Available online at www.sciencedirect.com

ScienceDirect

Procedia Chemistry 13 (2014) 218 – 220

Procedia Chemistry

International Seminar on Natural Product Medicines, ISNPM 2012

Anti-Inflammatory Activity of *Calophyllum inophyllum* Fruits Extracts

Muhamad Bin Zakaria[a]*, Vijayasekaran[a], Zul Ilham[a], Nur Airina Muhamad[a]

[a]*Institute of Biological Science, University of Malaya 50603 Kuala Lumpur, Malaysia*

Abstract

In the present work, we are reporting the isolation. characterisation and bioactvity of inophyllums, xanthones and other compounds found in the fruits of *Callophyllum inophyllum*. 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-inflamatory agent. Phytochemical studies was also conducted on the fruits. Inophyllum A, inophyllum C, inophyllum E, calophylloide, calophynic 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 idetified. On the HPLC chromatogram, at least 18 compounds can be detected.

Peer-review under responsibility of the School of Pharmacy, Bandung Institute of Technology

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[1]. The oleoresin from the bark of *C. inophyllum*is known as balsamand used as cicatrisant[2]. The decoction of the leaves has traditionally been used to relieve eye irritation and conjunctivitis[3,4].

In addition, Kashman *et al*[5] 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.*

* Corresponding author.
*E-mail address:*mzak@um.edu.my

Peer-review under responsibility of the School of Pharmacy, Bandung Institute of Technology
doi:10.1016/j.proche.2014.12.031

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 aintiinflammatory 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 ^{o}C for 7 days. Then, those were grinded 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 $CHCl_3$ and methanol, followed by method where sample was extracted directly by boiling in water , filtered, cooled and extracted with $CHCl_3$ 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.$CHCl_3$-Hexane (hexane between 10 - 50 %) b**.** $CHCl_3$ only.

2.2.3. Bioassays

The cyclooxygenase and 5-lipooxygenase 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 complicatesthe 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 isoprenylatedcoumarins 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 in vitro experiments. In the present work we are reporting the isolation and characterisation of inophyllums, 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**), calophylloide, calophynic acid, 11,12-anhydroinophyllum,1,7-dihydroxy-6-methoxyxanthone (**4**), n-nonacosane and sitosterol-3-O-β-D-glucopyranoside. Observations showed that morecoumarins can be obtained from fruits compared to the leaves.

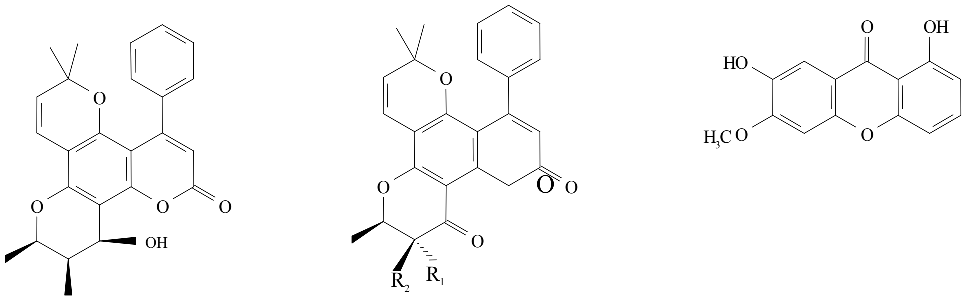

Fig. 1. Chemical structure of isolated compound sfrom Callophyllum inophyllum (**1**) inopyllum A (**2**) inophyllum C where R1 = Me; R2 = H ; (**3**) inophyllum E where R1 = H;R2 = Me, (**4**) calophylloide

The HPLC chromatogram showed the presence of at least 18 compounds. Compound peaks after calophylicacid 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 calophynic acid and derivatives are 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_4H_7 (1 unsaturation) and formation of a tropyllium ion (base peak at m/e = 369). This fragmentation indicates a C5 and a C10 chain attached to a single quaternary carbon[8]. For other isoprenylatedcoumarin 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-inflamatory activity. In-vitro assays demonstrated that the crude extract at concentration of 50 ug/ml inhibited 77% and 88% cyclooxygenase and lipooxygenase activities, respectively, indicating its potential as anti-inflamatory agent. This supported the traditional used of this oil for relieving pains.

References

1. Steiner RP, editor. *Folk medicine: the art and the science*. Washington, D.C.: American Chemical Society; 1986. p. 75.
2. Iinuma M, Tosa H, Tanaka T, Yonemori S. Two xanthones from root bark of *Calophyllum inophyllum*. *Phytochem* 1994;**35**:527–32.
3. Burkill Birtwistle, William,, Foxworthy, Frederick William,, Scrivenor, John Brooke,, Watson, James Gilbert,, IH. *A dictionary of the economic products of the Malay peninsulavol I*. Kuala Lumpur, Malaysia: Published on behalf of the governments of Malaysia and Singapore by the Ministry of Agriculture and cooperatives; 1966.
4. Kartikar KR, Basu B Das. *Indian medicinal plants Vol. 1*. *Second Edition*. Delhi: Jayed Press; 1975. p. 270-271.
5. Kashman Y, Gustafson KR, Fuller RW, Cardellina JH, McMahon JB, Currens MJ, et al. HIV inhibitory natural products. Part 7. The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum*. *J Med Chem* 1992;**35**:2735–43.
6. Redl K, Breu W, Davis B, Bauer R. Anti-Inflammatory Active Polyacetylenes from *Bidens campylotheca*. *Planta Med* 1994;**60**:58–62.
7. Kuhl P, Shiloh P, Jha H, Murawski U, Zilliken F. 6,7,4′-trihydroxyisoflavan: A potent and selective inhibitor of 5-lipoxygenase in human and porcine peripheral blood leukocytes. *Prostaglandins* 1984;**28**:783–804.
8. Gautier J, Kunesch G, Polonsky J. Structure of calophynic acid, A novel constituent of. *Tetrahedron Lett* 1972;**13**:2715–8.