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Anti-Inflammatory Activity of *Calophyllum inophyllum* Fruits Extracts

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Abstract

In the present work, we are reporting the isolation, characterisation and bioactivity of inophyllums, xanthones and other compounds found in the fruits of *Calophyllum inophyllum*. In-vitro assays demonstrated that the crude extract at concentration of 50 µg/ml inhibited 77% and 88% cyclooxygenase and lipoxygenase activities, respectively, indicating its potential as anti-inflammatory agent. Phytochemical studies was also conducted on the fruits. Inophyllum A, inophyllum C, inophyllum E, calophylloide, calophylic acid, 11,12-anhydroinophyllum A, 1,7-dihydroxy-6-methoxyxanthone, potocatechuic acid, gallic acid, n-nonacosane, β-sitosterol and sitosterol-3-O-β-D-glucopyranoside were isolated and identified. On the HPLC chromatogram, at least 18 compounds can be detected.

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Keywords: *Calophyllum inophyllum*; antiinflammatory; xanthone; isoprenylated coumarin, sitosterol

1. Introduction

Bintangor is the local Malaysian name for *Calophyllum* species and this large bitter-sweet kernel afforded greenish oil is used as liniment and valued for those who suffered from rheumatism, pains in the joints and bruises. The oleoresin from the bark of *C. inophyllum* is known as balsam and used as cicatrisant. The decoction of the leaves has traditionally been used to relieve eye irritation and conjunctivitis. In addition, Kashman *et al.* reported that the anti-HIV activity from *Calophyllum lanigerum* was shown to be attributed to the presence of calanolides. Isoprenylcoumarins isolated from the leaves of *C. lanigerum* and *C.*

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Inophyllum are reported to be the active substances in inhibiting the HIV-1 reversed transcriptase activity. The present work reported the isolation of chemical constituents of the fruits of *C. inophyllum* and its anti-inflammatory activities.

2. Experiments

2.1. Materials

The fruits of *C. inophyllum* were collected between November-December 2011 within the University of Malaya campus, Malaysia. The fresh samples were separated into nuts and endocarps before finely cut and dried in oven at 40 °C for 7 days. Then, those were ground to fine powder using Wiley Laboratory Mill.

2.2. Methods

2.2.1. Extraction method

Two methods were used for extraction, solvent extraction where sample was consecutively extracted with CHCl₃ and methanol, followed by method where sample was extracted directly by boiling in water, filtered, cooled and extracted with CHCl₃ then evaporated.

2.2.2. Isolation

The chemical constituents from the crude extracts were separated by column chromatography using silica gel (60-120 mesh) and PTLC on silica gel. The following solvent systems were used as eluants: a. CHCl₃-Hexane (hexane between 10 - 50 %) b. CHCl₃ only.

2.2.3. Bioassays

The cyclooxygenase and 5-lipoxygenase tests were conducted as described by Redl et al. and Kuhl et al.⁶,⁷.

3. Results and Discussion

In this study, 12 metabolites were isolated from the fruits of *C. inophyllum*. Based on our previous experience, solvent extraction gave a gummy extract which complicates the separation of metabolites on column chromatography. However, boiling the nuts in hot water produces oil which separated out on the top layer. After cooling, the layer contains isoprenylated coumarins that can be easily separated by column chromatography. This is the traditional method of producing the oil used for medicinal ointment.

Isoprenylcoumarins isolated from the leaves of *C. lanigerum* and *C. inophyllum* were reported to be the most active substances in inhibiting the HIV-1 reversed transcriptase activity. The chloroform extract of *C. inophyllum* showed anti-cyclooxygenase activity in vitro experiments. In the present work, we are reporting the isolation and characterisation of isoprenylcoumarins, xanthone and other compounds found in the fruits of *C. inophyllum*. We have isolated and identified 3,4-dihydroxy-benzoate, inophyllum A (1), inophyllum C (2), inophyllum E (3), calophyloide, calophylic acid, 11,12-anhydroinophyllum, 1,7-dihydroxy-6-methoxyxanthone (4), n-nonacosane and sitosterol-3-O-β-D-glucopyranoside. Observations showed that more coumarins can be obtained from fruits compared to the leaves.
Fig. 1. Chemical structure of isolated compounds from *Callophyllum inophyllum* (1) inophyllum A (2) inophyllum C where R1 = Me; R2 = H; (3) inophyllum E where R1 = H; R2 = Me, (4) calophylloide.

The HPLC chromatogram showed the presence of at least 18 compounds. Compound peaks after calophylic acid have not been isolated in the present work. The UV absorption spectra of these compounds peaks have spectra pattern similar to calophylic acid, thus, suggesting they are having basic structural chromophores similar to calophylic acid. The mass spectra of calophylic acid and derivatives are a very useful tool for the structure determination. The most important fragment is the loss of C\(_{10}\)H\(_{16}\) (2 unsaturations) due to a McLafferty rearrangement followed by loss of C\(_{6}\)H\(_{7}\) (1 unsaturation) and formation of a tropolium ion (base peak at m/e = 369). This fragmentation indicates a C5 and a C10 chain attached to a single quaternary carbon. For other isoprenylated coumarin structures, those can be easily characterized mainly from rigorous NMR analysis.

Protocatechuic acid, gallic acid, 1,7-dihydroxy-6-methoxy-xanthone, n-nonacosane and sitosterol-3-O-β-D-glucopyranoside are reported for the first time from this species. n-Nonacosane is the major component of the fruits.

4. Conclusion

The inhibition of the crude extract on lipooxygenase and cyclooxygenase activities indicates its anti-inflammatory activity. In-vitro assays demonstrated that the crude extract at concentration of 50 μg/ml inhibited 77% and 88% cyclooxygenase and lipooxygenase activities, respectively, indicating its potential as anti-inflammatory agent. This supported the traditional used of this oil for relieving pains.

References