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Lipoxygenase and Hyaluronidase Inhibitory Activities of Constituents from *Phyllagathis rotundifolia* and *Carallia brachiata*

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ABSTRACT Lipoxygenase is known to be responsible for a number of pathophysiological processes such as asthma, psoriasis, cancer metastasis and artherosclerosis, while hyaluronidase is involved in allergic effects, migration of cancer cells, inflammation and the increase in permeability of vascular system. There is considerable interest in searching for compounds that can inhibit the activities of these enzymes. As part of the chemical studies on medicinal plants of Malaysia, we have isolated a total of 50 compounds from *Phyllagathis rotundifolia* (Melastomataceae) and *Carallia brachiata* (Rhizophoraceae), including eight compounds which were new. Twenty-five of the isolated compounds were evaluated for their inhibitory effects on lipoxygenase and hyaluronidase activities. The relationship between the number of phenol groups on the test compounds and their IC_{50} values were also investigated.

ABSTRAK Lipoksigenas adalah bertanggungjawab terhadap beberapa proses pato-fisiologikal seperti sakit lelah, psoriasis, metastasis kanser dan arteroskerosis, sementara hialuronidas pula terlibat di dalam alergi, migrasi sel kanser, inflammasi dan peningkatan ketelapan sistem vascular. Minat mendalam telah ditumpukan untuk mencari kompaun-kompaun yang berupaya merencat aktiviti enzim-enzim tersebut. Kajian kimia yang dijalankan terhadap tumbuhan-tumbuhan ubatan di Malaysia telah berjaya mengasing dan mengenalpasti sebanyak 50 jenis kompaun daripada *Phyllagathis rotundifolia* (Melastomataceae) dan *Carallia brachiata* (Rhizophoraceae), di mana lapan daripadanya adalah kompaun yang baru ditemui. Selanjutnya, dua-puluh lima kompaun di antaranya dipilih untuk menjalani ujian perencatan terhadap enzim lipoksigenas dan hyaluronidas. Hubungan di antara bilangan kumpulan fenol pada kompaun ujian dan nilai IC_{50}nya juga diperhati dan dibincang.

(*Phyllagathis rotundifolia*, *Carallia brachiata*, lipoxygenase, hyaluronidase, enzyme inhibition)

INTRODUCTION

Phyllagathis rotundifolia (Jack) Bl., known as "Tapak Sulaiman" in Malaysia, is a creeper with short stem and heart-shaped leaves, and is commonly found on the damp forest floor throughout Malaysia and Sumatra. All parts of this plant are used in the form of a decoction for the treatment of malaria, fever and stomachache, and for childbirth and as tonic, in the traditional medicinal system of Malaysia [1, 2]. On the other hand, *Carallia brachiata* (Lour.) Merr. (Rhizophoraceae) is a medium-sized tree widely distributed in Madagascar, Sri Lanka, India, Southeast Asia, through to northern Australia and Melanesia. It is found in the lowlands including swamps, and occasionally in hill forests [3, 4]. However, unlike most of the other Rhizophoraceous species, it is not a mangrove species. In Malaysia, the plant is better known as "Meransi", which yields hard timber for furniture and interior finishings [5]. In the traditional medicinal system, the leaves are used to prepare tea, and they are also taken together with mixtures of turmeric, benzoin and rice-dust for the treatment of sapraemia, while the bark is used in the treatment of itch [6]. The leaves were reported to contain (+)-hygroline as the major alkaloid [7].

Previously, we have isolated and identified a total of 50 compounds from *P. rotundifolia* and *C. brachiata* [8, 9], of which eight were reported for the first time. As a continuation of the study on Malaysian medicinal plants, we evaluated the biological activities of 25 compounds isolated

from these plants. Here we report the lipoxygenase and hyaluronidase inhibitory activities of these compounds as well as the relationship between their IC_{50} values and the number of phenol substituents.

METHODOLOGY

General

Lipoxygenase (soybean, type 1-B), linoleic acid, hyaluronidase (bovine testes, type 1-S), hyaluronic acid (human umbilical cord, sodium salt), and bovine serum albumin were purchased from Sigma Chemical Co. Fisetin was purchased from Wako Chemical Co. Apigenin was obtained through acid hydrolysis of apiin, which was isolated from parsley.

Plant material

P. rotundifolia was collected from the Pasoh Forest Reserve, Negeri Sembilan, Malaysia in March 2000, whereas *C. brachiata* was collected from the Forest Research Institute Malaysia (FRIM), Kuala Lumpur, Malaysia. Voucher specimens of each species (FRI 45415 and FRI 45992, respectively) were deposited at the herbarium of FRIM, Kuala Lumpur, Malaysia.

Extraction and isolation

The extraction of the plant samples and subsequent fractionation and isolation of the pure compounds were described previously [8, 9].

Lipoxygenase Assay

This assay was performed according to the procedure described by Sigma, with slight modifications [10]. Briefly, the enzyme solution was preincubated with the test sample for 5 min at 25 °C, followed by addition of substrate solution and borate buffer to the final volume of 1.5 ml. The enzyme activity was calculated as the rate of change of absorbance per unit time. The enzyme inhibitory activity was expressed as the percentage ratio of the difference in enzyme activity between the test sample and control vs enzyme activity in the control experiment. Samples were tested at maximum concentration of 200 μM in the final volume of assay mixture. The concentration reducing enzyme activity by 50% with respect to the control was estimated from graphic plots of a concentration dependent study and was defined as IC_{50} expressed in μM. The values were presented as means ± S.D. of at least three determinations in duplicates. Fisetin was employed as positive controls and added as DMSO solution.

Hyaluronidase Assay

The assay was performed according to the Sigma protocol with slight modifications [10]. Briefly, hyaluronidase solution was preincubated with the test compound for 10 min at 37 °C. Then the assay was commenced by adding hyaluronic acid to the incubation mixture and incubated for a further 45 min at 37 °C. The undigested hyaluronic acid was precipitated with acid albumin solution. After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The absorbance in the absence of enzyme was used as the reference value for maximum inhibition. The inhibitory activity of test compound was calculated as the percentage ratio of the absorbance in the presence of test compound vs absorbance in the absence of enzyme. The performance of the assay was verified using apigenin as a reference under exactly the same experimental conditions. Compounds were tested at a maximum concentration of 50×10^2 μM in the final reaction mixture. The results were expressed as mean of the IC_{50} values ± S.D. of three separate experiments measured in triplicates.

RESULTS AND DISCUSSION

Our previous phytochemical study on the methanolic extracts of the leaves, stems and roots of *Phyllagathis rotundifolia* [8] has yielded six new galloylated cyanogenic glucosides based on prunasin, which were identified as prunasin 2′,6′-di-*O*-gallate, prunasin 3′,6′-di-*O*-gallate **3**, prunasin 4′,6′-di-*O*-gallate, prunasin 2′,3′,6′-tri-*O*-gallate, prunasin 3′,4′,6′-tri-*O*-gallate **4**, and prunasin 2′,3′,4′,6′-tetra-*O*-gallate; and one new alkyl glycoside, oct-1-en-3-yl α - arabinofuranosyl - (1→6)-β-glucopyranoside. Additionally, 13 known compounds were isolated and identified as prunasin **1**, prunasin 6′-*O*-gallate **2**, gallic acid **5** and its methyl ester **6**, β-glucogallin **7**, 3,6-di-*O*-galloyl-D-glucose **8**, 1,2,3,6-tetra-*O*-galloyl-β-D-glucose **9**, strictinin, 6-*O*-galloyl-2,3-*O*-(*S*)-hexahydroxydiphenoyl-D-glucose **10**, praecoxin B **11**, pterocarinin C **12**, 3-oxo-α-ionol 9-*O*-β-D-glucopyranoside and roseoside [8]. Similarly, we have isolated 30 compounds from the leaves of *Carallia brachiata* [9], including one new compound identified as 3-

hydroxy-5,6-epoxy-β-ionol 3-*O*-β-apiofuranosyl-(1″→6′) - β- glucopyranoside. The known compounds were consisted of corchoionoside A, vomifoliol, cassipoureamide-A **13**, 3-*O*-caffeoylquinic acid **14**, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, syringin, 3,4,5-trimethoxyphenol 1-*O*-β-D-apiosyl-(1″→6′)-β-D-glucopyranoside, (Z) 4-*O*-β-D-glucopyranosyl-*p*-coumaric acid **15**, (+)-catechin **16**, (+)-catechin 3-*O*-α-L-rhamnoside **17**, procyanidin B-1 **18**, procyanidin B-3 3-*O*-α-L-rhamnoside **19**, AC trimer, apigenin, luteolin **20**, apigenin-7-*O*-α-rhamnosyl-(1‴→2″)-β-glucopyranoside, luteolin -7- *O* – β- glucopyranoside, luteolin – 7 - *O* - α-rhamnosy - (1‴→2″) – β- glucopyranoside **21**, apigenin-5-*O*-β-glucopyranoside, apigenin-5-*O*-α-rhamnosyl-(1‴→2″)-β-glucopyranoside **22**, luteolin – 5 – *O* – α – rhamnosyl - (1‴→2″) - β-glucopyranoside, isovitexin **23**, isoorientin **24**, orientin **25**, isoswertisin, lariciresinol-9-*O*-β-D-glucopyranoside, 1, 2 – dilinolenyl – 3 – *O* - β-galactopyranosyl-glycerol and 1,2-dilinolenyl-3-*O* - (α-D-galactopyranosyl - (1″→6′) – *O* – β - D-galactopyranosyl)-*sn*-glycerol.

Arachidonic acid metabolism through lipoxygenase pathway generates various biologically active lipids such as leukotrienes that play important roles in many pathophysiological processes, e.g. thrombosis and tumor progression. Angiogenesis, the formation of new capillary vessels from preexisting ones, supports a number of physiological processes and participates in the development of several pathological conditions such as arthritis and cancer [11]. Lipoxygenases are therefore attractive targets for drug intervention and the discovery of mechanism-based inhibitors for the treatment of a variety of disorders such as bronchial asthma, psoriasis, inflammation, cancer, and autoimmune diseases. Hyaluronidase is an enzyme that degrades hyaluronic acid and chondroitin sulfate, which are components of the extracellular matrix of connective tissue. By degrading the components of connective tissue, hyaluronidase promotes the spread of inflammatory mediators throughout these tissues, thereby contributing to the pathogenesis of inflammatory diseases such as allergic effects, migration of cancer cells, inflammation and the increase in permeability of vascular system [12, 13]. The anti-hyaluronidase activity of compounds may therefore contribute to the treatment of these diseases by localizing and preventing their spread to other areas of the body.

Twenty-five of the isolated compounds (Figure 1) were evaluated for their effects on lipoxygenase and hyaluronidase activities. The results showed that many of the test compounds are potent inhibitors of lipoxygenase and hyaluronidase as indicated by their IC_{50} values which are lower than those of positive controls (fisetin and apigenin, respectively) (Table 1). Most of these compounds contain a number of phenolic substitutions. In order to provide a better understanding of the inhibitory effects of these compounds, the relationship between the number of phenol groups on the test compounds and their IC_{50} values were investigated (Figure 2). Generally, the IC_{50} values decrease with increase in the number of phenol groups on the test compounds for both the lipoxygenase and hyaluronidase inhibitory activities. The result shows that the phenolic substituents are essential for contributing to the enzyme inhibition. The enzyme inhibition may have resulted through conformational changes or denaturation of the enzyme, caused by hydrogen bonding or hydrophobic interaction between the enzyme and the phenol groups [14].

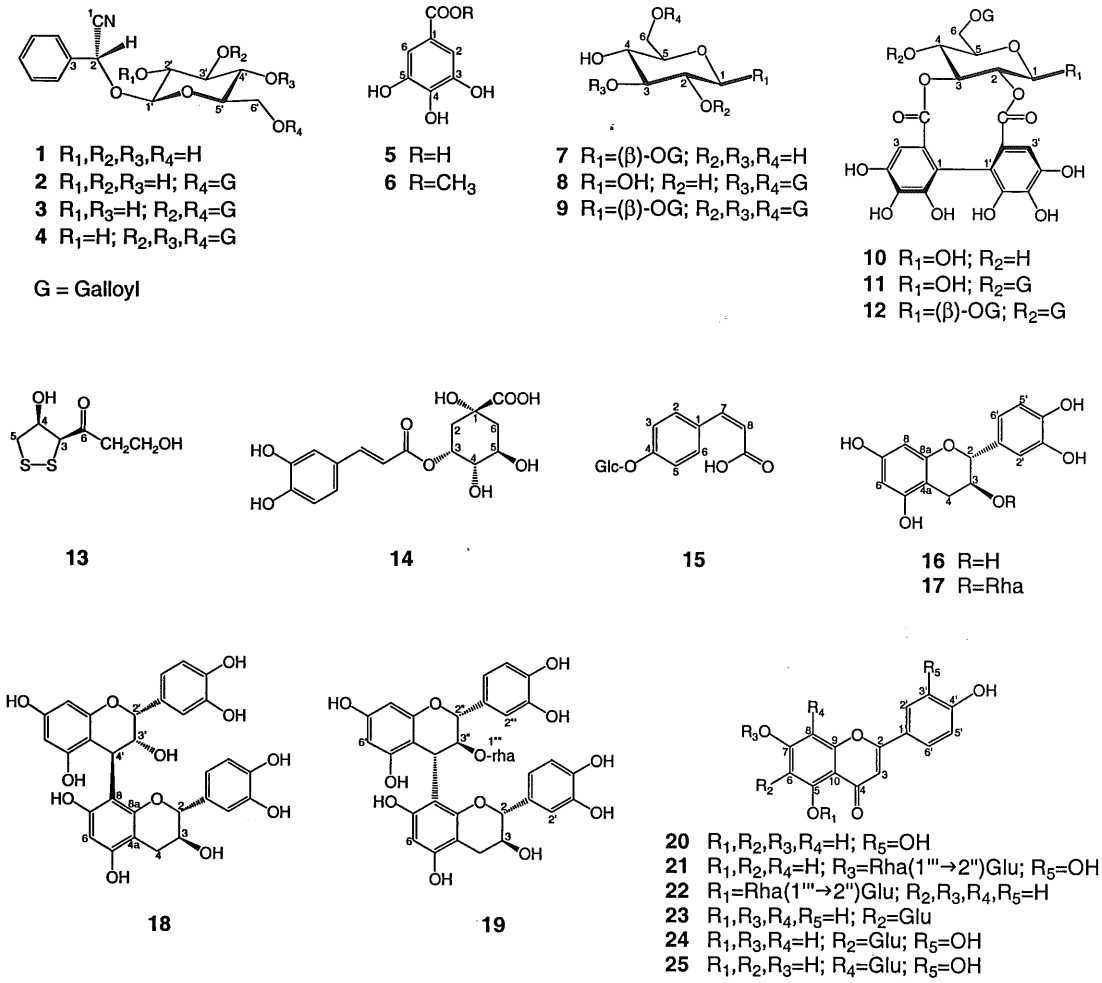

Figure 1. Chemical structures of compounds selected for evaluation of biological activities

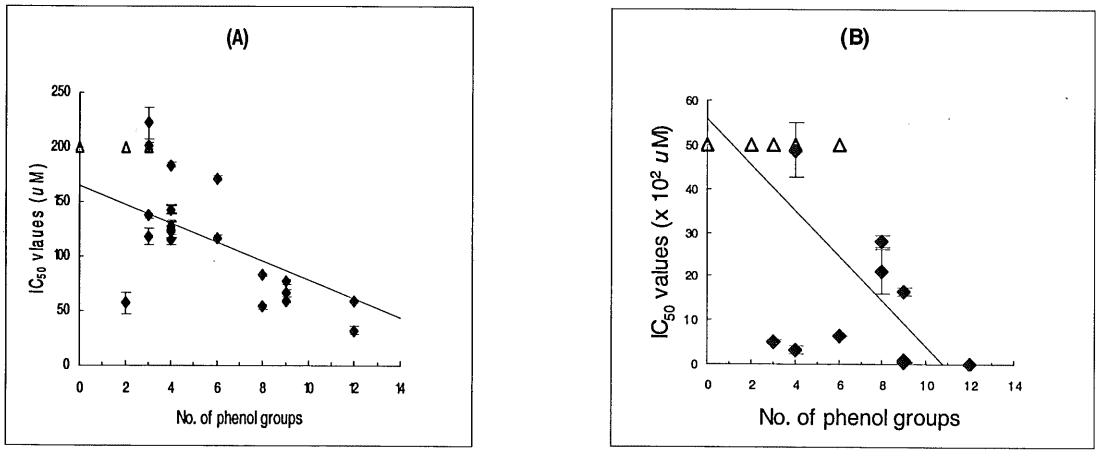

Figure 2. Relationship between the number of phenol groups on test compound and their inhibitory activities expressed as IC_{50} on lipoxygenase (**A**), and hyaluronidase (**B**). IC_{50} values > 200 μM for (**A**), and > 50 x 10^2 μM for (**B**) (△).

Table 1. Lipoxygenase and hyaluronidase inhibitory activities of compounds isolated from *Phyllagathis rotundifolia* and *Carallia brachiata*.

Compounds	No. of phenol groups	IC_{50} (□M)	
		Lipoxygenase [a)]	Hyaluronidase [b)]
Prunasin **1**	0	NA	NA
Prunasin 6-*O*-gallate **2**	3	201.5±3.5	NA
Prunasin 3′,6′-di-*O*-gallate **3**	6	117.3±2.3	6.25±0.04
Prunasin 3′,4′,6′-tri-*O*-gallate **4**	9	77.0±2.0	0.75±0.07
Gallic acid **5**	3	118.0±7.1	>50
Gallic acid methyl ester **6**	3	222.0±14.4	NA
β-Glucogallin **7**	3	>200	NA
3,6-Di-*O*-galloyl-D-glucose **8**	6	171.8±2.0	>50
1,2,3,6-Tetra-*O*-galloyl-β-D-glucose **9**	12	58.7±1.5	0.15±0.00
6-*O*-Galloyl-2,3-*O*-(S)-hexahydroxydiphenoyl-D-glucose **10**	9	59.5±2.1	16.50±0.70
Praecoxin B **11**	9	66.7±3.1	0.28±0.02
Pterocarinin C **12**	12	32.5±4.0	0.05±0.00
Cassipoureamide A **13**	0	NA	>50
3-*O*-Caffeoylquinic acid **14**	2	57.2±9.5	NA
(Z) 4-*O*-β-D-Glucopyranosyl-*p*-coumaric acid **15**	0	>200	>50
(+)-Catechin **16**	4	183.7±2.3	48.70±6.10
(+)-Catechin 3-*O*-rhamnoside **17**	4	114.7±4.6	>50
Procyanidin B-1 **18**	8	83.0±1.0	21.00±5.00
Procyanidin B-3 3-*O*-rhamnoside **19**	8	54.0±2.6	28.00±1.40
Luteolin **20**	4	127.3±5.5	3.28±1.06
Luteolin-7-*O*-rhamnosyl-(1‴→2″)-glucopyranoside **21**	3	>200	>50
Apigenin-5-*O*-rhamnosyl-(1‴→2″)-glucopyranoside **22**	2	>200	>50
Isovitexin **23**	3	137.3±2.1	>50
Isoorientin **24**	4	122.7±7.5	>50
Orientin **25**	4	142.7±4.0	>50

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