & Taylor, 1979; Picker et al., 1973). Now we report the isolation, identification and antimicrobial activity of the active constituents of the root bark of *Eupomatia laurina.*

In Papua New Guinea, the stem bark and the leaves of *Eupomatia laurina* are boiled in water and the filtered extract is popularly used to cure dysentery and diarrhoea (private communication from a local herbal healer to M.R.K.).

Materials and methods

Plant materials

The root bark of *Eupomatia laurina* was collected in May 2000, from Lae Botanical Gardens, Morobe Province, Papua New Guinea (PNG). The plant was identified at the PNG Forest Research Institute in Lae, where a voucher specimen is deposited.

Extraction and isolation of the alkaloids

Air-dried and ground root bark (150 g) was Soxhlet extracted for 1 h with methanol (700 ml); after filteration, the process was repeated four times. The combined fiterate, on evapora-

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tion under reduced pressure, deposited 34.8 g of residue. The crude extract was dissolved in a small amount of methanol followed by addition of distilled water (300 ml). The mixture was extracted three times with dichloromethane (150 ml) and the combined fraction was then extracted with 2 N HCl (150 ml). The aqueous fraction was basified with sodium bicarbonate and then extracted three-times with dichloromethane (150 ml). The organic layer, after drying (Na_2SO_4) and evaporation, yielded 4.8 g alkaloids mixture. Thin-layer chromatography on silica gel using CH_2Cl_2: MeOH : $MeNH_2$ (9 : 1 : 0.1) as the mobile phase gave one yellow spot (R_f 0.56). TLC on Alumina TypeE, double elution with $CHCl_3$ showed three yellow spots, E1 (R_f 0.49), E2 (R_f 0.32) and E3 (R_f 0.20). The chromatogram was subjected to antibacterial screening using *E. coli* as an indicator organism. The three spots exhibited antibacterial activity. The mixture of alkaloids (4.5 g) were chromatographed on a basic alumina column (100 g) (2.5 × 50 cm) eluting with CH_2Cl_2, collecting 3 ml fractions resulted in the isolation of three fractions: E1 (48.0 mg), E2 (90.0 mg) and E3 (traces).

Fractions E1 and E2 were separated into pure compounds by HPLC, on C18 Varian μ-Bond column (1.9 × 30 cm) using MeOH : H_2O (75 : 25) at a flow rate of 5.0 ml/min. as mobile phase. From E1 were isolated compounds **I** (5.1 mg) and **II** (5.2 mg) while from E2 were isolated **III** (21.6 mg) and **IV** (9.5 mg) (see Fig. 1).

The structural determination of the four compounds was achieved by comparison of the NMR, MS and mp. with the reported data.

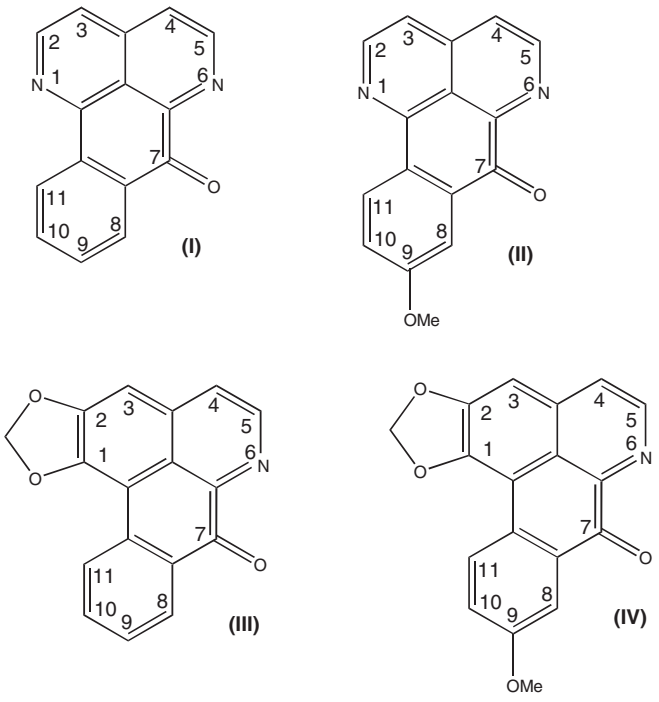

Figure 1. Compounds of fractions E1 and E2.

Sampangine (I)

^{1}H NMR ($CDCl_3$ at 270.17 MHz): δ 7.70 (ddd, 7.7, 7.7. 1.3 Hz. 9-H or 10-H), 7.84 (ddd, 7.6, 7.6, 1.3 Hz. 9-H or 10-H), 7.92 (d, 5.3 Hz. 3-H), 8.48 (dd, 7.7, 1.3 Hz. 8-H), 8.86 (dd, 7.9, 1.3 Hz. 11-H), 8.90 (d, 5.1 Hz. 5-H), 9.14 (d, 5.6 Hz. 2-H). MS (20 eV) *m/e* (%): 232 [M^+] (100), 204 (26), 167 (12), 149 (20). Mp. 209–211.6 °C. (Lit 210 °C) (Rao et al., 1986).

Eupomatidine-1 (II)

^{1}H NMR ($CDCl_3$ at 270.17 MHz): δ 4.01 (s, 9-OMe), 7.36 (dd, 8.7, 2.9 Hz. 10-H), 7.64 (d, 5.6 Hz. 4-H), 7.89 (d, 5.3 Hz. 3-H), 7.91 (d, 2.9 Hz. 8-H), 8.76 (d, 8.9 Hz. 11-H), 8.82 (d, 5.6 Hz. 5-H), 9.12 (d, 5.3 Hz. 2-H). MS (20 eV) *m/e* (%): 262 [M^+] (100%), 232 (10), 204 (5), 191 (5). (Kitahara et al., 1997).

Liriodenine (III)

^{1}H NMR ($CDCl_3$ at 270.17 MHz): δ 6.35 (s, 1,2-OCH_2O), 7.16 (s, 3-H), 7.56 (broad dd, 7.9, 7.6 Hz. 9-H or 10-H), 7.73 (ddd, 7.9, 7.6, 1.6 Hz. 9-H or 10-H), 7.74 (d, 5.3 Hz. 4-H), 8.57 (dd, 7.9, 1.7 Hz. 8-H or 11-H), 8.61 (broad d, 7.9 Hz. 8-H or 11-H), 8.86 (d, 4.9 Hz. 5-H). MS (20 eV) *m/e* (%): 275 [M+] (100), 247 (8), 219 (4), 217 (3). Mp. 270.6–272.4 °C. (Lit. 275 °C) (Rao et al., 1986).

Lanuginosine (IV)

^{1}H NMR ($CDCl_3$ at 270.17 MHz): δ 4.00 (s, 9-OMe), 6.33 (s, 1,2-OCH_2O), 7.14 (s, 3-H), 7.51 (dd, 8.9, 3.0 Hz. 10-H), 7.76 (d, 5.3 Hz. 4-H), 8.04 (d, 3.0 Hz. 8-H or 11-H), 8.57 (d, 8.9, 1.7 Hz. 8-H or 11-H), 8.80 (d, 5.3 Hz. 5-H). MS (20 eV) *m/e* (%): 305 [M+] (100), 275 (15), 247 (4), 234 (4). Mp. 310–31356 °C. (Lit. > 300 °C) (Wijeratne et al., 1996).

Tested materials

The ethyl acetate fraction of the stem bark (SE), the dichloromethane fraction of the root bark (RD) (obtained earlier) (Khan et al., 2001e) and the four alkaloids; **I**, **II**, **III** and **IV**.

Anti-microbial test

Anti-microbial activity was determined by the disc diffusion technique (Barry, 1976; Bauer et al., 1966; Cruickshank, 1968). The microorganisms used (Table 1 and 2) were obtained from the stock cultures of the Microbiology Laboratory of the Department of Applied Sciences in Lae.

Results and discussion

The result of the screening for anti-bacterial activity is given in Table 1 and for antifungal in Table 2. Four atimicrobial

Table 1. Antimicrobial activity of the alkaloids of *Eupomatia laurina*.[a]

Microorganizms		MIC on EI					RD	SE	I	II	III	IV	Chl
		10 µg	5 µg	1 µg	0.5 µg	0.1 µg	4 mg	4 mg	10 µg	10 µg	10 µg	10 µg	10 µg
Bacillus cereus	G+	14	12	10	10	6	8	18	16	20	14	12	16
B. coagulans	G+	26	20	18	12	8	16	20	–	26	18	16	18
B. megatarium	G+	20	12	10	8	0	18	18	–	20	16	12	16
B. subtilis	G+	16	8	6	6	0	16	18	12	18	14	12	16
Lactobacillus casei	G+	18	10	6	6	0	16	18	–	16	16	14	18
Micrococcus luteus	G+	20	10	6	6	0	12	20	14	20	18	14	16
M. roseus	G+	18	10	8	8	0	18	18	16	18	16	16	6
Staphylococcus albus	G+	20	10	8	6	0	18	18	16	20	16	14	16
S. aureus	G+	18	10	6	6	0	18	20	12	18	14	10	18
S. epidermidis	G+	12	10	8	0	0	16	18	12	12	12	10	00
Streptococcus faecalis	G+	18	8	8	6	0	20	18	16	20	14	14	00
S. pneumoniae	G+	18	10	8	6	0	16	18	–	16	16	14	18
S. mutans	G+	20	8	0	0	0	10	18	–	18	14	12	18
Agrobacterium tumefaciens	G–	28	18	16	10	0	14	20	–	14	18	16	12
Citrobacter freundii	G–	20	14	10	8	6	16	18	14	18	14	12	16
Enterobacter aerogenes	G–	20	8	0	0	0	16	16	–	18	12	14	18
Escherichia coli	G–	16	12	8	6	0	18	20	14	16	14	10	18
Klebsiella pneumonia	G–	18	6	0	0	0	14	18	–	18	18	14	0
Neisseria gonorrhoeae	G–	16	10	6	6	0	16	20	–	20	12	8	18
Proteus mirabilis	G–	20	12	10	8	0	16	18	12	18	18	14	16
P. vulgaris	G–	22	10	8	6	0	18	16	–	22	14	12	18
Pseudomonas aeruginosa	G–	20	16	12	8	0	16	18	–	20	16	12	24
Salmonella typhi	G–	12	10	6	0	0	18	20	12	16	16	12	16
S. typhymurium	G–	18	10	0	0	0	10	8	14	18	14	10	16
Serratia marcescens	G–	16	8	6	0	0	18	18	–	14	12	8	18
Trichomonas vaginalis	Pz	22	8	6	6	0	18	20	16	20	18	18	16

[a] Values are inhibition zone (mm) and an average of triplicates. MIC, minimum inhibitor concentration; EI, fraction which deposited compound I and II; RD, dichloromethane fraction of root bark; SE, ethylacetate fraction of stem bark; I, sampangin; II, eupomatidine-1; III, liriodine; IV, lanuginosine; –, not tested; G, gram reaction; Pz, protozoa; Chl, reference chloramphenicol (10 µg disc Oxoid B42960).

Table 2. Antifungal activity of the alkaloids of *Eupomatia laurina*.[a]

Mold	MIC on EI		RD	SE	I	II	III	IV	Ket	Gri
	10 µg	5 µg	4 mg	4 mg	10 µg	10 µg	10 µg	10 µg	20 mg	25 mg
Aspergillus niger	18	0	10	10	0	0	0	0	18	10
A. versicolor	16	–	8	14	–	–	–	–	22	18
A. vitis	10	–	10	8	–	–	–	–	20	12
Candida albican	12	0	10	8	0	0	0	0	18	10
C. tropicalis	14	0	8	10	0	0	0	0	18	12
Cladosporium cladoporoides	18	–	8	0	–	–	–	–	16	8
Penicillium notatum	16	–	8	8	–	–	–	–		
Trichophyton mentagrophytes	14	0	8	8	0	0	0	0	18	10
T. tonsurum	14	–	8	10	–	–	–	–	20	12

[a] Values are inhibition zone (mm) and an average of triplicates. MIC, minimum inhibitor concentration; EI, fraction which deposited compound I and II; RD, dichloromethane fraction of root bark; SE, ethylacetate fraction of stem bark; I, sampangin; II, eupomatidine-1; III, liriodine; IV, lanuginosine; –, not tested; ket, reference ketoconozole; Gri, reference griseofulvin.

alkaloids; sampangine (**I**), eupomatine-1 (**II**), liriodenine (**III**) and lanuginosine (**IV**) were isolated from the root bark of *Eupomatia laurina.* The isolated alkaloids, the fraction EI (mixture of **I** + **II**), the dichloromethane fraction of the root bark (RD) and the ethyl acetate fraction of the stem bark (SE) were screened against 13 $Gram^+$ and 12 $Gram^-$ bacteria, a protozoan and nine fungi. All demonstrated excellent level of activity against most of the bacteria and protozoan while fractions EI, RD and SE exhibited very good activity agaisnt all the fungi. In antibacterial screening, compound **II** and fraction EI were found to be far superior to the standard (Chl) used, both in broadness and level of activity. MIC of EI was in most cases 0.5 µg and in some down to 0.1 µg. Broad spectrum antifungal activity was exhibited by fractions EI (10 µg disc), RD (4 mg disc) and SE (4 mg disc). MIC of EI was 10 µg. The antifungal activity of the three fractions (EI, RD and SE) was far superior to the standand ketoconozole (20 mg disc) and griseofulvin (25 mg disc).

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