

# Effect of the Fractions of *Coccinia grandis* on Lipid Peroxidation and Antioxidant Enzymes in Oxonate-induced Hyperuricaemic Mice

Muthuswamy Umamaheswari • Tapan Kumar Chatterjee\*

Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India

Corresponding author: \*tkchatterjee81@yahoo.co.in

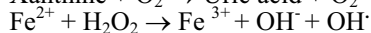
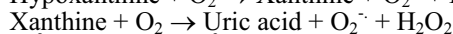
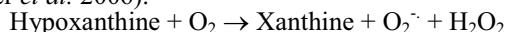
## ABSTRACT

The present study investigated the effect of several fractions of *Coccinia grandis* L. Voigt on lipid peroxidation and antioxidant enzymes in oxonate-induced hyperuricaemic mice. The leaves of *C. grandis*, belonging to the family Cucurbitaceae, are used in Indian traditional medicine for the treatment of gout, rheumatism, jaundice, eye infections, bronchitis, fever, skin eruptions, wounds, etc. The petroleum ether, chloroform, ethyl acetate, and residual fractions of the hydromethanolic extract of the leaves of *C. grandis* at a dose of 200 mg/kg b.w. were given orally to Swiss albino mice for 7 days. On the 7<sup>th</sup> day, potassium oxonate, an uricase inhibitor was injected intraperitoneally (280 mg/kg) to induce hyperuricemia. The end products of lipid peroxidation, viz. malondialdehyde (MDA) and lipid hydroperoxides (LH) and the levels of tissue protein, enzymatic and non-enzymatic antioxidants were estimated in the liver. Allopurinol (10 mg/kg p.o.) was used as the standard. There was a significant ( $P < 0.01$ ) elevation in MDA and LH and a decrease in total protein and antioxidant enzymes in hyperuricaemic mice compared to normal control. Pre-treatment with the pet-ether, chloroform and ethyl acetate fractions of *C. grandis* prevented a rise in MDA and LH significantly ( $P < 0.01$ ) and enhanced the total protein content and antioxidant enzymes. Among the fractions tested, the chloroform fraction exhibited highest activity followed by the pet-ether and ethyl acetate fractions. These results suggest that the use of leaves of *C. grandis* for the treatment of gout and related inflammatory disorders could be attributed to its antioxidant property.

**Keywords:** antioxidant activity, Cucurbitaceae, lipid hydroperoxides, malondialdehyde, rheumatism

## INTRODUCTION

Gout is a common metabolic disorder in human, characterized by an elevated serum uric acid level, resulting in the deposition of urate crystals in the joints and kidneys, causing inflammation as well as gouty arthritis and uric acid nephrolithiasis (Burke *et al.* 2006). The increased risk of hyperuricaemia has been also linked with the development of hypertension, hyperlipidaemia, cancer, diabetes and obesity (Emmerson 1998; Lin *et al.* 2000). The enzyme xanthine oxidase (XO) catalyses the oxidation of hypoxanthine to xanthine and then to uric acid, the final reactions in the metabolism of purine bases (Fukunari *et al.* 2004). The over-activity of this enzyme results in gout (Arromede *et al.* 2002; Liote 2003). Both XO and XDH remove hydrogen from the substrate using oxygen as hydrogen acceptor, and get reduced. During reoxygenation (i.e. reperfusion phase) it reacts with molecular oxygen, thereby releasing superoxide anion radicals, hydrogen peroxide ( $H_2O_2$ ), and further hydroxyl radicals (Borges *et al.* 2002). The XO pathway has been implicated as an important route in the oxidative injury to tissue, especially after ischemia-reperfusion (Pacher *et al.* 2006).



Historically plants have been used in folk medicine to treat various diseases and are rich natural sources of antioxidants. Many researchers have examined the effect of plants used traditionally by indigenous people to treat various diseases. *Coccinia grandis* L. Voigt., belonging to the family Cucurbitaceae is commonly known as “ivy gourd” in English. It is a tropical plant found in many countries of

Asia and Africa. The roots, stems, leaves, and whole plant of *C. grandis* are used in the treatment of eye infections, jaundice, bronchitis, skin eruptions, burns, insect bites, fever, indigestion, nausea, allergy, syphilis, gonorrhoea, etc. (Kirthikar and Basu 1987; Wasantwisut and Viriyapanich 2003). The leaves of this species are widely used in Indian folk medicine for reducing the amount of sugar in urine of patients suffering from diabetes mellitus. Venkateswaran and Pari (2003) reported the use of this plant in the treatment of diabetes. The crude hydromethanol extract of the leaves of *C. grandis* has been reported for its XO inhibitory and hypouricaemic activities (Umamaheswari *et al.* 2007). The various fractions of the hydromethanol extract of the leaves of *C. grandis* possessed significant *in vitro* antioxidant activity (Umamaheswari and Chatterjee 2008a), *in vitro* XO inhibitory activity (Umamaheswari and Chatterjee 2008b) and anti-cataract activity against naphthalene-induced cataractogenesis in rats (Umamaheswari and Chatterjee 2008c). To our knowledge, there are no available reports on the effect of leaves of *C. grandis* against oxonate-induced oxidative stress. Hence, the objective of the present work was to study the antioxidant activity of the various fractions of *C. grandis* hydromethanol leaf extract against oxonate-induced hyperuricaemia in mice.

## MATERIALS AND METHODS

### Plant material

The plant material consisted of dried powdered leaves of *C. grandis*. Leaves of *C. grandis* were collected from approximately 6-month old plants in vegetative season from Coimbatore district, Tamil Nadu, during May, 2006. The plant was identified and au-

thenticated by Dr. G.V.S Murthy, Joint Director, Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore (Ref No BSI/SC/5/23/06-07/Tech 1951).

### Preparation of the extract and fractionation

Fresh leaves of the plant were dried in the shade at room temperature and powdered mechanically and sieved through a No. 20 mesh sieve. About 500 g of the leaf powder was extracted with 2.5 L of methanol: water (7:3) at room temperature for 4 h using a mechanical shaker. The hydromethanol extract (27%w/v) was partitioned separately against petroleum ether, chloroform and ethyl acetate separately in the order of increasing polarity. The fractions were dried at 40°C under vacuum and the percentage yield of the fractions was petroleum ether (2%), chloroform (1.2%), ethyl acetate (1.4%) and residual fractions (18%).

### Experimental animals

Swiss albino mice of either sex weighing between 25-30 g were used for the study. The animals were housed in polypropylene cages inside a well-ventilated room. The room temperature was maintained at  $23 \pm 2^\circ\text{C}$  with a 12 h light/dark cycle. The animals were fed with commercial feed pellets and provided drinking water *ad libitum*. All animal procedures were approved by University animal ethical committee in accordance with animal experimentation and care.

### Drugs and chemicals

Oxonic acid potassium salt was obtained from Sigma-Aldrich, USA. Allopurinol, 2-deoxy-2-ribose, thiobarbituric acid, butylated hydroxytoluene, oxidized glutathione, epinephrine and 5,5'-dithio-bis nitrobenzoic acid (DTNB) were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. 2,2'-dipyridyl and *O*-dianisidine were obtained from HiMedia Laboratories Ltd., Mumbai, India. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

### Animal treatment

Mice were divided into seven groups consisting of six animals each. Group I received 0.5% carboxyl methylcellulose (10 ml/kg b.w., orally) and served as the solvent control. Group II received potassium oxonate (280 mg/kg b.w., i.p) and served as hyperuricemic control. Groups III to VI received the petroleum ether, chloroform, ethyl acetate and residual fractions of *C. grandis*, respectively at a dose of 200 mg/kg orally (Umamaheswari and Chatterjee 2008c). Group VII received allopurinol (10 mg/kg, orally) and served as the positive control. The fractions and the reference drug were suspended in 0.5% carboxyl methylcellulose and administered orally for 7 days. On the 7<sup>th</sup> day potassium oxonate (280 mg/kg b.w.) was injected intraperitoneally one hour before the final drug administration to induce hyperuricemia (Wang *et al.* 2004; Zhu *et al.* 2004).

### Preparation of liver homogenate

After one hour of the final drug administration, animals were sacrificed by cervical dislocation and the liver was dissected out and washed with ice-cold saline to remove as much blood as possible. Liver homogenate (5%, w/v) was prepared in cold 50 mM phosphate buffer (pH 7.4) using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 5000 × g for 10 min and the resulting supernatant was used for the estimation of lipid peroxidation, enzymatic and non-enzymatic antioxidants.

### Estimation of protein content

Protein content of the tissue homogenate was assayed by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

### Estimation of malondialdehyde

Lipid peroxidation as evidenced by the formation of TBARS and LH were measured by the method of Nichans and Samuelson (1986). About 0.1 ml of tissue homogenate was treated with 2 ml of 0.37% thiobarbituric acid (TBA) and 15% trichloroacetic acid (TCA) reagent and placed in a water bath for 15 min, cooled and centrifuged at room temperature for 10 min at 1000 rpm. The absorbance of the clear supernatant was measured against a reference blank at 535 nm. The values are expressed as  $\mu\text{moles}$  of malondialdehyde (MDA) formed /min/mg protein.

### Estimation of lipid hydroperoxides

About 0.1 ml of tissue homogenate was treated with 0.9 ml of Fox reagent (188 mg butylated hydroxytoluene (BHT), 7.6 mg xylenol orange and 9.8 mg ammonium ion sulphate were added to 90 ml of methanol and 10 ml 250 mM sulphuric acid) and incubated for 30 min. The colour developed was read at 560 nm using a colorimeter. The values were expressed as  $\mu\text{mol}$  of LH formed/min/mg tissue protein (Nichans and Samuelson 1986).

### Determination of enzymatic antioxidants

#### Estimation of superoxide dismutase (SOD)

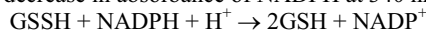
SOD activity was determined by the inhibition of auto catalyzed adrenochrome formation in the presence of liver homogenate at 480 nm. The reaction mixture contained 150  $\mu\text{l}$  of liver homogenate, 1.8 ml of carbonate buffer (30 mM, pH 10.2), and 0.7 ml of distilled water and 400  $\mu\text{l}$  of epinephrine (45 mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate. Activity was expressed as  $\mu\text{moles}/\text{min}/\text{mg}$  protein (Kakkar *et al.* 1984).

#### Estimation of catalase (CAT)

Catalase (CAT) activity was measured by the catalysis of  $\text{H}_2\text{O}_2$  to  $\text{H}_2\text{O}$  in an incubation mixture adjusted to pH 7.0 was recorded at 254 nm. The reaction mixture contained 2.6 ml of 25 mM potassium phosphate buffer pH 7.0 and 0.1ml of tissue homogenate and was incubated at 37°C for 15 min and the reaction was started with the addition of 0.1 ml of 10 mM  $\text{H}_2\text{O}_2$ . The time required for the decrease in absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for the calculation of enzyme activity. One unit of catalase activity was defined as the amount of enzyme causing the decomposition of  $\mu\text{mol}$   $\text{H}_2\text{O}_2/\text{mg}$  protein/min (Sinha 1972).

#### Estimation of glutathione reductase (GSSH)

The enzyme activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm.



The reaction mixture contained 2.1 ml of 0.25 mM potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidized glutathione, 0.1 ml of bovine serum albumin (10 mg/ml). The reaction was started by the addition of 0.02 ml of tissue homogenate with mixing and the decrease in the absorbance at 340 nm was measured for 3 minutes against a blank. Glutathione reductase activity was expressed as  $\mu\text{mol}$  NADPH oxidized /min/mg protein (Racker 1955).

#### Estimation of peroxidase (Px)

Peroxidase (Px) activity was measured spectrophotometrically by the change in absorbance at 460 nm due to *O*-dianisidine oxidation in the presence of  $\text{H}_2\text{O}_2$  and enzyme. Reaction mixture contained 0.2 ml of 15 mM *O*-dianisidine, 0.1ml of tissue homogenate and 2.5 ml of 0.1 M potassium phosphate buffer pH 5.0 and were incubated at 37°C for 15 minutes and the reaction was started with the addition of 0.2 ml of  $\text{H}_2\text{O}_2$  and the absorbance at 460 nm was followed against a blank, spectrophotometrically for about 3-5 min at 37°C. Unit of enzyme activity defined as  $\mu\text{moles}$  of *O*-dianisidine/min/mg protein (Lobarzewski and Ginalska 1995).

## Estimation of glutathione peroxidase (GPx)

Glutathione peroxidase (GPx) activity was measured by the procedure of Paglia and Valentine (1967). The enzyme catalyses the reaction:



The reaction mixture consisted of 0.2 ml of 0.4 M Tris buffer, 0.1 ml of sodium azide, 0.1 ml of  $\text{H}_2\text{O}_2$ , 0.2 ml of glutathione and 0.2 ml of supernatant which is incubated at  $37^\circ\text{C}$  for 10 min. The reaction was arrested by the addition of 10% TCA and the absorbance was measured at 340 nm. Activity was expressed as nmoles/min/mg protein (Paglia and Valentine 1967).

## Determination of non-enzymatic antioxidants

### Estimation of reduced glutathione (GSH)

The method was based on the reaction of reduced glutathione with 5,5'-dithiobisnitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. To the homogenate 0.1 ml of 10% TCA was added and centrifuged. About 0.1 ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of DTNB in 100 ml of 0.1% sodium nitrate) and 3.0 ml of 0.2 M phosphate buffer (pH 8.0) and the absorbance was read at 412 nm. Activity was expressed as  $\mu\text{moles/min/mg protein}$  (Ellman 1959).

### Statistical analysis

Statistical analysis was carried out using GraphPad software (GraphPad InStat) by one-way analysis of variance (ANOVA) followed by Dunnett's test. Results are expressed as mean  $\pm$  SEM from six mice in each group. P values  $< 0.05$  were considered significant.

## RESULTS

There was a significant ( $P < 0.01$ ) increase in the end products of lipid peroxidation namely, MDA and LH and a significant ( $P < 0.01$ ) decrease in the total protein content in the liver of oxonate-treated mice when compared to the normal control. Mice pre-treated with the pet-ether, chloroform and ethyl acetate fractions of *C. grandis* orally for seven days produced a significant ( $P < 0.05$ ) decrease in the levels of MDA and LH and a significant ( $P < 0.01$ ) increase in total protein content when compared to oxonate control. The effect produced by the residual fraction on the above parameters was insignificant ( $P > 0.05$ ) compared to oxonate control. The activity produced by the standard drug allopurinol was found to be highest among the groups tested (Table 1).

The activities of the enzymatic antioxidants like SOD, CAT, GSSH, GPx and Px and the non-enzymatic antioxidant GSH in liver tissue of mice treated with potassium oxonate were significantly ( $P < 0.01$ ) decreased when compared with control mice. Mice pre-treated with the pet-ether, chloroform and ethyl acetate fractions of *C. grandis* produced a significant ( $P < 0.05$ ) increase in all the enzymatic and non enzymatic antioxidant activities compared to oxonate control. The activity exhibited by the chloroform fraction was found to be the highest among the fractions tested and was almost comparable to the allopurinol-treated group ( $P < 0.01$ ). The effect produced by the residual fraction on the above parameters was insignificant ( $P > 0.05$ ) compared to hyperuricaemic control (Table 2).

**Table 1** Effect of fractions of *Coccinia grandis* on tissue protein, MDA and LH in control and experimental animals.

Treatment	Protein (nmoles/min/mg wet tissue)	MDA (nmoles/min/mg protein)	LH (nmoles/min/mg protein)
Normal control (0.5% CMC)	94.98 $\pm$ 3.64 b	0.15 $\pm$ 0.01 b	0.26 $\pm$ 0.01 b
Potassium oxonate (280 mg/kg b.w.)	44.48 $\pm$ 0.83 a	0.45 $\pm$ 0.03 a	0.42 $\pm$ 0.03 a
PEF (200 mg/kg b.w.)	82.95 $\pm$ 1.67 b	0.28 $\pm$ 0.01 c	0.29 $\pm$ 0.01 c
CF (200 mg/kg b.w.)	76.39 $\pm$ 2.20 b	0.25 $\pm$ 0.01 b	0.25 $\pm$ 0.01 b
EAF (200 mg/kg b.w.)	68.07 $\pm$ 3.86 b	0.29 $\pm$ 0.02 c	0.30 $\pm$ 0.02 c
RF (200 mg/kg b.w.)	49.28 $\pm$ 1.27 d	0.38 $\pm$ 0.08 d	0.35 $\pm$ 0.01 d
Allopurinol (10 mg/kg b.w.)	87.14 $\pm$ 2.81 b	0.18 $\pm$ 0.06 b	0.27 $\pm$ 0.06 b

Values are mean  $\pm$  SEM; n = 6 in each group. All drugs were given orally and potassium oxonate injected intraperitoneally.

<sup>a</sup>P < 0.01 compared to normal control; <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.05 and <sup>d</sup>P > 0.05 compared to hyperuricaemic control (One way ANOVA followed by Dunnett's test)

**Table 2** Effect of fractions of *Coccinia grandis* on tissue enzymatic and non-enzymatic antioxidants in control and experimental animals.

Treatment	SOD (nmoles/min/mg protein)	CAT ( $\mu\text{moles/min/mg protein}$ )	GSH (nmoles/min/mg protein)	GPx (nmoles/min/mg protein)	GSSH (nmoles/min/mg protein)	Peroxidase (nmoles/min/mg protein)
Normal control (0.5% CMC)	3.49 $\pm$ 0.22 b	1.58 $\pm$ 0.13 b	2.76 $\pm$ 0.24 b	3.26 $\pm$ 0.07 b	2.4 $\pm$ 0.27 b	1.27 $\pm$ 0.06 b
Potassium oxonate (280 mg/kg b.w.)	2.07 $\pm$ 0.22 a	0.55 $\pm$ 0.04 a	1.41 $\pm$ 0.07 a	1.57 $\pm$ 0.07 a	0.66 $\pm$ 0.1 a	0.75 $\pm$ 0.06 a
PEF (200 mg/kg b.w.)	3.02 $\pm$ 0.34 c	2.85 $\pm$ 0.34 b	2.04 $\pm$ 0.06 b	2.21 $\pm$ 0.16 b	1.98 $\pm$ 0.1 b	0.95 $\pm$ 0.04 c
CF (200 mg/kg b.w.)	3.22 $\pm$ 0.22 b	1.24 $\pm$ 0.05 b	2.1 $\pm$ 0.12 b	2.14 $\pm$ 0.05 b	2.20 $\pm$ 0.12 b	1.02 $\pm$ 0.03 c
EAF (200 mg/kg b.w.)	2.94 $\pm$ 0.12 c	1.14 $\pm$ 0.06 c	1.91 $\pm$ 0.05 c	1.98 $\pm$ 0.06 b	1.02 $\pm$ 0.08 c	1.01 $\pm$ 0.03 c
RF (200 mg/kg b.w.)	2.27 $\pm$ 0.13 d	0.94 $\pm$ 0.01 d	1.57 $\pm$ 0.01 d	1.71 $\pm$ 0.07 d	1.22 $\pm$ 0.08 d	0.95 $\pm$ 0.01 d
Allopurinol (10 mg/kg b.w.)	3.36 $\pm$ 0.15 b	1.35 $\pm$ 0.05 b	2.06 $\pm$ 0.01 b	2.17 $\pm$ 0.03 b	2.19 $\pm$ 0.10 b	1.06 $\pm$ 0.12 c

Values are mean  $\pm$  SEM; n = 6 in each group. All drugs were given orally and potassium oxonate injected intraperitoneally.

<sup>a</sup>P < 0.01 compared to normal control; <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.05 and <sup>d</sup>P > 0.05 compared to hyperuricaemic control (One way ANOVA followed by Dunnett's test)

## DISCUSSION

Lipid peroxidation (LPO) is an autocatalytic process, which is a common cause of cell death. Reactive oxygen species and particularly free radical-induced lipid peroxidation has been implicated in the pathogenesis of various diseases (Bandopadhyay *et al.* 1999). The determination of MDA and LH, the end products of lipid peroxidation is one of the most commonly used methods for monitoring LPO. Our result suggests that there was a dramatic increase in lipid peroxidation after oxonate treatment which was significantly inhibited by the treatment with the pet ether, chloroform and ethyl acetate fractions of the plant extract, which proves its potent activity towards inhibition of lipid peroxidation.

XO is an important source of oxygen-derived free radicals. The enzyme catalyses the reduction of oxygen (during reperfusion phase), leading to the formation of superoxide anion,  $H_2O_2$ , as well as hydroxyl radicals. It has been proposed as a central mechanism of oxidative injury in some situations (Berry and Hare 2004). Thus, the determination of the *in vivo* antioxidant enzymes was carried out.

The first enzyme involved in the antioxidant defence is SOD. It is a metalloprotein found in both prokaryotic and eukaryotic cells. The oxygen radicals, generated by the interaction of  $Fe^{2+}$  and  $H_2O_2$ , are the species responsible for the oxidation of epinephrine and are strongly inhibited by SOD (Misra and Fridovich 1972). GPx has a major role in degrading the levels of  $H_2O_2$  in cells. Since GPx acts on hydroperoxides of unsaturated fatty acids, the enzyme plays an important role in protecting membrane lipids, and thus the cell membranes from oxidative disintegration (Rotruck *et al.* 1973). CAT plays a major role in cellular antioxidant defense system by decomposing  $H_2O_2$ , thereby preventing the generation of hydroxyl radical through the Fenton reaction (Halliwell and Gutteridge 1989). In hyperuricaemic mice, significant ( $P < 0.01$ ) diminution was observed in liver CAT activity. Therefore it is likely that CAT inhibition and resulting elevation of extracellular  $H_2O_2$  level by increased blood uric acid causes oxidative stress. The fractions of *C. grandis* significantly increased ( $P < 0.05$ ) the CAT level in hyperuricaemic mice liver which may be a compensatory mechanism for the decline of blood uric acid level as well as  $H_2O_2$ . The levels GPx, GSSH, SOD, and Px in oxonate treated groups were significantly decreased when compared to normal control. The chloroform fraction of *C. grandis* significantly ( $P < 0.01$ ) increased the level of antioxidant enzymes such as GPx, GSSH, Px and SOD. GSH is widely distributed in cells. GSH is an intracellular reductant and plays a major role in catalysis, metabolism and transport. It protects the cells against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects. The pet ether, chloroform and ethyl acetate fractions of *C. grandis* significantly ( $P < 0.01$ ) increased the level of non enzymatic antioxidant GSH when compared to oxonate treated mice.

To conclude, the study suggested that the fractions of *C. grandis* possessed XO inhibitory and antioxidant activities, which might be helpful in preventing or slowing the progress of gout-related diseases. The results obtained from the present study indicate that the leaves of *C. grandis* chloroform fraction exhibited higher activities compared to the other fractions. Further investigations on the isolation and identification of active compounds present in the leaves are in progress to identify a potential chemical entity for clinical use in the prevention and treatment of gout and related inflammatory disorders.

## REFERENCES

Arromede E, Michet CJ, Crowson CS, O'Fallon WM, Gabriel SE (2002) Epidemiology of gout: Is the incidence rising? *Journal of Rheumatology* **29**, 2403-2406

Bandopadhyay U, Das D, Banerjee RK (1999) Reactive oxygen species oxidative damage and pathogenesis. *Current Science* **77**, 658-666

Berry CE, Hare JM (2004) Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications. *Journal of Physiology* **555**, 589-606

Borges F, Fernandes E, Roleira F (2002) Progress towards the discovery of xanthine oxidase inhibitors. *Current Medicinal Chemistry* **9**, 195-217

Burke A, Smyth E, FitzGerald GA (2006) Analgesic-antipyretic agents; Pharmacotherapy of gout. In: Brunton LL, Lazo JS, Parker KL (Eds) *Goodman & Gilman's the Pharmacological Basis of Therapeutics* (11<sup>th</sup> Edn), McGraw-Hill Medical Publishing Division, New York, pp 706-710

Ellman GL (1959) Tissue sulphhydryl groups. *Archives of Biochemistry and Biophysics* **82**, 70-77

Emmerson BT (1998) Hyperlipidaemia in hyperuricaemia and gout. *Annals of Rheumatic Diseases* **57**, 509-510

Fukunari A, Okamoto K, Nishino T, Eger BT, Pai EF, Kamezawa M, Yamada I, Kato N (2004) Y-700 [1-[3-cyano-4-(2,2-dimethylpropoxy)phenyl]-1H pyrazole-4-carboxylic acid]: a potent xanthine oxidoreductase inhibitor with hepatic excretion. *Journal of Pharmacology and Experimental Therapeutics* **311**, 519-528

Halliwell B, Gutteridge JMC (1989) *Free Radicals in Biology and Medicine* (2<sup>nd</sup> Edn), Oxford University Press, Oxford, pp 134-140

Kakkar P, Das B, Viswanathan PN (1984) A modified spectrophotometric assay of SOD. *Indian Journal of Biochemistry and Biophysics* **2**, 130-132

Krithikar KR, Basu RD (1981) *Indian Medicinal Plants* (2<sup>nd</sup> Edn), International Book Distributors, Dehradun 781 pp

Lin KC, Lin HY, Chou P (2000) The interaction between uric acid level and other risk factors on the development of gout among asymptomatic hyperuricaemic men in a prospective study. *Journal of Rheumatology* **27**, 1501-1505

Liote F (2003) Hyperuricaemia and gout. *Current Rheumatology Reports* **5**, 227-234

Lobarzewski J, Ginalska G (1995) Industrial use of soluble or immobilized plant peroxidases. *Plant Peroxidase Newsletter* **6**, 3-7

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. *The Journal of Biological Chemistry* **193**, 265-275

Misra HP, Fridovich I (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry* **247**, 3170-3175

Nichans WG, Samuelson B (1986) Formation of MDA from phospholipids arachidonate during microsomal lipid peroxidation. *European Journal of Biochemistry* **6**, 126-130

Packer P, Nivorozhkin A, Szabo C (2006) Therapeutic effects of xanthine oxidase inhibitors: Renaissance half a century after the discovery of allopurinol. *Pharmacological Reviews* **58**, 87-114

Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidases. *Journal of Laboratory and Clinical Medicine* **70**, 158-159

Racker E (1955) Glutathione reductase from bakers' yeast and beef liver. *The Journal of Biological Chemistry* **217**, 855-866

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973) Selenium: Biochemical role as a component of glutathione peroxidase. *Science* **179**, 588-590

Sinha AK (1972) Colorimetric assay of catalase. *Analytical Biochemistry* **47**, 389-394

Umamaheswari M, Asokkumar K, Somasundaram A, Sivashanmugam T, Subhadradevi V, Ravi TK (2007) Xanthine oxidase inhibitory activity of some Indian medicinal plants. *Journal of Ethnopharmacology* **109**, 547-551

Umamaheswari M, Chatterjee TK (2008a) *In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. *African Journal of Traditional Complementary and Alternative Medicine* **5**, 61-73

Umamaheswari M, Chatterjee TK (2008b) Hypouricemic and xanthine oxidase inhibitory activities of the fractions of *Coccinia grandis* L. Voigt. *Oriental Pharmacy and Experimental Medicine* **7**, 477-484

Umamaheswari M, Chatterjee TK (2008c) Effect of the fractions of *Coccinia grandis* on naphthalene-induced cataractogenesis in rats. *International Journal of Biomedical and Pharmaceutical Sciences* **2**, 70-74

Venkateswaran S, Pari L (2003) Effect of *Coccinia indica* leaves on antioxidant status in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology* **84**, 163-168

Wang Y, Zhu JX, Kong LD, Yang C, Cheng CH, Zhang X (2004) Administration of procyanidins from grape seeds reduces serum uric acid levels and decreases hepatic xanthine dehydrogenase/oxidase activities in oxonate-treated mice. *Basic and Clinical Pharmacology and Toxicology* **94**, 232-237

Wasantwisut E, Viriyapanich T (2003) Ivy gourd (*Coccinia grandis* Voigt., *Coccinia cordifolia*, *Coccinia indica*) in human nutrition and traditional applications. In: Simopoulos AP, Gopalan C (Eds) *Plants in Human Health and Nutrition Policy: World Reviews of Nutrition and Diets*, Karger, Basel, pp 60-66

Zhu JX, Wang Y, Kong LD, Yang C, Zhang X (2004) Effects of *Biota orientalis* extract and its flavonoid constituents, quercetin and rutin on serum uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. *Journal of Ethnopharmacology* **93**, 133-140