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## Antioxidant and Antiinflammatory Properties of *Citrus sinensis* Peel Extract

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**In the present study, the antioxidant and antiinflammatory properties of methanolic extract of *Citrus sinensis* peel was investigated. Qualitative tests confirmed the presence of flavanoids and free phenolic compounds in the extract. The antioxidant property of the extract was tested qualitatively by thin layer chromatography using  $\beta$ -carotene-linoleate oxidation. The antiinflammatory activity of the extract was performed on carrageenan- induced acute pedal paw edema model and Freund's complete adjuvant-induced chronic inflammatory model. In both acute and chronic anti-inflammatory models, the extract exhibited significant antiinflammatory activity at two doses of 150 and 300 mg/kg.**

Recently, there is considerable interest in the food industry, preventive medicine and cosmeticology in the development of natural antioxidants from plant material<sup>1-3</sup>. Natural antioxidants were reported to be present in citrus peel<sup>4</sup>, various vegetables<sup>5</sup>, soyabean<sup>6</sup>, sesame seed<sup>7</sup>, olives<sup>8</sup>, carob pod<sup>9</sup> and green tea leaves<sup>10</sup>.

The antioxidant properties of citrus juices<sup>11</sup> have been reported, but to our knowledge, the antioxidant and antiinflammatory properties of *Citrus sinensis* peel was not investigated. In the present study, *Citrus sinensis* peel was extracted by cold percolation using methanol and the extract was vacuum dried. Qualitative tests were performed on the extract to confirm the presence of flavonoids and free phenolic compounds. The antioxidant property of the extract was determined qualitatively by thin layer chromatography using  $\beta$ -carotene-linoleate oxidation method<sup>12,13</sup>. The antiinflammatory activity of the extract was studied in acute and chronic models of inflammation.

*Citrus sinensis* peel was collected, dried at room temperature under shade, powdered and extracted with methanol by cold percolation method<sup>14</sup>. The vacuum dried extract was used for antioxidant and antiinflammatory studies.  $\beta$ -Carotene, linoleic acid, carrageenan and Freund's complete adjuvant was purchased from Sigma Chemical Co., St. Louis MO, USA. Diclofenac sodium was generously

gifted by Madras Analytical and Research Laboratories, Chennai.

Wistar rats of either sex (150-200 g) were obtained from King Institute, Chennai. They were housed in cages at  $25 \pm 2^\circ$ , relative humidity of 45-55%, maintained under 12 h light and dark cycle and were fed with standard animal feed. All the animals were acclimatized for a week before use. Indigenously fabricated plethysmograph filled with mercury was used to measure the rat paw volume before and after the administration of extract.

Preliminary chemical tests<sup>15</sup> were performed to detect the presence of flavonoids and free phenolic compounds. The qualitative chemical tests performed were, Shinoda test, ammonia fuming test, lead acetate test, ferric chloride test, chalcones test, boric acid test, zirconium oxychloride test, Gibbs test, p-benzoquinone test and o-dinitrobenzene test. All these tests confirmed the presence of flavonoids and free phenolic compounds.

Dried *Citrus sinensis* peel extract<sup>15</sup> was solubilized in methanol and subjected to TLC on 20x20 cm glass plates precoated with silica gel-G. Solvents used for development were chloroform:methanol (9:1) for flavonoids and chloroform:ethyl acetate:formic acid (5:4:1) for free phenolic compounds. The location of the spots was marked under UV light.  $\beta$ -carotene-linoleate (a mixture of  $\beta$ -carotene in 30 ml of chloroform and 2 ml of purified linoleic acid in 60

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ml of 95% ethanol) was sprayed uniformly on the plates and exposed to daylight for about 4 h. The back-ground was bleached and the spots which contained the flavonoids and phenolic compounds retained the yellow colour which is indicative of antioxidant activity.

Acute antiinflammatory activity<sup>16</sup> of the extract was evaluated by carrageenan- induced pedal paw edema method. Wistar rats of either sex (150-200 g) selected by random sampling technique were employed for the study. Each group consisted of six animals. Hind paw edema was induced by injecting 0.1 ml of 1% carrageenan in normal saline on the sub plantar region. *Citrus sinensis* peel extract was administered as a suspension in 1% carboxy methyl cellulose (CMC) orally at 2 dose levels (150 and 300 mg/kg) 1 h prior to carrageenan administration. Paw volume was measured at 15, 30, 60, 120 and 240 min after the administration of carrageenan. One milliliter of 1% CMC served as control and diclofenac sodium (12.5 mg/kg) as standard. The data are presented in Table 1.

Chronic antiinflammatory activity<sup>17</sup> of the extract was evaluated by Freund's complete adjuvant-induced chronic inflammatory method. Wistar rats of either sex (150-200 g)

were selected by random sampling technique for the study. Each group consisted of six animals. Chronic inflammation was induced by subcutaneous injection of 0.1 ml of Freund's complete adjuvant into the right hind plantar region. *Citrus sinensis* peel extract was fed orally (1 w pretreatment before Freund's complete adjuvant administration) at two dose levels (150 and 300 mg/kg) at 12 h intervals for 14 d. Paw volume after 18 h of adjuvant injection was taken as subacute phase of inflammation and paw volume on the day 14 was observed as an index of chronic inflammation. Unpaired student-t test ( $P < 0.05$ ) was performed to ascertain the significance of the pharmacological data. The data is presented in Table 2.

The flavonoids and free phenolic compounds present in *Citrus sinensis* peel extract possess antioxidant property which was evident from the non bleaching of  $\beta$ -carotene-linoleate reagent from thin layer chromatographic studies. *Citrus sinensis* peel extract exhibited significant antiinflammatory activity against acute and chronic antiinflammatory models. The extract exhibited a graded dose response. The relationship between antiinflammatory activity and antioxidant property for flavonoids has been reported<sup>18,19</sup>. Thus, the antiinflammatory property of the

TABLE 1: ACUTE ANTIINFLAMMATORY ACTIVITY OF *CITRUS SINENSIS* PEEL EXTRACT.

Compound	Dose (mg/kg)	% Reduction of edema (Mean $\pm$ SEM)				
		15 min	30 min	60 min	120 min	240 min
Extract	150	-	-	15.15 $\pm$ 0.019	15.66 $\pm$ 0.015*	16.01 $\pm$ 0.020*
	300	-	15.15 $\pm$ 0.015*	22.51 $\pm$ 0.015*	37.78 $\pm$ 0.028*	56.69 $\pm$ 0.019*
Diclofenac	12.5	-	22.85 $\pm$ 0.023*	40.75 $\pm$ 0.015*	65.71 $\pm$ 0.030*	78.23 $\pm$ 0.023*

\* Level of significance  $P < 0.05$  compared to control.

TABLE 2: CHRONIC ANTIINFLAMMATORY ACTIVITY OF *CITRUS SINENSIS* PEEL EXTRACT.

Compound	Dose(mg/kg)	% Reduction of edema(Mean $\pm$ SEM)	
		18 <sup>h</sup> h	14 <sup>h</sup> d
Extract	150	25.65 $\pm$ 0.0281*	14.85 $\pm$ 0.0204*
	300	61.99 $\pm$ 0.0281*	56.62 $\pm$ 0.0304*
Diclofenac	12.5	79.33 $\pm$ 0.0544*	60.15 $\pm$ 0.0304*

\* Level of significance  $P < 0.05$  compared to control.

extract may be due to the antioxidant property of the flavonoids and free phenolic compounds present in the extract.

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## Spectrophotometric Determination of Propranolol Hydrochloride in Pharmaceutical Preparations

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Two simple, sensitive and accurate spectrophotometric methods are described for the determination of propranolol hydrochloride either in pure form or in pharmaceutical preparations. Each method involves nitration of the drug with uranyl nitrate or thorium nitrate in sulphuric acid medium. The yellow coloured nitro derivative has an absorption maximum at 377 nm. The nitro derivative obeys Beer's Law in the concentration range of 2-32 µg/ml and 1-30 µg/ml for uranyl nitrate and thorium nitrate respectively. The optimum reaction conditions and other analytical parameters are evaluated. The influence of substrates commonly employed as excipients with propranolol drug has been studied. Results of analysis of pure drug and its dosage forms by the proposed methods are in good agreement with those of the official method.

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