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## ***In vivo* Anti-inflammatory and *in vitro* Antioxidant Activities of *Peperomia pellucida***

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**Abstract:** *Peperomia pellucida* is widely used in the traditional medicine such as in the treatment of inflammation, gout, arthritis and wound healing. The present study was performed to evaluate the anti-inflammatory and antioxidant effects of petroleum ether, chloroform and methanol extracts of *P. pellucida*. The carrageenan-induced rat hind paw edema method was performed to evaluate the anti-inflammatory effect *in vivo* and the determination of free radical scavenging activity method was carried out to assess the antioxidant effect *in vitro*. For the anti-inflammatory activity, 1000 mg kg<sup>-1</sup> of petroleum ether extract significantly reduced carrageenan-induced hind paw edema (p<0.05) compared with the control (p<0.01). However, there was no significant anti-inflammatory activity for chloroform and methanol extracts. The antioxidant effect was evaluated by determining the free radical scavenging activity of the three extracts. The total phenol content was determined for the methanol extract (6.93%) that showed the strongest free radical scavenging activity (0.083 mg mL<sup>-1</sup> ± 0.008). Petroleum ether and chloroform showed low free radical scavenging activity compared to methanol extract. The results obtained from this study suggest that this plant is a good natural source for anti-inflammatory and antioxidant therapy.

**Key words:** Piperaceae, traditional medicine, carrageenan, DPPH, total phenolics

### **INTRODUCTION**

The inflammatory response is a physiological characteristic attributed to the defence response by the organism to a wide variety of external and indigenous stimuli (Rang *et al.*, 1999; Sosa *et al.*, 2002). The foremost inflammatory stimuli are the pro-oxidants generated from activated neutrophils and macrophages have been reported to play a pivotal role in the pathogenesis of various inflammation-related diseases, including neurodegenerative disorders, cancer and atherosclerosis (Winrow *et al.*, 1993; Confortia *et al.*, 2007).

*Peperomia pellucida* (L.) HBK is a traditional medicinal plant belongs to the family Piperaceae. Traditionally it has been used for variety of inflammatory ailments. Traditional medicinal practitioners used to prepare infusion and decoction from the leaves and stems of the plant to treat gout, arthritis and to heal old wounds. The plant is also used as topical medicine for the skin related problems such as acne, pimples and abnormal complexion. Pounded whole plant is used as warm poultice for boils, pustules and pimples (De Fatima *et al.*, 2004). In the other parts of Asia such as Bangladesh, the aerial parts including leaves of the plant have been used by local traditional healers to treat the excited mental

disorders (Khan *et al.*, 2008). Mamila Medical Society reported that *P. pellucida* is useful to relieve rheumatoid arthritic joint pains, but it may manifest some CNS depression side effects (Khan *et al.*, 2008). Several scientific studies reported the antibacterial and analgesic activities of *P. pellucida* (Aziba *et al.*, 2001; Khan and Omoloso, 2002) and some other studies reported the isolation of bioactive phytoconstituents with strong antifungal and anticancer properties from *P. pellucida* (Ragasa *et al.*, 1998; Xu *et al.*, 2006).

Owing to safety concerns, the public prefer to use natural treatments from edible materials such as fruits, spices, herbs and vegetables rather than using synthetic agents. Thus, the effective agents of natural origin are preferred to be developed and utilized. In the view of these scientific reports and the traditional usages of *P. pellucida*, the present study was aimed to investigate the anti-inflammatory and the antioxidant activities of *P. pellucida* extracts to validate its ethnomedicinal use of the plant.

### **MATERIALS AND METHODS**

**Materials:** Carrageenan type IV (lambda), indomethacin, sodium chloride (NaCl), Tween20, 1,1-diphenyl-2-

picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT) and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteu (Fc) reagent was purchased from Merck (Darmstadt, Germany). Petroleum ether (40-60), chloroform and methanol were purchased from Fisher Scientific (UK).

**The plant:** The plant was collected with the flowers in the month of March, 2009 and taxonomic authentication of the plant was done in the Department of Botany, School of Biological Sciences, Universiti Sains Malaysia, where its herbarium is deposited. The herbarium voucher number is 11154.

This project was conducted from April 2009 to October 2009 at School of Pharmaceutical Sciences, Universiti Sains Malaysia.

**Preparation of plant extract:** The plant was dried at 45°C in an oven (Memmert, Germany) and then ground into powder. Three hundred and fifty gram of the powdered plant material was placed in a thimble and serially extracted with 5 L of petroleum ether, chloroform and methanol in a soxhlet extractor, each for 3 days. The extract was concentrated and subsequently lyophilized. The yield of petroleum ether, chloroform and methanol extracts obtained were found to be 11.1, 5.92 and 21.26 g, respectively.

**Experimental animals:** Male Sprague Dawley (SD) rats (180-250 g weight) were used for the experiment. The animals were kept under standard conditions (26-30°C) with free access to food (normal laboratory chow, Gold Coin Feed Mills Sdn Bhd, Malaysia) and tap water. The animals were acclimatized to laboratory conditions for 7 days before commencement of experiments and starved for 10 h prior to the experiments. All the experimental procedures on animals were carried out in accordance with the guidelines and regulations of the Animal Ethics Committee, Universiti Sains Malaysia.

**Carrageenan-induced rat hind paw edema:** According to the method described by Winter *et al.* (1962), the anti-inflammatory activity was evaluated. A total of 30 male rats were used in the experiment. They were divided into five groups, each of six rats. Prior to the experiment, carrageenan was freshly prepared as 1% (w/v) suspension in sterile 0.9% NaCl. A volume of 0.1 mL of carrageenan was injected into the plantar tissue of the rat right hind paw. 1000 mg kg<sup>-1</sup> of petroleum ether extract, chloroform extract, methanol extract and 10 mg kg<sup>-1</sup> of

indomethacin were administered orally 1 h before carrageenan injection. The animals in the control group received 4% tween20 (v/v) in distilled water. Measuring the footpad thickness was performed by placing the foot of the unanesthetized animal between the anvil and spindle of peacock dial thickness gauge of a micrometer (Ozaki Ltd., Japan). The readings were taken before injection and at 2, 4 and 6 h after injection of carrageenan. Using the equation No.1, the percentage in thickness changes of the hind paw was calculated:

$$\text{Increase in thickness (\%)} = \frac{C_t - C_0}{C_0} \times 100 \quad (1)$$

where,  $C_t$  is the thickness of hind paw at t hour and  $C_0$  is the thickness of hind paw before carrageenan injection.

**Determination of DPPH scavenging activity:** The free radical scavenging activity of *P. pellucida* extracts and BHT were measured in terms of radical scavenging ability or hydrogen donating by using the stable DPPH (Kumaran and Karunakaran, 2006). Using 96 well plates, 100 µL each of 0.015625, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 2 and 4 mg mL<sup>-1</sup> *P. pellucida* extracts and BHT (in methanol) were added. To this mixture, 200 µL of 0.1 mM DPPH was added. After 30 min incubation at room temperature, absorbance was measured at 517 nm by using the Power Wave, 340 ELISA micro-plate reader (Bio-Tek Instruments, Inc. USA) against methanol as a blank. The control contained 100 µL methanol and 200 µL of 0.1 mM DPPH. According to the equation No.2, free radical scavenging activity was determined:

$$\text{Free radical scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100 \quad (2)$$

where,  $A_s$  is the absorbance of sample.  $A_c$  is the absorbance of control.

**Determination of total phenolic content:** The total phenolic content of the methanol extract was determined according to the Folin-Ciocalteu method with some modification (Slinkard and Singleton, 1977). Twenty microlitres of 4 mg mL<sup>-1</sup> extract (in methanol) was added to 100 µL 2 N Folin-Ciocalteu reagent. After 8 min of incubation at room temperature, 300 µL of 20% (w/v) sodium carbonate was added followed by 1.58 mL distilled water. Absorbance of the mixture, after 2 h of incubation at room temperature, was measured at 760 nm using

spectrometer. A calibration curve using gallic acid in a concentration range of 0.0625-0.5 mg mL<sup>-1</sup> was prepared. The total phenolic content of the sample was expressed as Gallic Acid Equivalents (GAE), which reflected the phenolic content as amount of gallic acid in sample. Experiments were performed in triplicate.

**Statistical analysis:** The results are reported as Mean±SEM (standard error mean). For comparison, One-way Analysis of Variance (ANOVA) followed by two-tailed Dunnett's multiple comparison test was used. The statistical methods were performed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). A p-value of <0.01 (\*\*) and <0.05 (\*) were set as statistical significant.

### RESULTS

**Carrageenan-induced rat hind paw edema:** The percentage changes of right hind paw thickness in the carrageenan-induced edema test by petroleum ether extract, chloroform extract, methanol extract and indomethacin are shown in Fig. 1. After 1 h of injection, carrageenan caused localized edema in the control group and increased progressively to after 6 h of carrageenan injection. Rats were treated with petroleum ether extract, chloroform extract and methanol extract at a dose of 1000 mg kg<sup>-1</sup>. The petroleum ether extract showed a significant inhibition (n = 6; p<0.05) in magnitude of swelling 4 h following carrageenan administration. However, no significant inhibition in carrageenan-induced paw edema was observed with chloroform and methanol extracts throughout the whole experimental period following the injection of carrageenan in rats. Indomethacin (10 mg kg<sup>-1</sup>) caused a significant attenuation in hind paw edema after 4 h (p<0.01) and 6 h (p<0.05) of carrageenan injection (n = 6).

**Determination of DPPH scavenging activity:** The scavenging activity of petroleum ether, chloroform and methanol extracts of *P. pellucida* was investigated in the present study and expressed in percentage of inhibition of DPPH free radicals using BHT as a standard reference agent. Since 50% and above inhibition of DPPH radical is considered to be significant (Omisore *et al.*, 2005). Methanol extract of *P. pellucida* showed free radical scavenging activity with IC<sub>50</sub> = 0.083 mg mL<sup>-1</sup>. BHT showed free radical scavenging activity with IC<sub>50</sub> = 0.027 mg mL<sup>-1</sup>. The results of free radical scavenging activity for all extracts and BHT are shown in Table 1.

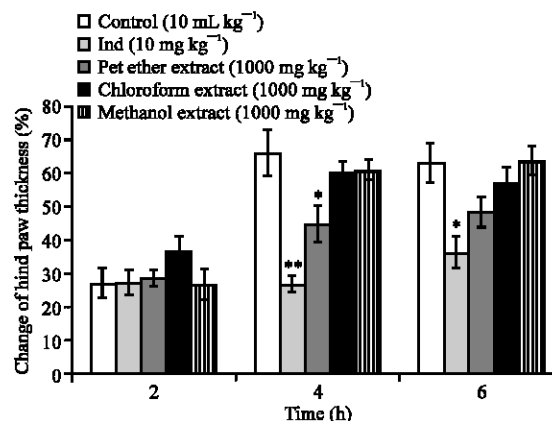


Fig. 1: Effect of the *P. pellucida* extracts and indomethacin on the carrageenan-induced hind paw edema. \*\*p<0.01 and \*p<0.05 from one-way analysis of variance (ANOVA) against control group

Table 1: The DPPH scavenging activity of *Peperomia pellucida* extracts and BHT

Sample	IC <sub>50</sub> ±SD
Petroleum ether extract	0.829000±0.314
Chloroform extract	0.160333±0.019
Methanol extract	0.083000±0.008
BHT	0.027000±0.004

SD = Standard deviation (n = 3)

### DISCUSSION

Carrageenan is a suitable model to study the acute inflammation in rats (Sawadogo *et al.*, 2006; Tandon and Gupta, 2006). The carrageenan-induced paw edema model is very much sensitive in evaluating orally active anti-inflammatory agents, particularly the natural products and it is very reliable for acute anti-inflammatory studies (Antonio and Brito, 1998). The intraplantar injection of carrageenan in rat paw leads to edema formation. The formation of edema is a biphasic event (Di Rosa, 1972; Vinegar *et al.*, 1996). The primary phase (0-2.5 h after injection of carrageenan) is mainly resulted by the release of inflammatory mediators such as histamine, serotonin and kinins from the surrounding damaged tissues. The second phase (3-6 h after injection of carrageenan) is sustained by release of prostaglandins, produced by macrophages and mediated by bradykinin and leukotrienes (Antonio and Brito, 1998). Oral administration of petroleum ether extract (1,000 mg kg<sup>-1</sup>) suppressed the edematous response after 4 h of carrageenan injection. The observed effect was quite similar to that exhibited by the group treated with indomethacin. These results indicate the probability that

petroleum extract of *P. pellucida* acts in the second phase by the production of arachidonic acid metabolites (Di Rosa *et al.*, 1971).

In the present study, the anti-inflammatory and antioxidant properties of *P. pellucida* were investigated. De Fatima *et al.* (2004) reported the anti-inflammatory activity of aqueous extract of *P. pellucida*. In this study, we assessed the anti-inflammatory activity of three different sequential organic extracts, nevertheless, the findings in this study were in agreement with that of De Fatima *et al.* (2004). So, far no antioxidant study was reported on the *P. pellucida*. Thus, we attempted to evaluate the potential antioxidant property of *P. pellucida* extracts. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). Because of the reliability of the DPPH assay, it has been used widely to evaluate the general antioxidant effect of plant extracts as well as pure compounds *in vitro* (Koleva *et al.*, 2002; Gonçalves *et al.*, 2005). The results have shown that methanolic extract of *P. pellucida* has DPPH scavenging effect *in vitro*. We observed that a dose-response relationship is found in DPPH radical scavenging activity for methanolic extract, as the concentration increased the activity increased.

Phenolic compounds are synthesized in plants as secondary metabolites. They exhibit several biological activities such as, antioxidation, anti-inflammation, anti-aging, as well as inhibition of angiogenesis and cell proliferation. Most of these biological activities have been associated with their intrinsic reducing capability towards pro-oxidants (Han *et al.*, 2007). It has been found that total phenolic content is highly associated with antioxidant properties (Chiang *et al.*, 1994; Kim *et al.*, 1999; Plumb *et al.*, 1999). The phenolic content of the methanolic extract is 6.93% (results not shown). Thus, the antioxidant activity of this plant may due to the presence of phenolic compounds in its methanolic extract.

### CONCLUSIONS

Determination of the natural compounds of plant extracts will help to develop new therapeutically agents. The results obtained from the present study show that this plant may be a good natural source for anti-inflammatory and antioxidant agents. Further investigation on the isolation and identification of anti-inflammatory and antioxidant components of this plant may lead to chemical entities for clinical use.

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