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Anti-inflammatory Action of Components from *Melastoma malabathricum*

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Abstract

Ethnic folklore or empirical therapeutic uses of plant parts have often provided the early indication of the possibility of discovering some pharmacologically active substance from a plant. *Melastoma malabathricum* L. (Melastomaceae), locally known as sendudok putih, is a small shrub. Traditional medicinal uses include diarrhea, dysentery, ulcers, wound care, and piles. In the search for natural compounds useful against anti-inflammatory activity, α-amyrin, betulinic acid, and isolated flavonoids, including quercetin and quercitrin, were assessed *in vitro* by determining their inhibitory effects on platelet activating factor (PAF) binding to rabbit platelets using ^{3}H-PAF as a ligand. The results indicated that quercetin, quercitrin, α-amyrin, and betulinic acid showed inhibition of PAF receptor binding with IC_{50} values of 33.0, 45.4, 20.0, and 22.2 μM, respectively. The IC_{50} values of these compounds were comparable to Cedrol (13.1 μM), which is a known PAF receptor antagonist. These results suggest that natural flavonoid and pentacyclic triterpenes from *M. malabathricum* possess selective antagonistic activity toward PAF and could be an attractive candidate as a natural anti-inflammatory compound.

Keywords: Anti-inflammatory activity, flavonoid, *Melastoma malabathricum*, pentacyclic triterpene, platelet activating factor (PAF) binding, receptor antagonist.

Introduction

In the last several years, the role of inflammation disease pathogenesis has become increasingly recognized by the medical community. Each inflammatory disease has distinct therapeutic needs that are not adequately served by current prevention and treatment strategies. Although inflammation is the unifying factor, the specific treatment approach required for each type of inflammatory disease may be unique to that patient population. One of the most widely used classes of agent for treating inflammation is the corticosteroid class (Hawker, 1997). While these drugs are very potent anti-inflammatory agents, their chronic use can lead to gastroduodenal ulcers (Simon & Strand, 1997; Wallace, 1997). Many of the therapies available today are palliative rather than curative, directed to the symptoms rather than to the underlying causes of inflammation. Fortunately, there are many natural substances that have powerful anti-inflammatory effects, and some of the nutrients even address the cause or aetiology of the condition.

The use of botanical and herbal medicines as a complementary approach for the treatment of inflammatory diseases has been steadily increasing, possibly because of the adverse effects associated with the use of nonsteroidal anti-inflammatory drugs. One approach to discover newer anti-inflammatory agents is to search for their presence in natural sources. *Melastoma malabathricum* L., locally known as sendudok putih, is a small shrub of the family Melastomaceae. The young leaves are eaten raw or cooked and taste sour. Leaves are used to treat diarrhea, dysentery, and ulcers; to prevent scarring from smallpox; and to treat piles. A decoction of roots and leaves is often given to women after childbirth (Burkill, 1966). In the course of our continuing search for natural products as anti-inflammatory agents, methanol extract of the leaves was found to be active in the platelet activating factor receptor antagonist binding assay. From bioactive extract, several compounds of pentacyclic triterpenes and flavonoids were obtained, and these compounds could

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Table 1. Inhibitory effects of compounds isolated from *M. malabathricum* on platelet activating factor (PAF) receptor binding with rabbit platelets (concentration of sample in reaction mixture = 18.2 μg/ml).

No.	Compound	Inhibitory potential (%)
1	α-Amyrin	67.3**
2	Betulinic acid	64.3**
3	Quercetin	57.4*
4	Quercitrin	45.4*
5	Cedrol (positive control)	79.6

*$P < 0.05$; **$P < 0.01$ as compared with control.

explain in part the anti-inflammatory activity of this species. Therefore, this study was undertaken to evaluate the anti-inflammatory potential of the compounds on the binding of 3H-PAF to washed rabbit platelets.

Materials and Methods

Preparation of samples for PAF assay

The compounds were identified by spectroscopic techniques as described earlier (Susanti et al., 2005). Each sample (1 mg) was dissolved in dimethyl sulfoxide (DMSO) and ethanol (1:1). The stock solution was diluted with normal saline to give final concentration of 200 μg/ml. The final concentration of DMSO in reaction mixture was fixed at 0.2% to avoid interference with the receptor binding studies. Reaction mixture with saline and 0.2% DMSO in saline was used as the control.

Reagents and buffers

Tris-tyrode buffer (10 mM, pH 7.0) was used as the medium for binding studies. ACD solution (0.15 M trisodium citrate, 0.075 M citric acid, pH 5.2) was used as the anticoagulant. Buffer A (20% ACD solution, 60% K_2HPO_4 buffer, 20% sodium citrate, pH 6.8) and buffer B (50 ml K_2HPO_4 buffer, 0.1 g bovine serum albumin, pH 7.0) were used for washing the platelets. Radiolabeled PAF (1-*O*-^{3}H-octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, 125 Ci/mmol) was purchased from Amersham, UK. Unlabeled PAF and Cedrol were obtained from Sigma Chemical Co., USA.

Preparation of rabbit platelets

Whole blood samples were drawn by cardiac puncture from healthy New Zealand white strain rabbits (3–4 kg). Six volumes of blood were mixed with one volume of ACD solution. The blood was centrifuged at 270 × g for 10 min at room temperature, and the top platelet-rich plasma was carefully removed. The latter was further centrifuged at 500× g for 15 min. The platelet pellets were then washed twice by means of centrifugation at 500× g (15 min) in Buffer A, followed once at 150× g (10 min) in Buffer B. The top whitish layer was removed and centrifuged at 500× g (15 min) to obtain the platelets. Platelets from different rabbits were usually pooled, a procedure that has not led to any behavioral differences from platelets prepared from a single animal. The final concentration for the platelets was adjusted to 3×10^8 platelets/ml.

PAF receptor binding inhibitory assay

The assay was carried out in triplicate according to the modified method (Valone et al., 1982). The reaction mixture consisted of 200 μl of washed rabbit suspension, 25 μl of ^{3}H-PAF (2.0 nM) with or without unlabeled PAF (2.0 μM), and 25 μl of sample. The final concentration of sample in the reaction mixture was 18.2 μg/ml. Cedrol, a known PAF receptor antagonist, was used as a standard in this bioassay. These reaction mixtures were incubated at room temperature for 1 h. The free and platelet-bound ligands were then separated by filtration technique using a glass microfiber filter in a cell harvester. Radioactivity was measured by scintillation counting. Specific binding of radiolabeled ligand is defined as the difference between total radioactivities of bound ^{3}H-PAF in the reaction mixture with the absence and presence of excess unlabeled PAF. Percentage inhibition of the sample was determined according to the equation:

$$\% \text{ Inhibition} = \frac{(\text{Tc} - \text{Nc}) - (\text{Ts} - \text{Ns})}{\text{Tc} - \text{Nc}} \times 100$$

Where

Tc = total binding of control
Ts = total binding of sample
Nc = nonspecific binding of control
Ns = nonspecific binding of sample (Yang et al., 1995).

The results of the assay are expressed as the mean of three distinct experiments.

Results and Discussion

Platelet activating factor (PAF) is a potent phospholipids mediator that is involved in a variety of inflammatory, respiratory, and cardiovascular disorders. Its structure has been identified as 1-*O*-alkyl-2-O-acetyl-*sn*-glycero-3-phosphocholine. The structural requirement for its biological actions is highly specific, suggesting that its function may be mediated through a receptor (Demopoulus et al., 1979; Hanahan, 1986). In an attempt to understand the mechanism of PAF actions and to further regulate this new mediator, we established a

Table 2. Percentage inhibition (%) of compounds on platelet activating factor (PAF) receptor binding to platelets at various concentrations and their IC_{50} values.

Compound	(μg/ml) 18.2	9.1	4.5	1.8	IC_{50} (μM) (mean ± SD)
Quercetin	58.9	33.6	19.1	4.3	33.0 ± 4.8
Quercitrin	44.8	29.3	18.4	2.5	45.4 ± 5.1
α-amyrin	70.4	51.2	30.3	17.9	20.0 ± 3.4
Betulinic acid	65.1	47.8	29.1	11.8	22.2 ± 4.2
Cedrol (ctrl)	73.5	65.2	55.9	43.5	13.1 ± 2.1

Statistical analysis: Results are expressed as mean percentage inhibition of control in the case of receptor binding test. IC_{50} were obtained from graphs relating Probit percentage inhibition (ordinate) against log dose. The IC_{50} is that dose of compound/drug that would inhibit receptor binding by 50%. Data represent mean ± SD of three independent experiments performed in triplicate.

receptor binding assay using isolated rabbit platelet membranes and tritium-labeled PAF to search for PAF receptor antagonists.

In view of the results on the anti-inflammatory activity of these compounds on platelet activating factor inhibitory binding assay, it appears (Table 1) that α-amyrin and betulinic acid gave significant inhibitory effects of 67.3 and 64.3%, respectively. The other two compounds, quercetin and quercitrin, appeared to demonstrate moderate inhibitory activity, with inhibitory percentages of 57.4 and 45.4%. Cedrol, a known PAF antagonist from natural sources (Yang et al., 1995), was used as a positive control in the bioassay. The percentage inhibitory effects of all these compounds at various concentrations and their IC_{50} values, with the mean values of three measurements, are shown in Table 2. The compounds showed dose-dependent responses, i.e., as the concentration of the compound increased, the percentage inhibition increased. The IC_{50} values of α-amyrin (**1**), betulinic acid (**2**), quercetin (**3**), and quercitrin (**4**) (Fig. 1), obtained from the leaves of *Melastoma malabathricum*, were 20.0, 22.2, 33.0, and 45.4 μM, respectively. The data depicted in Table 2 reveal that the IC_{50} values of these compounds showed levels comparable to but higher than that of cedrol (13.1 μM). On the basis of our results, it can be seen that the compounds were relatively strong PAF receptor binding inhibitors. Further phytochemical studies need to be carried out to investigate further the structure-activity relationship of the compounds and to find derivatives with maximum receptor binding inhibitory activity.

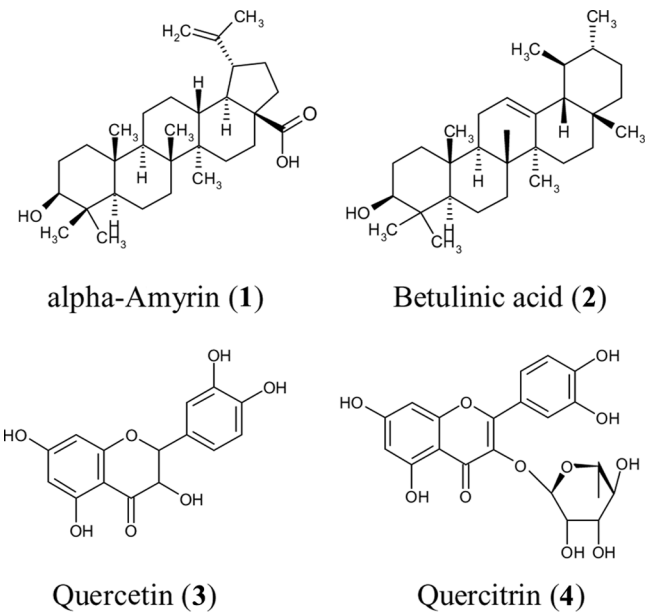

Figure 1. Chemical structure of compounds isolated from *Melastoma malabathricum*.

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