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Preliminary Studies on Lipoxygenase Inhibitory Activity of Selected Malaysian Medicinal Plants

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ABSTRACT

A potential source for new lipoxygenase inhibitors is undoubtedly provided by the abundance of medicinal plants used in traditional medicine. As part of screening programme for biologically active plants, 20 extracts of different parts of 10 Malaysian medicinal plants belonging to four families were evaluated for their inhibitory activity on soybean 15-lipoxygenase (15-sLo). The leaves of *Peronema canescens* Jack. (Verbenaceae) were shown to be the most potent inhibitor with an IC₅₀ value of 25.64 µg/ml. While the extracts of *P. canescens* stem and *Stereospermum fibricatatum* DC. (Bignoniaceae) leaves were found to possess profound inhibitory activity with IC₅₀ values of 33.65 and 39.41 µg/ml, respectively. The methanol extracts obtained from *Stenolobium stans* (L.) D. Don (Bignoniaceae) leaves and stems, *Piper ribesoides* Wall. (Piperaceae) stems and *S. fibricatatum* stems were moderately active with IC₅₀ values ranging from 40 to 60 µg/ml. While the remaining samples showed low lipoxygenase inhibitory activity at concentration of 100 µg/ml.

KEY WORDS: Malaysian medicinal plants, methanolic extracts, 15-lipoxygenase inhibitory activity

INTRODUCTION

Medicinal plants have been used since times immemorial as remedies for human diseases because they contain components of therapeutic value. Plants have been important sources for providing many pharmacological agents with novel structures and unique mechanisms. Plants with a history of use in traditional medicine constitute an obvious starting point in the search for new therapeutically active drugs. A scientific evaluation of medicinal plants according to their traditional claims could be incorporated into the complementary and alternative medicine system. In the course of our investigations of pharmacologically active substances from natural resources, ten plants which have been used in folk medicine for the treatment of various diseases including inflammatory associated illnesses were evaluated for their anti-inflammatory properties by

using soybean 15-lipoxygenase inhibitory activity as a model.

Lipoxygenases are a family of non-heme iron-containing dioxygenases that widely distributed in plants and animals. Lipoxygenases giving rise to 5-, 8-, 12-, and 15-oxygenated derivatives, named for their positional specificity on arachidonic acid have been demonstrated to produce a wide range of physiologically active metabolites. These enzymes catalyze the first step of the arachidonic pathway leading to a wide variety of bioactive lipids including hydroxy- and hydroperoxy- derivatives (1). These products play a role in a variety of disorders such as allergies, asthma, psoriasis, atherosclerosis and also have profound influence on the development of several human cancers (2, 3). Hence inhibitors against this group of enzymes have great potential for rational drug design and discovery in health sectors. In

addition, scientific evaluation of these local ethnomedicinal plants may provide a rational basis for their traditional uses and a suitable alternative for those who cannot afford the benefits of modern medicines (4). Therefore in the present investigation, the effects of ten selected medicinal plants on soybean 15-lipoxygenase inhibitory activity were studied.

MATERIALS AND METHODS

Plant material and preparation of extract

The plant material used in this study consisted of leaves and stems of *Jacaranda obsutifolia* Humb. Bonpl., *Myristica fragrans* Houtt., *Peronema canescens* Jack., *Piper mucronatum* C.DC., *Piper ribesoides* Wall., *Stenolobium stans* (L.) D.Don., *Stereospermum fibracatum* DC., *Tabebuia corymbosa* (Jacq.) G. Nicholson, *Tabebuia pallida* Miers. and *Tabebuia rosea* DC. were collected from January 2005 to March 2005 from various places of Peninsula Malaysia. The plant material was taxonomically identified by Cik Zainon Abu Samah, a botanist of the Forest Research Institute Malaysia. The voucher specimens were deposited in the department herbarium for future reference. The plant materials were air dried and ground to mesh size 40-60 using a grinding machine. The dried pulverized materials were extracted (by simple soaking) in methanol (10 times the amount of plant material) over 72h. The methanol extracts were filtered and the solvent removed by distillation under reduced pressure using rotary evaporator.

In vitro anti-inflammatory assay

The assay was carried out as previously described (5). In brief, enzyme activity was measured spectrophotometrically using a spectrophotometer, in borate buffer (0.2 M, pH 9.0) by the increase in absorbance at 234 nm, 25°C between 50-210 s after addition of lipoxygenase (167 U/ml. final concentration), using linoleic acid (134 µM) as substrate. The enzyme solution was kept in ice, and controls (100% enzyme activity) were measured before the test samples. Enzyme inhibitory activities were calculated from the values for absorption increase per time unit, as supplied from the software of the spectrophotometer. For the test, 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 3.0 ml. Three or more parallels for samples were measured. 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. Fisetin was employed as positive control and added as dimethylsulfoxide (DMSO) solution.

RESULTS AND DISCUSSION

The pharmacological screening was carried out in order to determine if the methanol extracts of the selected medicinal plants had any other activity that might be considered of interest and to establish general effects of the extracts. In the course of our search for potential anti-inflammatory from plants, we screened 20 methanol extracts of 10 species of Malaysian medicinal plants for their inhibitory action against lipoxygenase activity using soybean lipoxygenase. The results of the pharmacological activity in terms of LOX inhibition are given in Table 1. The screening concentration was 100 µg/ml test volume. It can be seen from the table that the extracts of the Malaysian medicinal plants showed widely varying activity with the extract of *Peronema canescens* Jack. leaves displaying the most active inhibitory activity with IC₅₀ value of 25.64 µg/ml. This result was almost comparable to that produced by fisetin (22.76 µg/ml). The stems of *P. canescens* and leaves of *Stereospermum fibracatum* DC., both with an IC₅₀ value of 33.65 and 39.41 µg/ml, respectively also exhibited potent inhibitory activity. A close similarity of moderate activity was displayed by the leaves (41.97 D.Don, indicating comparable biological activities between aerial plant parts. The stems of *P. ribesoides* and *S. fibracatum* were moderately active with IC₅₀ values of 46.25 and 46.31 µg/ml, respectively. All the IC₅₀ values of these extracts showed comparable but higher than that of fisetin (22.76 µg/ml). While the vast proportion of remaining extracts tested did not exhibit promising lipoxygenase inhibitory activity. However, considering that all the samples are crude extracts, the possibility that the active principle, when eventually isolated pure, could be of high potency cannot be ruled out, especially if such principles are present in small amounts.

In conclusion, the evaluation of 10 Malaysian medicinal plants has demonstrated varying degree of anti-inflammatory activity with two species of *P. canescens* and *S. fibracatum* exhibited potent activity in the lipoxygenase assay. These preliminary results suggest that these plants may possess some anti-inflammatory properties through the inhibition of leukotrienes production by lipoxygenase. From our investigation of screening different plant species, the results obtained

Table 1. Inhibition of soybean 15-lipoxygenase by methanol extracts of Malaysian medicinal plants

Name	Family	Part	IC ₅₀ (µg/ml) ^a
<i>Jacaranda obtusifolia</i> Humb. & Bonpl.	Bignoniaceae	Leaf	>100
		Stem	>100
<i>Myristica fragrans</i> Houtt.	Myristicaceae	Leaf	62.62
		Stem	63.86
<i>Peronema canescens</i> Jack.	Verbenaceae	Leaf	25.64
		Stem	33.65
<i>Piper mucronatum</i> C.DC.	Piperaceae	Leaf	>100
		Stem	^b
<i>Piper ribesioides</i> Wall.	Piperaceae	Leaf	^b
		Stem	46.25
<i>Stenolobium stans</i> (L.) D.Don	Bignoniaceae	Leaf	41.97
		Stem	40.63
<i>Stereospermum fibracatum</i> DC.	Bignoniaceae	Leaf	39.41
		Stem	46.31
<i>Tabebuia caryantha</i> (Jacq.) G. Nicholson	Bignoniaceae	Leaf	60.71
		Stem	59.43
<i>Tabebuia pallida</i> Miers.	Bignoniaceae	Leaf	^b
		Stem	^b
<i>Tabebuia rosea</i> DC.	Bignoniaceae	Leaf	>100
		Stem	53.48

^aInhibition of soybean 15-lipoxygenase; n = 3 or more, inhibition was significantly different with respect to control, P < 0.01. ^bInhibition was not significantly different with respect to control at concentration up to 100 µg/ml. Fisetin (positive control) had an IC₅₀ value of 22.76 µg/ml in this system.

form a good basis for selection of candidate species for further phytochemical and pharmacological investigation which may find use in treating inflammation, asthma, atherosclerosis, tumour angiogenesis and cancer. Further studies are warranted to assess other pharmacological mechanisms through which the plants might mediate anti-inflammatory effects. In addition, these results also support the folkloric usage of some plants used in traditional medicine in the treatment of inflammation.

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