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Effect of total phenolics from *Laggera alata* on acute and chronic inflammation models

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Abstract

The anti-inflammatory effect of total phenolics from *Laggera alata* (TPLA) was evaluated with various *in vivo* models of both acute and chronic inflammations. In the acute inflammation tests, TPLA inhibited significantly xylene-induced mouse ear oedema, carrageenan-induced rat paw oedema and acetic acid-induced mouse vascular permeability. In the carrageenan-induced rat pleurisy model, TPLA significantly suppressed inflammatory exudate and leukocyte migration, reduced the serum levels of lysozyme (LZM) and malondialdehyde (MDA), increased the serum levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX), and also decreased the contents of total protein, nitric oxide (NO) and prostaglandin E_2 (PGE_2) in the pleural exudates. In the chronic inflammation experiment, TPLA inhibited significantly cotton pellet-induced rat granuloma. These results indicated that TPLA possesses potent anti-inflammatory activity on acute and chronic inflammation models. Its anti-inflammatory mechanisms are probably associated with the inhibition of prostaglandin formation, the influence on the antioxidant systems, and the suppression of LZM release. Furthermore, the total phenolic content of *Laggera alata* and its main component type was quantified, and its principle components were isolated and authenticated. Acute toxicity studies revealed that TPLA up to an oral dose of 8.5 g/kg body weight was almost nontoxic in mice.

Keywords: *Laggera alata*; Folk medicine; Anti-inflammation; Total phenolics

1. Introduction

Of the 20 species of *Laggera* genus (Asteraceae), distributing mainly in tropical Africa and Southeast Asia, *Laggera alata* (D. Don) Sch.-Bip ex Olivier and *Laggera pterodonta* (DC.) Benth are the only two *Laggera* species found in China. Both are employed as folk medicines for the treatment of inflammatory disorders. *Laggera alata* was given wide attention in the last few decades due to its remarkable curative effect, especially for rheumatic arthritis, faucitis, bronchitis, tonsillitis, nephritis, etc. However, most of them focused on folk use and phytochemical work (Bohlmann et al., 1985; Li et al., 1998; Raharivelomanana et al., 1998). Contrarily, no systematic bioactivity studies were carried out to the folk medicine. Based on our previous researches on phytochemistry of this genus (Zheng et al., 2003a,b), we initiate a program aiming at validating the anti-inflammatory activity of the title species. The results of prescreening showed that the aqueous extract of *Laggera alata* has potent anti-inflammatory activity. In addition, the total phenolics of *Laggera alata* (TPLA) was found to be the principle components. Since the anti-inflammatory activity of TPLA had not

Abbreviations: TPLA, total phenolics from *Laggera alata*; LZM, lysozyme; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; NO, nitric oxide; PGE_2, prostaglandin E_2; PGs, prostaglandins; DEX, dexamethasone; HPLC, high performance liquid chromatography; GAE, gallic acid equivalents

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been reported, the present study was carried out and the results are reported hereby.

The focus of this paper is on the inhibitory effects of TPLA on acute and chronic inflammation models, such as xylene-induced ear oedema, carrageenan-induced paw oedema, acetic acid-induced vascular permeability, carrageenan-induced pleurisy and cotton pellet-induced granuloma. The results indicated that TPLA possesses potent anti-inflammatory activity on the acute and chronic inflammation models that we have employed.

2. Materials and methods

2.1. Plant materials

The whole herb of *Laggera alata* (D. Don) Sch.-Bip ex Olivier was collected from Tengchong county, Yunnan Province, China, in August 2003, and authentified by Professor Liurong Chen, Department of Traditional Chinese Medicine and Natural Drug Research, College of Pharmaceutical Sciences, Zhejiang University, China. A voucher specimen (No. ZY982003LA) was deposited in the herbarium of College of Pharmaceutical Sciences, Zhejiang University, China.

2.2. Preparation of TPLA

The aerial parts of the whole herb of *Laggera alata* was dried in shade. Then according to the method of Yao (2001), the dried materials (10 kg) was cut into segments of 0.5–2.0 cm in length and extracted three times with 95% ethanol. The extract was combined and concentrated under reduced pressure to give a dark-green tarry mass (445 g), which was dissolved in hot water. This solution was basified up to pH 9–10 with 5% sodium carbonate, followed by repeated extraction with ethyl acetate to remove lipophilic constituents. The aqueous extract left was further partitioned with *n*-butanol after acidification to pH 4 using 1N hydrochloric acid. The *n*-butanol fraction was washed with water to pH 7 and condensed under reduced pressure to afford a dark-brown powder (150 g), showing intense reactions with both magnesium powder–hydrochloric acid and 5% ethnolic ferric chloride, which was named total phenolics of *Laggera alata* (TPLA) (yield: 1.5%). TPLA was dissolved in normal saline to be administered to the tested animals.

2.3. Quantification of phenolic compounds in the aqueous extracts

The above-mentioned TPLA suspension was filtered and the supernatant was lyophilized. The total phenolic content was determined as described previously (Spanos et al., 1990). Gallic acid was used as standard sample. The extracts (100 mg) were dissolved in 5 ml of 0.3% hydrochloric acid in methanol/water (60:40, v/v). The resulting solution (100 ml) was added to 2 ml of 2% sodium carbonate. After 2 min, 50% Folin-Ciocalteu reagent (100 ml) was added to the mixture, which was then left for 30 min. Absorbance was measured at 750 nm using a spectrophotometer. The result was expressed as g of gallic acid equivalents (GAE) per 100 g of lyophilized extract (g GAE/100 g extract). The results of quantification indicated that the aqueous extract of *Laggera alata* has a high content of phenolic compounds that made up half of the extract (52.6 g GAE/100 g extract).

HPLC (Waters 2695, USA) was used to analyze the aqueous extract of *Laggera alata*. A Zorbax SB-C_{18} (5 μm, particle size) column (250 mm × 4.6 mm) fitted with A Zorbax SB-C_{18} guard column (5 μm particle size; 7.5 mm × 4.6 mm) was used. The mobile phase consisted of two eluents: 0.1% acetic acid (B) and acetonitrile (A). To achieve separation, flow rate was set at 0.8 ml/min and 5 μl sample were injected. The method of gradient elution was adopted: initial 84% B and 16% A maintained for 15; 35 min linear change to 15% B and 85% A. The column temperature was set at 30 °C. Detection was at 280 nm. The results indicated that dicaffeoylquinic acids were the major phenolic components in the aqueous extract whose content attached to 51.0% (Fig. 1).

Two grams of the aqueous extracts powder was dissolved with water and the solute was chromatographed on Sephadex LH-20 with 30% methanol yielding eight fractions. Repeated chromatography on Sephadex LH-20 of fraction 6 with methanol afforded 4,5-*O*-dicaffeoylquinic acid (**1**) (35 mg), 3,5-*O*-dicaffeoylquinic acid (**2**) (23 mg) and 3,4-*O*-dicaffeoylquinic acid (**3**) (44 mg). The structures of dicaffeoylquinic acids were

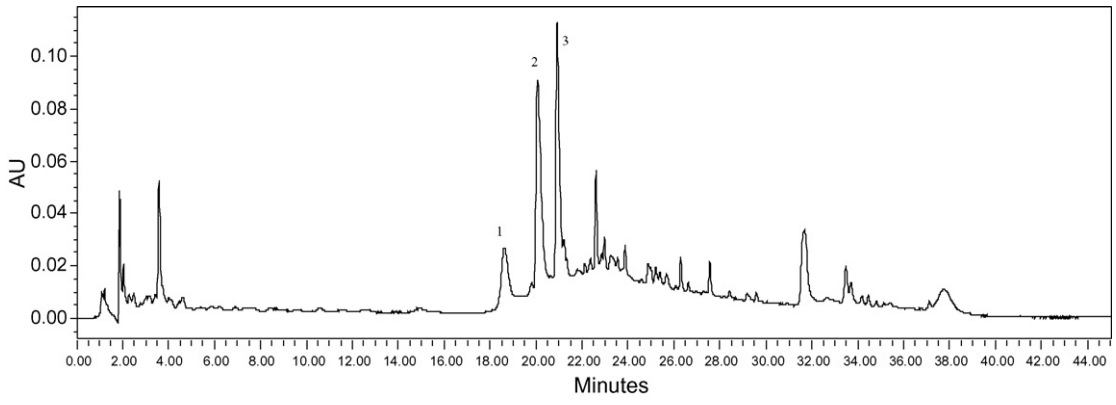
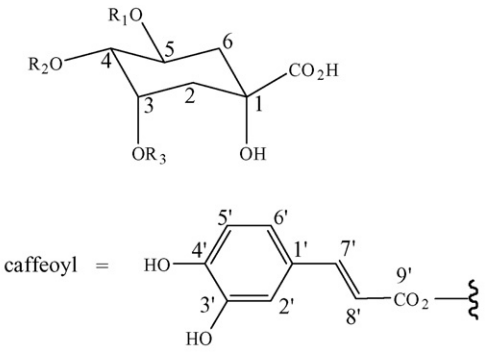


Fig. 1. Trace of reverse phase HPLC analysis of *Laggera alata* aqueous extract. Numbers in figure indicate the main phenolics of 4,5-*O*-dicaffeoylquinic acid (**1**), 3,5-*O*-dicaffeoylquinic acid (**2**) and 3,4-*O*-dicaffeoylquinic acid (**3**).



Compound	R_1	R_2	R_3
4,5-*O*-Dicaffeoylquinic acid (**1**)	caffeoyl	caffeoyl	H
3,5-*O*-Dicaffeoylquinic acid (**2**)	caffeoyl	H	caffeoyl
3,4-*O*-Dicaffeoylquinic acid (**3**)	H	caffeoyl	caffeoyl

Fig. 2. Structures of dicaffeoylquinic acids.

characterized as described in literature (Merfort, 1992; Um et al., 2002) and shown in Fig. 2.

2.4. Animals

Male standard ICR strain mice weighing 20–25 g and male standard Sprague-Dawley strain rats weighing 180–220 g were bred in the standard animal house. The animals were kept in a room maintained at 22 ± 2 °C and at relative humidity between 40% and 70%. The animals had free access to food and water. The experimental protocol was approved by the Animal Ethics Committee of Zhejiang University, in accordance with "Principles of Laboratory Animal Care and Use in Research" (Ministry of Health, Beijing, China).

2.5. Chemicals

Carrageenan and Evans' blue were obtained from Sigma Chemical Co. (St Louis, MO, USA). Nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), lysozyme (LZM) and total protein kits were purchased from Nanjing Jiancheng Bioengineering Institute (China). All other reagents were of the highest commercial grade available.

2.6. Anti-inflammatory studies

2.6.1. Xylene-induced ear oedema in mice

The method described by Vogel and Vogel (1997) was employed. Male ICR mice were divided into six groups. Group A: normal saline; Group B: dexamethasone (2.5 mg/kg); Group C: TPLA-25 (25 mg/kg); Group D: TPLA-50 (50 mg/kg); Group E: TPLA-100 (100 mg/kg); Group F: TPLA-200 (200 mg/kg). The vehicle and drugs were administered orally to the groups of mice, respectively, once per day for 3 days. Group A received the same volume of normal saline orally as vehicle control. One hour after the last administration of drugs, the ventral and dorsal sides of the right ears of mice both received 10 μl of xylene by topical application. One hour after xylene applied to the right ears of mice, the mice were sacrificed and 9 mm punches were made in the right and the left ear by the borer. Each ear disc was weighed and the differences in weights of the right and left ear discs of mice were recorded as oedema level.

2.6.2. Acetic acid-induced vascular permeability in mice

This test was followed by the method described by Whittle (1964) with some modification. Male ICR mice were divided into three groups. TPLA at 100 mg/kg was administered orally to the test group of mice. The positive and negative control groups of mice were given dexamethasone (2.5 mg/kg) and the same volume of normal saline, respectively. The mice received orally drugs once per day for 7 days. One hour after the last administration of drug, 0.2% Evan's blue in normal saline was injected intravenously into the tail vein at a dose of 0.1 ml/10 g body weight. Thirty minutes later, each mouse was injected intraperitoneally with 0.2 ml of 0.6% acetic acid in normal saline. After 1 h, the mice were sacrificed and the abdominal wall was cut to expose the entrails. The abdominal cavity was washed using 5 ml of normal saline to collect pigments in a test tube. After centrifuging the contents of the tube to eliminate contaminants, the solution was subjected to colorimetry using a spectrophotometer at a wavelength of 590 nm. Control mice were treated similarly. The vascular permeability effects were expressed as the aborbance (A), which represented the total amount of dye leaked into the intraperitoneal cavity.

2.6.3. Carrrageenan-induced paw oedema in rats

This animal model was made according to the report of Winter et al. (1962). The rats were divided into three groups. The drug control group received orally dexamethasone at a dose of 2.5 mg/kg. The same volume of normal saline was administered orally to the vehicle control group of rats, while TPLA at a dose of 100 mg/kg was given orally to the test group of animals. The drugs and vehicle were respectively given to experimental animals once per day for a period of 7 days. One hour after the last administration, acute paw oedema was induced by subplantar injection of 0.1 ml of 1% freshly prepared carrageenan suspension in normal saline into the right hind paw of each rat. The left hind paw was injected with 0.1 ml of normal saline. The paw volume was measured before (0 h) and at intervals of 1, 2, 3, 4, 5 and 6 h after carrageenan injection using a plethysmometer.

2.6.4. Carrageenan-induced pleurisy in rats

The method of Mikami and Miyasaka (1983) was employed. The rats were divided into four groups. The experimental and positive drug groups of animals were treated with TPLA at a dose of 100 mg/kg and dexamethasone at a dose of 2.5 mg/kg, respectively. The same volume of normal saline was respectively administered to the vehicle and model groups of rats. The drugs were given orally once per day for 7 days. One hour after the last administration of drugs, rats were lightly anaesthetized under ether and then 0.2 ml of normal saline alone or containing 1% carrageenan were injected into the pleural cavity of each rat. Four hours after the injection of carrageenan, rats were slightly anaesthetized and blood samples were taken from the eyepit. The serum was separated and stored at −20 °C for the measurement of LZM, MDA, SOD and GSH-PX. The animals were then sacrificed under an overdose of ether and the pleural cavities were exposed. The exudate volume was measured and the pleural cavity was washed with 2 ml of ice-cold phosphate-buffered saline (pH 7.2) with heparin (5 U/ml). The exudates and washing were combined as the pleural exudates for the measurement of NO, PGE_2 and total protein. Exudates contaminated with blood were discarded. The total leukocyte number in the pleural exudates was counted in a Neubauer Chamber.

The levels of LZM, MDA, SOD and GSH-PX in the pleural rat serum were assayed with LZM, MDA, SOD and GSH-PX kits, respectively. The production of NO and total protein in the pleural exudate was measured with NO and total protein kits, respectively. The measurement of PGE_2 in the pleural exudates was performed according to the chemical method previously described by Wu (1991).

2.6.5. Cotton pellet-induced granuloma in rats

The experiment was carried out using the method of Winter and Porter (1957). The rats were divided into five groups. Under ether anesthesia, sterile cotton pellets weighting 10 ± 1 mg were implanted subcutaneously in both the axilla region of each rat through a single needle incision, one on each side. The second group was served as drug control and received dexamethasone daily at a dose of 2.5 mg/kg orally for 7 days. The same volume of normal saline was given orally to the first group of animals as vehicle control. TPLA at doses of 50, 100 and 200 mg/kg were administered orally to the other three groups of rats, respectively, for 7 days from the day of cotton pellet implantation. On the eighth day, the granuloma tissue was dissected out carefully and dried at 60 °C to constant weight. The increase in dry weight of the pellets was taken as the measure of granuloma formation.

2.7. Acute toxicity

Different doses of TPLA were made in a 0.5% CMC-Na solution which were homogenized with high speed homogeneous equipment, and were given orally to groups of 10 mice each. During the 7 days after treatment, all the animals were observed and recorded daily, and dead animals would be subjected to postmortem examination for determination of the cause of death.

2.8. Statistical analysis

Values are expressed as mean ± standard deviations of the mean (S.D.) and analyzed statistically by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test or Tamhane's T2. $P < 0.05$ was chosen as the criterion of statistical significance.

3. Results

3.1. Effect of TPLA on xylene-induced ear oedema in mice

The oral administration of TPLA suppressed significantly xylene-induced ear oedema in mice (Table 1). The oedema inhibitory rates of TPLA were 29.6%, 43.0%, 53.2% and 64.9% at doses of 25, 50, 100 and 200 mg/kg, respectively. Whereas dexamethasone (2.5 mg/kg), used as a reference drug, produced 76.1% inhibitory rate compared to control.

3.2. Effect of TPLA on acetic acid-induced vascular permeability in mice

A dose of 100 mg/kg TPLA was applied. Under these conditions, the effect of TPLA on vascular permeability was stronger than that of 2.5 mg/kg of dexamethasone and the differences of both groups against the vehicle group showed statistical significance (Fig. 3). TPLA and the standard drug used for comparison

Table 1
Effects of total phenolics from *Laggera alata* (TPLA) and dexamethasone (DEX) on xylene-induced ear oedema in mice

Group	Dose (mg/kg, p.o.)	Oedema degree (mg)	Inhibition rate (%)
Vehicle	–	24.65 ± 2.47	–
DEX	2.5	5.90 ± 1.41***	76.1
TPLA	25	17.36 ± 1.96***	29.6
	50	14.06 ± 1.84***	43.0
	100	11.53 ± 1.77***	53.2
	200	8.65 ± 1.32***	64.9

Values are mean ± S.D. of differences in weight between right and left ear of animals (n). $n = 10$, ***$P < 0.001$ compared with vehicle control group (one-way ANOVA, Dunnett's *t*-test as the post hoc test).

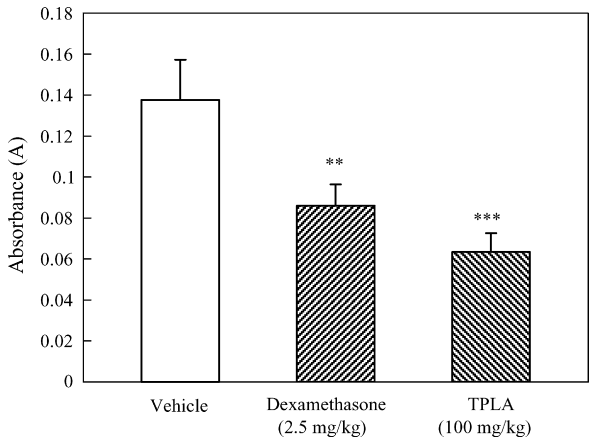


Fig. 3. Effect of total phenolics from *Laggera alata* (TPLA) (100 mg/kg) and dexamethasone (DEX) (2.5 mg/kg) on acetic acid-induced increased vascular permeability in mice. Values are mean ± S.D., $n = 8$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with vehicle control group (one-way ANOVA, Dunnett's *t*-test as the post hoc test).

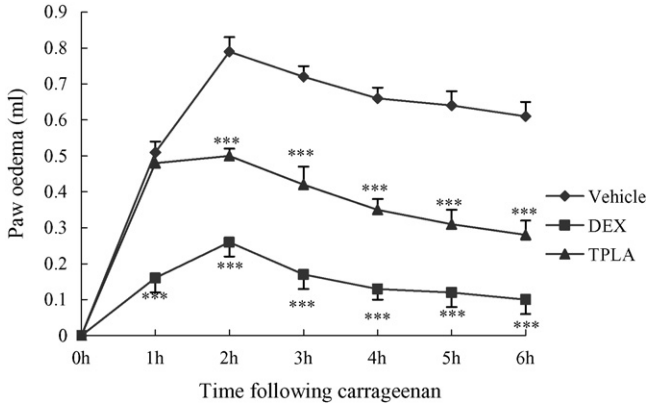


Fig. 4. Effect of total phenolics from *Laggera alata* (TPLA) (100 mg/kg) and dexamethasone (DEX) (2.5 mg/kg) on carrageenan-induced paw oedema in rats. Values are mean ± S.D., $n = 8$, $^{***}P < 0.001$ compared with vehicle control group (one-way ANOVA, Dunnett's *t*-test as the post hoc test).

produced 54.0% and 37.5% inhibition of dye leakage, respectively.

3.3. Effect of TPLA on carrageenan-induced paw oedema in rats

The oral treatment with TPLA inhibited significantly carrageenan-induced paw oedema in rats (Fig. 4). At a dose of 100 mg/kg of TPLA, oedematization was suppressed and inhibitory rates were 25.5–54.4% at 1–6 h after carrageenan treatment. At a dose of 2.5 mg/kg, dexamethasone, as a standard drug, produced a greater inhibition of oedema development by 48.5–75.2% at 1–6 h after carrageenan injection.

3.4. Effect of TPLA on carrageenan-induced pleurisy in rats

At a dose of 100 mg/kg, the oral pre-treatment of TPLA significantly reduced the pleural exudate volume and the total leukocyte migration, inhibited the production of total protein and two inflammatory mediators (NO and PGE_2) in the pleural exudates (Table 2), increased the serum levels of SOD and GSH-PX, and also decreased the serum levels of LZM and MDA (Table 3). Dexamethasone used as the standard drug indicated the similar effect.

3.5. Effect of TPLA on cotton pellet-induced granuloma in rats

The effects of TPLA and dexamethasone on cotton pellet-induced granuloma in rats were showed in Table 4. At doses of 50, 100 and 200 mg/kg, TPLA as well as dexamethasone (2.5 mg/kg) inhibited markedly granuloma formation surrounding the pellets compared with vehicle control group. Two hundred milligrams per kilogram of TPLA produced a maximum inhibition of 54.8%, while 50 and 100 mg/kg of TPLA produced 29.1% and 45.8% inhibition in granuloma weight, respectively, when compared to 69.6% for dexamethasone.

3.6. Acute toxicity

No animal died during acute toxicity test, neither apparent adverse effects were observed for tested animals with different doses of TPLA. The result revealed that the extract TPLA up to an oral dose of 8.5 g/kg body weight was almost nontoxic in mice.

4. Discussion

Traditional Chinese Medicines especially the medicinal plants are known to possess a diversity of components or secondary metabolites which have various biological activities. However, to identify the active substances and to elucidate their

Table 2
Effect of total phenolics from *Laggera alata* (TPLA) and dexamethasone (DEX) on exudate volume, leukocyte migration, total protein content, NO and PGE_2 levels of pleural rats induced by carrageenan

Group	Dose (mg/kg, p.o.)	Exudate (ml)	Total leukocyte ($\times 10^7$ ml^{-1})	Total protein (g/l)	NO (μmol/l)	PGE_2 (A)
Vehicle	–	$0.03 \pm 0.01^{***}$	$0.91 \pm 0.18^{***}$	$19.95 \pm 1.29^{***}$	$47.67 \pm 7.66^{***}$	$0.254 \pm 0.038^{***}$
Model	–	1.88 ± 0.21	3.77 ± 0.40	40.29 ± 3.08	129.22 ± 9.83	0.729 ± 0.118
DEX	2.5	$0.58 \pm 0.15^{***}$	$1.18 \pm 0.24^{***}$	$25.09 \pm 2.38^{***}$	$70.92 \pm 6.83^{***}$	$0.446 \pm 0.061^{**}$
TPLA	100	$0.91 \pm 0.11^{***}$	$1.80 \pm 0.30^{***}$	$22.61 \pm 3.01^{***}$	$77.70 \pm 6.24^{***}$	$0.346 \pm 0.051^{***}$

Values are mean ± S.D., $n = 8$, $^{**}P < 0.01$ and $^{***}P < 0.001$ with respect to model control group, exudate volume, total leukocyte and PGE_2 were analyzed statistically by Tamhane's T2, while total protein and NO were done by Dunnett's *t*-test, one-way ANOVA.

Table 3
Effects of total phenolics from *Laggera alata* (TPLA) and dexamethasone (DEX) on the serum levels of lysozyme, MDA, SOD and GSH-PX in the pleural rats induced by carrageenan

Group	Dose (mg/kg, p.o.)	Lysozyme (μg/ml)	MDA (nmol/ml)	SOD (U/ml)	GSH-PX (μmol/l)
Vehicle	–	38.20 ± 4.57***	8.36 ± 0.68***	254.72 ± 8.02***	388.36 ± 8.34***
Model	–	58.56 ± 5.82	13.70 ± 1.28	202.57 ± 14.28	343.10 ± 14.19
DEX	2.5	41.64 ± 4.84***	8.79 ± 1.19***	229.06 ± 15.90**	359.47 ± 10.81*
TPLA	100	41.67 ± 3.79***	9.02 ± 0.92***	240.35 ± 9.51***	364.47 ± 16.19**

Values are mean ± S.D., $n = 8$, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with model control group (one-way ANOVA, Dunnett's *t*-test as the post hoc test).

mechanism of action unambiguous is a facing task. *Laggera alata,* as a folk medicine to treat a variety of inflammatory diseases in China, has been widely used for several centuries, but the pharmacological studies on this herbal medicine have never been carried out. In our preliminary investigations, *Laggera alata* was bio-guided fractionated to check the anti-inflammatory activities against xylene-induced mouse ear oedema model. The results showed that total phenolics of *Laggera alata* (TPLA) exhibited the strongest potency of the inflammation model employed. Our current investigation verifies for the first time the anti-inflammatory action of *Laggera alata* in experimental models as well as the main chemical components responsible for the anti-inflammatory potential of *Laggera alata*. All the results in this paper show that TPLA possesses potent anti-inflammatory activity against acute and chronic inflammations in mice and rats. Therefore, pharmacologically experimental evidences provide scientific explanation for the folkloric uses of *Laggera alata* in the treatment of some ailments associated with inflammation.

In order to estimate the effect of TPLA on acute inflammation, four experimental models were chosen in our study, which represent the essential features of acute inflammation. Amongst them, acetic acid-induced increased vascular permeability in mouse model is a typical capillary permeability assay (Winter et al., 1962). TPLA significantly reduced the increased peritoneal vascular permeability, indicating the suppression of the vascular response in the process of acute inflammation. Xylene-induced mouse ear oedema and carrageenan-induced rat paw oedema reflect the oedematization during the early stages of acute inflammation (Matsuda and Tanihata, 1992; Vogel and Vogel, 1997). In our pilot test, TPLA was found to exhibit stronger anti-inflammatory effect on xylene-induced ear oedema than other extracts from *Laggera alata* (data not shown). Subsequently, the activity of TPLA against the inflammatory oedema was ascertained in the rat model of carrageenan-induced paw oedema. Moreover, another well-characterized acute inflammation model, carrageenan-induced pleurisy in rat, permits the quantification and correlation of both exudates and cellular migration with changes of other inflammatory parameters (Vinegar et al., 1982). The findings further confirmed the anti-inflammatory activity of TPLA, which included the reduction in the volume of pleural exudates and the content of total protein as well as the inhibition of the leukocyte migration. As a model of chronic inflammation, on the other hand, cotton pellet-induced granuloma in rats was utilized in the present study. The model is thought to be a valuable way to assess the action of anti-inflammatory drugs on the proliferation phase of inflammation (Selye, 1953). The observation showed that TPLA reduced the formation of granuloma tissue in a dose-dependent manner, which represented an ability of TPLA to inhibit the proliferation phase of inflammatory process.

The data obtained from our current study indicated that several factors may contribute to the anti-inflammatory action of TPLA. First, one possible mechanism involves the inhibition of prostaglandin formation at the site of inflammation. The increase in the formation of prostaglandins (PGs) corresponds to the release of arachidonic acid from membrane phospholipids and the up-regulation of cyclooxygenase-2 (Subbaramaiah et al., 1997). Among different PGs, PG_2 plays an important role in plasma exudation of carrageenan-induced rat pleurisy (Harada et al., 1982). The result showed that TPLA significantly decreased the content of PGE_2 in the pleural exudates, thereby suggesting TPLA's interference with the cyclooxygenase pathways of arachidonate metabolism.

Second, the anti-inflammatory mechanism of TPLA is partially associated with its influence on the antioxidant systems. It has been reported that in the process of carrageenan-induced inflammation, the early phase is related to the production of histamine, leukotrienes, platelet-activating factor and possibly cyclooxygenase products, while the delayed phase is linked to neutrophil infiltration and the production of neutrophil-derived free radicals, such as hydrogen peroxide, superoxide and hydroxyl radicals, as well as to the release of other neutrophil-

Table 4
Effect of oral treatment with total phenolics from *Laggera alata* (TPLA) and dexamethasone (DEX) on cotton pellet-induced granuloma in rats

Group	Dose (mg/kg, p.o.)	Animals (*n*)	Granuloma weight (mg/100 g body weight)	Inhibition rate (%)
Vehicle	–	8	10.76 ± 0.76	–
DEX	2.5	8	3.27 ± 0.34***	69.6
	50	8	7.63 ± 0.58***	29.1
TPLA	100	8	5.83 ± 1.06***	45.8
	200	8	4.86 ± 1.04***	54.8

Values are mean ± S.D., $^{***}P < 0.001$ compared to vehicle control group (one-way ANOVA, Tamhane's T2 as the post hoc test).

derived mediators (Oh-ishi et al., 1989; Dawson et al., 1991; Peskar et al., 1991; Cuzzocrea et al., 1997). In addition to oxyradicals, overproduction of NO also plays an important role in various models of inflammation (Moncada et al., 1991; Nanthan, 1996; Cuzzocrea et al., 1998). The reaction of NO with superoxide anion forms peroxynitrite (Beckman et al., 1990), a potent cytotoxic oxidant eliciting lipid peroxidation and cellular damage (Rubbo et al., 1994). As an indicator of lipid peroxidation, the serum level of MDA was measured in the current study. Furthermore, the serum levels of SOD and GSH-PX, both of which are antioxidant enzymes, were also detected for evaluating the ability to scavenge radicals. As a consequence, TPLA reduced NO production, lowered the serum level of MDA and increased the serum levels of SOD and GSH-PX, implying that TPLA could not only inhibit the lipid peroxidation but also scavenge radicals by enhancing the activities of the antioxidant enzymes. Taken together, the anti-oxidative properties of TPLA may contribute to the alleviation of inflammatory response.

Finally, other anti-inflammatory mechanism of TPLA is, at least in part, due to the inhibition of LZM release. It is known that the degranulation occurs after neutrophils reach the injured tissue by margination, adhesion, and emigration. As a result, LZM is discharged from lysosomes of neutrophils. Released LZM destroys not only phagosomes but also the tissue itself, which aggravates the responses to inflammation (Heiman et al., 1989; Ronca et al., 1998). In our study, TPLA markedly attenuated the serum level of LZM. The inhibitory effect of TPLA on LZM release hence protects tissue from damage induced by inflammation.

Phytochemical and HPLC assay of the aqueous extract of *Laggera alata* led to a conclusion that this active extract part contains plenty of phenolic compounds, especially dicaffeoylquinic acids. This is in agreement with our prediction. Phenolic compounds are substances that possess an aromatic ring bearing hydroxyl substituents, including their functional derivatives such as esters, methoxy compounds and glycosides. They exhibit several pharmacological functions such as anti-inflammatory, antioxidate and antiviral, etc. (Kimura et al., 1987; McDougall et al., 1998; Melzig et al., 2001; Gongora et al., 2002, 2003). Our results obtained in this paper clearly indicated that total phenolics of *Laggera alata* have pronounced inhibitory potential on all five tested inflammation models. Furthermore, the anti-inflammatory effects of the prepared pure dicaffeoylquinic acids have been confirmed both *in vitro* and *in vivo*. Our findings with the effect of TPLA agree with the description of the pharmacological actions of phenolic compounds in the aforementioned references. These described results approved the extract of *Laggera alata* which containing mainly dicaffeoylquinic acid type phenolic compounds possess a good efficacy and safety for medicinal utilization.

In conclusion, the total phenolics of *Laggera alata* (TPLA) which containing mainly dicaffeoylquinic acid type phenolic compounds have been identified to possess potent anti-inflammatory activity on acute and chronic inflammation models. The anti-inflammatory mechanisms of TPLA are probably associated with the inhibition of prostaglandin formation, the influence on the antioxidant systems, and the suppression of LZM release. Furthermore, The total phenolic content of *Laggera alata* and its main component type was quantified, and its principle components were isolated and authenticated. These results may help understanding the popular planted phenomenon and the long history of its medicinal application by the local people, and it afforded some basic background of the structure–activity relationships for further detailed investigation. Meanwhile, our study also demonstrated that *Laggera alata* is a good candidate for the development of new anti-inflammatory medicine.

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