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Antimicrobial and anti-inflammatory activities of *Piper porphyrophyllum* (Fam. Piperaceae)



Farediah Ahmad ^{a,*}, Emrizal ^a, Hasnah M. Sirat ^a, Fadzureena Jamaludin ^b,
Nik Musa'adah Mustapha ^b, Rasadah M. Ali ^b, Dayar Arbain ^c,
Hassan Y. Aboul-Enein ^{d,*}

^a Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, Skudai 81310, Johor Bahru, Malaysia

^b Forest Research Institute Malaysia (FRIM), Kepong, 52109 Kuala Lumpur, Malaysia

^c Department of Pharmacy, Faculty of Science, Andalas University, Padang, Indonesia

^d Pharmaceutical and Medicinal Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, Cairo 12311, Egypt

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Abstract The crude extracts and isolated compounds of *Piper porphyrophyllum* (Piperaceae) were evaluated for antibacterial and anti-inflammatory activities. The ethyl acetate extract and 3',4',5,7-tetramethoxyflavone exhibited the highest activity against *Staphylococcus aureus* giving values of MIC = 62.5 and 250 µg/mL, respectively. 5-Hydroxy-7-methoxyflavanone and 4',5-dihydroxy-3',7-dimethoxyflavone were active against *Pseudomonas aeruginosa*, both with MIC value 125 µg/mL. The hexane extract and 4',5-dihydroxy-3',7-dimethoxyflavone gave the highest anti-inflammatory activity in *in vitro* quantitative lipoxygenase inhibition assay with inhibitory activity of (IE) 99.72% and 91.81%, respectively.

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1. Introduction

Piper porphyrophyllum of Piperaceae or locally known as Lada hutan, Sireh rimau, Kerakap rimau, and Akar bugu was reported to contain seven flavonoids identified as 5-hydroxy-7-methoxyflavanone (1), 5,7-dimethoxyflavone (2), 4',5,7-trimethoxyflavone (3), 3',4',5,7-tetramethoxyflavone (4), 4'-hydroxy-3',5,7-trimethoxyflavone (5), 5-hydroxy-3',4',7-trimethoxyflavone (6), and 4',5-dihydroxy-3',7-dimethoxyflavone (7) (Rajudin et al., 2010).

Compounds 1–4 isolated from the hexane extract while 5–7 were obtained from the ethyl acetate extract.

* Corresponding authors.

E-mail addresses: farediah@kimia.fs.utm.my (F. Ahmad), eneina@gawab.com (H.Y. Aboul-Enein).

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Re-investigation of the ethyl acetate extract resulted in the isolation of cubebin (**8**) (Pascoli et al., 2006; Bastos et al., 2001) and 3,3',4'-trihydroxy-5,7-dimethoxyflavone (**9**) (Daniel et al., 1999). Compound **9** is reported for the first time in genus *Piper*.

Herein we report the antimicrobial and anti-inflammatory activities of the extracts and isolated compounds.

The ethyl acetate extract showed potent antibacterial activity against *S. aureus* followed by compound (**4**) each with MIC values of 62.5 and 250 µg/mL, respectively. Compound (**1**) and (**7**) were active against the Gram-negative bacteria (*P. aeruginosa*) both with MIC = 125 µg/mL. As for the remaining compounds, they are moderate or weak inhibitors. The antibacterial activity against *S. aureus*, a major human pathogen, which can colonize many different diseases is significant (Schwarz-Linek et al., 2006). The significant antibacterial activity exhibited by (**2**) and (**7**) against Gram-negative strain, *P. aeruginosa* showed that these compounds have the ability to penetrate the outer membrane of the bacteria which is a barrier to many substances, including antibiotics (Palombo and Sempé, 2001).

At a dose of 2 mg per ear, the hexane extract showed the highest inhibition in TPA-induced edema with IE $92.12 \pm 9.1\%$ while the ethyl acetate extract only reduced $64.13 \pm 4.9\%$ of the edema compared to indomethacin (the standard) which gave $85.50 \pm 5.01\%$ inhibition. At a dose of 0.5 mg per ear, (**4**) and (**2**) which were isolated from the hexane extract, exhibited significant activity by inducing 53.64 ± 5.0 and $50.13 \pm 7.0\%$ reduction in the edema, respectively. In contrast, the other compounds showed low activity (in the range of 27–24%) or inactive in this anti-inflammatory model. In the *in vitro* quantitative lipoxygenase inhibition assay, the hexane extract again displayed the highest inhibition ($99.72 \pm 0.02\%$) while the ethyl acetate extract was found to be inactive. 4',5-Dihydroxy-3',7-dimethoxyflavone (**7**) which was obtained from the ethyl acetate extract exhibited the highest inhibition with IE $91.81 \pm 0.05\%$. Compound (**1**) gave moderate activity (53.31 ± 0.04) followed by (**5**) ($48.1 \pm 0.0\%$) and (**8**) ($42.92 \pm 0.05\%$) when compared to the standard, phenidone ($89.39 \pm 0.04\%$).

In the (12-O-tetradecanoylphorbol-13-acetate) TPA-induced edema, the active extracts and compounds were probably inhibited protein kinase C (PKC), the principle target of TPA, thus blocked the production of phospholipase A₂. In the case of *in vitro* quantitative lipoxygenase assay, the active extract and compounds inhibited leukotriene synthesis on lipoxygenase pathway (Daniel et al., 1999).

In this study, synergic effects of the isolated compounds might be the caused for the antimicrobial and anti-inflammatory activities of the ethyl acetate and hexane extracts, respectively. However, further study is necessary to assess the interaction of both extract and isolated compounds with other model of experiments, in order to verify their mechanisms of action.

2. Experimental

2.1. Plant material

P. porphyrophyllum (AZ 6382) was collected from Pasir Putih, Kelantan, Malaysia in June 2004. The plant was identified by

Mr. Ahmed Zainuddin Ibrahim and the voucher specimen was deposited at the Herbarium of the Forest Research Institute, Malaysia.

2.2. Isolation and purification

The air dried and powdered sample (760.3 g) was extracted using Soxhlet extractor with hexane and ethyl acetate sequentially. Evaporation of the respective solvents gave hexane (2.64 g, 0.35%) and ethyl acetate (3.87 g, 0.51%) extracts. Fractionation and purification of the hexane extract by vacuum liquid chromatography and multiple column chromatography afforded four compounds (**1–4**) while purification of the ethyl acetate extract using the same methodology as the hexane gave five pure compounds (**5–9**). The structural elucidations of the compounds were based on the spectroscopic data and comparison of the spectral data with the literatures.

2.3. Microorganisms and medium

The bacterial stains were provided from the Biology Laboratory, Faculty of Bioscience and Bioengineering, UTM and acquired from The American Type Culture Collection (ATCC). The bacterial strains were *S. aureus* (ATCC 25923), *P. aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). The nutrient broth was suspended in distilled water and autoclaved for 20 min at 121 °C. Each of the strains was suspended on nutrient broth in sterilized test tubes. The inocula were prepared by adjusting the turbidity of the suspension to match 0.5 McFarland standard in barium salts suspension. Streptomycin sulphate was used as the positive control.

2.4. Minimum inhibition concentration (MIC)

The MIC was determined by the micro-titer broth dilution method (Zavala et al., 1997). The test was performed in sterile 96-well micro-titer plates. The methanolic solution of samples were properly prepared and transferred to each microplate well in order to obtain a twofold serial dilution of the original solution (1:2–1:128 starting from the concentration of 1000 µg/mL). The inoculate (100 µL) containing 150×10^6 colony/unit of each microorganism were added to each well. A number of wells were reserved for methanol effect. Plates were incubated at 37 ± 1 °C for 24 h. MIC was defined as the lowest concentration that had no macroscopically visible growth.

2.5. Minimum bactericidal concentration (MBC)

The determination of MBC was according to the method by Arias et al. (2004). All wells in the MIC study, which did not show any growth of bacteria after incubation were first diluted in fresh nutrient broth (1:4) and sub-cultured onto the surface of freshly prepared nutrient agar plates (θ , 15 mm). The plates were incubated for 18 h at 37 ± 1 °C. The MBC was recorded as the lowest concentration of the sample that did not permit any visible growth of bacteria colony on the agar plate after incubation period.

2.6. TPA-induced mouse ear edema model and *in vitro* quantitative lipxygenase inhibition assay

Both experiments were carried following the previous methods reported (Rajudin et al., 2008).

The results were expressed as mean \pm standard deviation obtained from five mice in the TPA-mouse ear edema and from three samples *in vitro* quantitative lipxygenase assay.

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