Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 15 (2008) 306–309
Phytomedicine
www.elsevier.de/phymed

Inhibitory effect of compounds from Zingiberaceae species on human platelet aggregation

I. Jantan[a,*], S.M. Raweh[a], H.M. Sirat[b], S. Jamil[b], Y.H. Mohd Yasin[a], J. Jalil[a], J.A. Jamal[a]

[a]*Department of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia*
[b]*Department of Chemistry, Universiti Teknologi Malaysia, Skudai, Johore Bahru, Malaysia*

Received 3 April 2007; accepted 9 August 2007

Abstract

Twelve compounds isolated from *Alpinia mutica* Roxb., *Kaempferia rotunda* Linn., *Curcuma xanthorhiza* Roxb., *Curcuma aromatica* Valeton and *Zingiber zerumbet* Smith (Family: Zingiberaceae) and three synthesized derivatives of xanthorrhizol were evaluated for their ability to inhibit arachidonic acid- (AA), collagen- and ADP-induced platelet aggregation in human whole blood. Antiplatelet activity of the compounds was measured *in vitro* by the Chrono Log whole blood aggregometer using an electrical impedance method. Among the compounds tested, curcumin from *C. aromatica*, cardamonin, pinocembrine and 5,6-dehydrokawain from *A. mutica* and 3-deacetylcrotepoxide from *K. rotunda* showed strong inhibition on platelet aggregation induced by AA with IC_{50} values of less than 84 μM. Curcumin was the most effective antiplatelet compound as it inhibited AA-, collagen- and ADP-induced platelet aggregation with IC_{50} values of 37.5, 60.9 and 45.7 μM, respectively.

Keywords: Zingiberaceae; Antiplatelet aggregation; Human whole blood; Curcumin; Xanthorrhizol; Arachidonic acid

Introduction

In a previous paper, we reported the ability of compounds from several Zingiberaceae species to displace [^{3}H]PAF-specific binding from washed rabbit platelets (Jantan et al., 2004). The methanol extracts of these species showed strong antiplatelet aggregation activity in human whole blood *in vitro*. This paper reports the antiplalelet aggregation activity of compounds previously isolated from five Zingiberaceae species, namely, *Alpinia mutica*, *Kaempferia rotunda*, *Curcuma xanthorrhiza*, *Curcuma aromatica* and *Zingiber zerumbet*, and structure–activity analysis of these compounds.

Materials and methods

The rhizomes of *C. xanthorrhiza* (AR 415), *C. aromatica* (AR 424), *K. rotunda* (AR 436) and *Z. zerumbet* (AR 478), and the fruit of *Alpinia mutica* (AR 457) were collected from Johore Bahru, Malaysia, and their voucher specimens were deposited at the Herbarium of the Department of Biology, Universiti Putra Malaysia. Twelve pure compounds were isolated from the chloroform extracts of the plants by repeated chromatography on silica gel (230–400 mesh) using petroleum ether–ether gradients of increasing

*Corresponding author. Tel.: +603 40405331; fax: +603 26983271.
E-mail address: ibj@medic.ukm.my (I. Jantan).

0944-7113/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2007.08.002

polarities. They were identified by spectroscopic techniques and by comparison with published data (Sirat et al., 1993; Itokawa et al., 1988; Shiobara et al., 1986; Janssen and Scheffer, 1985). Xanthorrhizol epoxide, 1-acetyl-2-methyl-5-(1′,5′-dimethylhex-4′-enyl)benzene and 1-methoxy-2-methyl-5-(1′,5′-dimethylhex-4′-enyl)benzene were prepared by epoxidation, acetylation and methylation of xanthorrhizol, respectively. Collagen, ADP and arachidonic acid (AA) were purchased from Sigma Chemical Co. (USA).

The use of human whole blood in this study was approved by the Ethics Committee of the Universiti Kebangsaan Malaysia. Blood was collected from volunteers who were selected based on the criteria that they were healthy, non-smokers, had not taken any medications, including aspirin, within the last 2 weeks and had not taken any food within the last 8 h. Whole blood (20 ml) was withdrawn from the right arm of a subject into a vacutainer containing 3.8% sodium citrate. The blood and the anticoagulant were thoroughly mixed by inverting the vacutainer several times. The blood sample was diluted with normal saline in the ratio of 1:1. The dried methanol extracts and the isolated compounds were each dissolved in dimethyl sulfoxide (DMSO) to obtain stock solutions of 20, 10, 5 and 2.5 μg/μl. Five microliters of the stock solutions was added to a cuvette containing the diluted whole blood and the mixture was allowed to incubate at 37 °C for 4 min prior to the addition of AA (0.5 mM), ADP (10 μM) or collagen (2 μg/ml). The total volume of the mixture was 1 ml. The final concentrations of the sample in the mixture were 100, 50, 25 and 12.5 μg/ml.

Platelet aggregation was measured by a Whole Blood Lumi-Aggregometer (Chrono-Log Corp., Havertown, PA) using an electrical impedance method (Ingerman-Wojenski and Silver, 1984). The mean platelet aggregation in whole blood was measured as a change in impedance over 6 min after the addition of inducers by comparison to that of a control group impedance (Challen et al., 1982). A mixture containing 0.5% DMSO in the diluted whole blood was used as control. Aspirin was used as the positive control. The final concentration of DMSO in the whole blood was 0.5% to eliminate the effect of the solvent on the aggregation (Dong and Chen, 1998).

Each sample was measured in triplicate and the data are presented as means ± SE. A one-way analysis of variance was used for multiple comparisons and if significant variation occurred between treatment groups, the mean values for inhibitors were compared with those for controls by Student's *t* test. $p < 0.05$ was considered to be statistically significant. The IC_{50} values of the compounds were obtained from at least three determinations.

Results and discussion

The methanol extracts of the fruit of *A. mutica* and the rhizomes of *K. rotunda*, *C. xanthorrhiza*, *C. domestica* and *Z. zerumbet* showed strong antiplatelet aggregation activity at 100 μg/ml in human whole blood *in vitro*, with all extracts exhibiting 100% inhibition. Twelve compounds isolated from these species were investigated for their effects on platelet aggregation of human whole blood (Fig. 1). Aspirin, a potent cyclooxygenase inhibitor, was used as a positive control in the bioassay (Lloyd and Bochner, 1996). Table 1 shows the % inhibitory effects of the isolated compounds and synthesized derivatives of xanthorrhizol at various concentrations. The compounds showed dose-dependent responses. Among all tested compounds, four compounds, i.e. zerumbone (**10**), xanthorrhizol (**11**), curcumin (**12**) and xanthorrhizol epoxide (**13**) showed strong inhibition on platelet aggregation caused by all three inducers (AA, collagen and ADP), with inhibitory effects ranging from 64.7% to 100% at 100 μg/ml.

The IC_{50} values of the active compounds with the mean values of three measurements are shown in Table 2. Curcumin (**12**) was the most effective antiplatelet compound; it inhibited AA-, collagen- and ADP-induced platelet aggregation with IC_{50} values of 37.5, 60.9 and 45.7 μM, respectively. The result for AA-induced aggregation by compound **12** was in

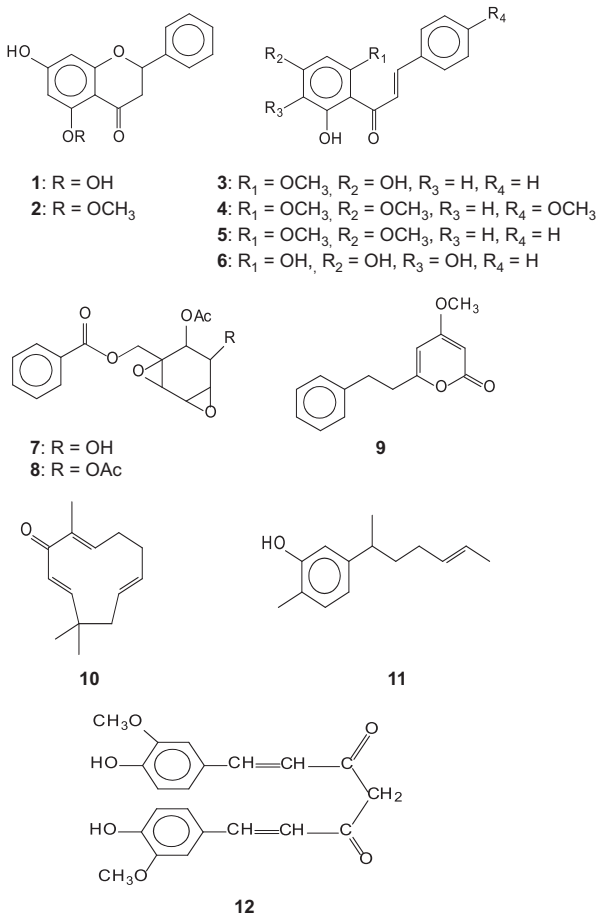

Fig. 1. Structures of compounds from Zingiberaceae species.