OPEM
www.opem.org

Oriental Pharmacy and Experimental Medicine 2008 **7(5)**, 477-484
DOI 10.3742/OPEM.2008.7.5.477

KHUP

Hypouricemic and xanthine oxidase inhibitory activities of the fractions of *Coccinia grandis* L. Voigt

M Umamaheswari and TK Chatterjee*

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

SUMMARY

The present study was aimed at investigating the hypouricemic and xanthine oxidase inhibitory activities of the various fractions of the hydromethanolic extract of the leaves of *Coccinia grandis* L. Voigt (Cucurbitaceae). The leaves of this species was used in traditional medicinal system for the treatment of gout, rheumatism, jaundice, bronchitis, fever, skin eruptions, wounds, etc. The degree of xanthine oxidase inhibition was determined *in vitro* by measuring the increase in absorbance at 295 nm associated with uric acid formation. Among the fractions tested, the chloroform fraction exhibited highest potency (IC_{50} 17.8 μg/ml). This was followed by the pet-ether (IC_{50} 29.7 μg/ml), ethyl acetate (IC_{50} 41.2 μg/ml) and residual (IC_{50} 47 μg/ml) fractions. The IC_{50} value of allopurinol was 6.1 μg/ml. In addition, the hypouricemic and hepatic xanthine oxidase (XO)/xanthine dehydrogenase (XDH) inhibitory activities of the fractions were examined *in vivo* using oxonate (280 mg/kg, i.p.) induced hyperuricemic mice. At a dose of 200 mg/kg orally for 7 days, the pet-ether, chloroform and ethyl acetate fractions produced a significant ($P < 0.01$) reduction in serum urate level and also inhibited hepatic XO/XDH activities when compared to hyperuricemic mice. These inhibitory effects were weaker than that observed for the standard drug, allopurinol (10 mg/kg, *p.o.*). Lineweaver-Burk analysis of the enzyme kinetics indicated that the mode of inhibition was of a mixed type. These results suggest that the use of *Coccinia grandis* leaves for the treatment of gout could be attributed to its XO inhibitory activity.

Key words: *Coccinia grandis*; Hyperuricemia; Xanthine oxidase; Xanthine dehydrogenase; Uric acid

INTRODUCTION

Xanthine oxidase (XO) is a highly versatile enzyme that is widely distributed among species (from bacteria to man) and among the various tissues of mammals. It is a member of the group of enzymes known as molybdenum-iron-sulfur-flavin hydroxylases. It 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 a condition, generally called as gout (Arromede *et al.*, 2002; Liote, 2003). Gout is one of the most common metabolic disorders affecting humans. It is characterized by marked hyperuricemia, leading to the deposition of monosodium urate crystals in the joints and kidney, resulting in gouty arthritis and uric acid nephrolithiasis (Kramer and Curhan, 2002). The deposition of needle shaped monosodium urate crystals in the synovial fluid of the major joints produces an extremely painful acute arthritis with repeated attacks of gout. The increased risk of hyperuricemia has been also linked with the development of hypertension, hyperlipidaemia,

*Correspondence: Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India. E-mail: tkchatterjee_81@rediffmail.com

cancer, diabetes and obesity (Emmerson, 1998; Lin *et al*., 2000). The treatment for gout is either increasing the excretion of uric acid or reducing the uric acid production (Emmerson, 1996). XO inhibitors are much useful, since they possess lesser side effects compared to uricosuric and anti-inflammatory agents. Allopurinol is the only clinically used XO inhibitor, which also suffers from many side effects such as hypersensitivity syndrome, Stevens–Johnson syndrome and renal toxicity (Burke *et al*., 2006). Thus, there is a need to develop compounds with XO inhibitory activity, which is devoid of the undesirable side effects of allopurinol. A potential source of such compounds can be obtained from medicinal plants (Theoduloz *et al.,* 1991; Gonzalez *et al.,* 1995; Kong *et al.,* 2000). Many Indian medicinal plants have been used for the prevention and treatment of gout and related inflammatory disorders (Kirthikar and Basu, 1987), but they lack sufficient scientific evidence.

Coccinia grandis L. Voigt commonly known as 'Ivy gourd' is a tropical plant belonging to the family Cucurbitaceae. It has been found in many countries in Asia and Africa. The leaves of this species has been used in the traditional medicine for the treatment of gout, rheumatism, jaundice, bronchitis, fever, skin eruptions, wounds, etc. (Wasantwisut and Viriyapanich, 2003). Literature suggests the use of the leaves and roots of this plant in the treatment of diabetes mellitus (Venkateswaran and Pari, 2003). Our published results showed that the various fractions of the hydromethanolic extract of the leaves of *Coccinia grandis* possessed significant *in vitro* antioxidant activity (Umamaheswari and Chatterjee, 2008). The putative therapeutic effect of many traditional medicinal plants has been ascribed to the presence of phytochemical constituents with antioxidant activity. Flavonoids, triterpenoids and polyphenolic crude extracts have been reported to possess XO inhibitory activity (Iio *et al.,* 1985; Chang *et al.,* 1993). Preliminary phytochemical screening of the hydromethanolic extract of the leaves of *Coccinia grandis* in our laboratory revealed the presence of many phytochemical constituents such as triterpenoids, flavonoids, saponins, tannins and phenolics. The objective of the present study is to screen the various fractions of the hydromethanolic extract of the leaves of *Coccinia grandis* for its *in vitro* XO inhibitory activity and *in vivo* hypouricemic and XO/xanthine dehydrogenase (XDH) inhibitory activities using oxonate-induced hyperuricemic mice.

MATERIALS AND METHODS

Plant material

The plant material consists of dried powdered leaves of *Coccinia grandis* L. Voigt. belonging to the family Cucurbitaceae. The leaves were collected in and around Coimbatore district, Tamil Nadu, India during the month of August 2006 and were authenticated 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

The air-dried powdered leaves of *Coccinia grandis* (2 kg) was extracted with methanol-water (7 : 3) mixture using a mechanical shaker for 4 h. The resultant extract was concentrated under reduced pressure to yield a residue. The hydromethanolic extract was then extracted successively with equal volumes of petroleum-ether, chloroform and ethyl acetate. Each fraction was then concentrated under reduced pressure to obtain the pet-ether fraction (PEF) chloroform fraction (CF), ethyl acetate fraction (EAF) and residual fraction (RF).

Drugs and chemicals

XO (source: microorganisms), xanthine and allopurinol were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Oxonic acid potassium salt was obtained from Sigma-Aldrich, USA. Uric acid level was determined using commercial kits

obtained from Agappe Diagnostics Pvt. Ltd., Kerala, India. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

***In vitro* XO inhibitory activity**

XO inhibitory activity was assayed spectrophotometrically under aerobic conditions using xanthine as the substrate (Owen and Johns, 1999). The assay mixture consisted of 1 ml of the fraction (5 - 100 μg/ml), 2.9 ml of phosphate buffer (pH 7.5) and 0.1 ml of XO enzyme solution (0.01 units/ml in phosphate buffer, pH 7.5), which was prepared immediately before use. After preincubation at 25°C for 15 min, the reaction was initiated by the addition of 2 ml of the substrate solution (150 μM xanthine in the same buffer). The assay mixture was incubated at 25°C for 30 min. The reaction was then stopped by the addition of 1 ml of 1N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer. Different concentrations of the fractions (5 - 100 μg/ml) were dissolved in dimethyl sulfoxide (DMSO) and the final concentration of DMSO was 5%, which did not affect the enzyme assay. Allopurinol (5 – 100 μg/ml), a known inhibitor of XO, was used as the positive control. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid/min at 25°C. XO inhibitory activity was expressed as the percentage inhibition of XO in the above assay system calculated as,

$$\% \text{ Inhibition} = \{(A - B) - (C - D)/(A - B)\} \times 100$$

where, A is the activity of the enzyme without the fraction, B is the control of A without the fraction and enzyme, C and D are the activities of the fraction with and without XO respectively. The assay was done in triplicate and IC_{50} values were calculated from the percentage inhibition.

Enzyme inhibition mechanism

To determine the mode of inhibition by the most active chloroform fraction, Lineweaver- Burk plot analysis was performed. The enzyme kinetics was carried out in the absence and presence of the choroform fraction with varying concentrations (10 - 150 mM) of xanthine as the substrate using the XO assay methodology. The mode of inhibition was compared with the positive control allopurinol. The Lineweaver-Burk transformed values were plotted to determine the mode of inhibition.

***In vivo* hypouricemic activity**

Experimental model of hyperuricemia was induced by the uricase inhibitor potassium oxonate (Stavric *et al.*, 1975). Mice were divided into seven groups consisting of six each. Group I received 0.5% carboxy methylcellulose (CMC) (10 ml/kg b.w., orally) and served as normal control. Group II received potassium oxonate (280 mg/kg b.w., i.p.) and served as hyperuricemic control. Groups III to VI received the PEF, CF, EAF and RF of *Coccinia grandis* respectively at a dose of 200 mg/kg orally. Group VII received allopurinol (10 mg/kg, b.w., orally) and served as the positive control. The extracts and the reference drug were suspended in 0.5% CMC and administered orally for 7 days. On the 7th day, animals in the groups II to VII were injected intraperitoneally with potassium oxonate at a dose of 280 mg/kg 1 h before the final drug administration to increase the serum urate level (Wang *et al.*, 2004). After one hour of the final drug administration, blood was collected by retro-orbital puncture under mild ether anesthesia. The blood was allowed to clot for approximately 1 h at room temperature and then centrifuged at 5000 g for 10 min to obtain the serum. The serum uric acid level was determined using standard kits.

Assay of XDH/XO activities in mouse liver

On the 7th day after blood collection, animals were killed by cervical dislocation and liver was excised, washed in cold 0.15 M KCl and homogenized (10% w/v) using 50 mM phosphate buffer, pH 7.5 (containing 0.5 mM EDTA). The homogenate was then centrifuged at 5,000 g for 10 min and the lipid

layer was carefully removed and the resulting supernatant was further centrifuged at 5,000 g for 10 min. The supernatant was used for the assay of XO and XDH enzyme activities (Hall *et al.,* 1990; Kong *et al.,* 2004). The reaction mixture contained 2 ml of 50 mM phosphate buffer, (pH 7.5), 0.5 ml of tissue homogenate and 1 ml of 1 mM potassium oxonate. For the assay of XDH activity, but not of XO activity, 200 μM NAD^+ was added as an electron acceptor to the above mixture. After preincubation for 15 min at 37°C, the reaction was initiated by the addition of 1 ml of 150 μM xanthine (dissolved in phosphate buffer, pH 7.5). After 10 min, the reaction was stopped by adding 0.5 ml of 0.58 M HCl and the solution was centrifuged at 5,000 g for 5 min. The absorbance of the supernatant was measured at 290 nm against the blank (50 mM phosphate buffer, pH 7.5, containing 0.5 mM EDTA). XO/XDH activities were expressed as nanomole uric acid formed/min/mg protein. Each assay was performed in triplicate.

Protein determination

Protein concentration in the tissue homogenate was determined by the Lowry method using bovine serum albumin as the standard (Lowry *et al*., 1951).

Statistical analysis

All data were expressed as mean ± standard error of mean (S.E.M.) and statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ were considered significant.

RESULTS

In vitro* XO inhibitory activity of the fractions of *Coccinia grandis

All the fractions of *Coccinia grandis* elicited a dose-dependent inhibition of XO enzyme activity. Inhibition of XO resulted in a decreased production of uric acid, which was measured spectrophotometrically. At a concentration of 100 μg/ml, highest activity was observed in the chloroform fraction (88.1 ± 0.2% inhibition). The percentage inhibition of the pet-ether fraction was 85.7 ± 0.8%, which was almost similar to the chloroform fraction. This was followed by the ethyl acetate (76.55 ± 0.75%) and residual (66.35 ± 1.05 %) fractions. The concentration of the fractions at 50% of the XO inhibition (IC_{50}) was calculated. These results were compared with the standard drug allopurinol, which showed 92.25 ± 0.05% inhibition at 100 μg/ml concentration with IC_{50} value 6.1 μg/ml (Table 1).

Enzyme inhibition mechanism

The inhibition mechanism of the chloroform fraction, which is a potent inhibitor of XO, was studied by kinetic analysis using double-reciprocal plotting. Lineweaver- Burk plots of reactions in the presence and absence of the chloroform fraction in a XO reaction mixture is shown in Fig. 1. The mode of inhibition was investigated and compared with the standard drug, allopurinol. The data indicates that the mode of XO inhibition is of mixed type

Table 1. *In vitro* xanthine oxidase inhibitory activity of the fractions of *Coccinia grandis*

Fraction	Yield of fraction (% w/w)	Percentage xanthine oxidase inhibition					IC_{50} (μg/ml)
		5 μg/ml	10 μg/ml	25 μg/ml	50 μg/ml	100 μg/ml	
Pet-ether	1.6	27.35 ± 0.94	37.35±0.85	46.6±0.5	64.72±0.57	85.7±0.8	29.75±0.25
Chloroform	1.8	31.35±0.65	44.29±0.99	57.34±0.94	71.95±0.85	88.1±0.2	17.87±2.37
Ethyl acetate	0.8	10.8±0.5	25.4±0.9	32.2±1.7	59.38±0.98	76.55±0.75	41.25±0.25
Residual	5.3	6.1±1.3	29.85±1.55	36.1±0.9	51.85±1.85	66.35±1.05	47.0±3.0
Allopurinol	-	44.91±1.88	66.91±0.88	75.25±1.45	85.46±0.83	92.25±0.05	6.1±0.3

Values are mean ± S.E.M. of three parallel measurements.

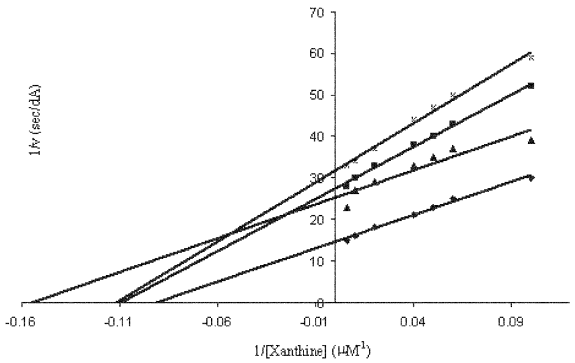

Fig. 1. Lineweaver-Burk plot of inhibition of xanthine oxidase by chloroform fraction of *Coccinia grandis* (▲), in the presence of 50 μM allopurinol (■) and in the presence of 100 μM allopurinol (×). The symbol (◆) represents negative control.

(between uncompetitive and non-competitive type of inhibition) similar to allopurinol. Therefore, both the fraction and allopurinol inhibited XO by binding either with the free enzyme or the enzyme-substrate complex.

Effect of the fractions of *Coccinia grandis* on serum urate level in hyperuricemic mice

Administration of potassium oxonate resulted in hyperuricemia as evidenced by a significant (P < 0.01) increase in the serum uric acid level (7.39 ± 0.42 mg/dl) when compared to the control group (3.72 ± 0.17 mg/dl). Pre-treatment with the pet-ether, chloroform and ethyl acetate fractions of *Coccinia grandis* for seven days reduced the serum urate levels significantly (P < 0.01) when compared with the hyperuricemic control. The reduction in urate level produced by the chloroform fraction (5.08 ± 0.20 mg/dl) was more potent among the fractions tested. Administration of the residual fraction did not produce any significant (P > 0.05) reduction in serum urate level. Allopurinol, at a dose of 10 mg/kg elicited a significant (P < 0.01) reduction in serum urate level (3.05 ± 0.13 mg/dl) compared to hyperuricemic control (Table 2).

Effect of the fractions of *Coccinia grandis* on serum urate level in normal mice

Animals were treated orally with the fractions at a dose of 200 mg/kg orally for 7 days to examine its effect on serum urate level in potassium oxonate untreated mice. Administration of the fractions to normal mice did not decrease the serum uric acid level and is almost similar to vehicle treated group. However, allopurinol significantly (P < 0.01) lowered the serum uric acid level in normal animals (Table 3). These results indicate that the fractions do not possess hypouricemic activity in normal mice.

Effect of the fractions of *Coccinia grandis* on XO/ XDH activities in mouse liver

Animals treated with the pet-ether, chloroform and ethyl acetate fractions at a dose of 200 mg/kg b.w. for seven days produced a significant (P < 0.01)

Table 2. Effect of the fractions of *Coccinia grandis* on serum urate level in oxonate-induced hyperuricemic mice

Treatment	Dose (mg/kg)	Serum urate level (mg/dl)	% Inhibition
0.5% CMC	10 ml/kg	3.72 ± 0.17	-
Potassium oxonate	280	7.39 ± 0.42[a]	-
Pet-ether fraction	200	5.55 ± 0.32[b]	24.84
Chloroform fraction	200	5.08 ± 0.20[b]	31.25
Ethyl acetate fraction	200	5.85 ± 0.21[b]	20.83
Residual fraction	200	6.97 ± 0.27[c]	5.68
Allopurinol	10	3.05 ± 0.13[b]	58.64

All drugs were given orally and potassium oxonate injected intraperitoneally.
% Inhibition = 100 [(hyperuricemic control - experiment)/hyperuricemic control].
Values are mean ± S.E.M. N = 6 in each group. [a]P < 0.01 vs normal control; [b]P < 0.01, [c]P > 0.05 vs hyperuricemic control.

Table 3. Effect of the fractions of *Coccinia grandis* on serum urate level in normal mice

Treatment	Dose (mg/kg)	Serum urate level (mg/dl)
0.5% CMC	10 ml/kg	3.72 ± 0.17
Pet-ether fraction	200	3.21 ± 0.14[a]
Chloroform fraction	200	3.17 ± 0.11[a]
Ethyl acetate fraction	200	3.95 ± 0.35[a]
Residual fraction	200	3.76 ± 0.16[a]
Allopurinol	10	2.82 ± 0.12[b]

Values are mean ± S.E.M. N = 6 in each group. [a]P > 0.01 and [b]P < 0.01 vs normal control.

inhibition towards XO (39.9%, 42.0% and 18.34%) and XDH (38.4%, 40.6% and 22.18%) respectively, when compared with the hyperuricemic control. The inhibition of the XO/XDH activities of the chloroform fraction was found to be the highest among the fractions tested. The inhibition of enzyme activities of the ethyl acetate fraction resulted similar to that of the pet-ether fraction. On the other hand, the action of the residual fraction on the inhibition of XO (13.6%) and XDH (9.37%) was non significant (P > 0.05) at the same dose. Allopurinol inhibited both XO (63.9%) and XDH (65.93%) activities at a dose of 10 mg/kg, exhibiting more potent activity than the fractions (Table 3).

DISCUSSION

The enzyme XO catalyses the oxidation of hypoxanthine to xanthine and then to uric acid, which plays a crucial role in gout (Bowman and Rand, 1980). XO is an important source of oxygen-derived free radicals, which catalyzes the reduction of oxygen (during reperfusion phase), leading to the formation of superoxide anion radicals and hydrogen peroxide, as well as hydroxyl radicals (Halliwell and Gutteridge, 2001). It has been proposed as a central mechanism of oxidative injury in some situations like gout, ischemia, renal damage, hypertension, diabetes, etc. (Mazzali *et al.,* 2001; Berry and Hare, 2004; Nakagawa *et al.,* 2006). Gout is characterized by high level of uric acid in the blood, which can result from a number of genetic disorders that cause either over production or impaired excretion of uric acid. An acute attack of gout occurs as a result of inflammatory reactions to monosodium urate crystals that are deposited in the joint tissue (Rang *et al.,* 2001). Recent findings show that the occurrence of gout is increasing worldwide, possibly due to the changes in dietary habits like intake of high-purine foods viz., organ meats, yeast, beer and other alcoholic beverages (Lewis and Doisy, 1918). The main therapeutic approach for gout is the use of XO inhibitors such as allopurinol, which block the final step in the

Table 4. Effect of the fractions of *Coccinia grandis* on liver XO/XDH activities in oxonate-induced hyperuricemic mice

Treatment	Dose (mg/kg)	nanomole uric acid formed/ min / mg protein		% Inhibition	
		XO	XDH	XO	XDH
0.5% CMC	10 ml/kg	1.33 ± 0.07	1.51 ± 0.04	-	-
Potassium oxonate	280	3.38 ± 0.11[a]	3.20 ± 0.12[a]	-	-
Pet-ether fraction	200	2.03 ± 0.15[b]	1.97 ± 0.12[b]	39.9	38.4
Chloroform fraction	200	1.96 ± 0.09[b]	1.91 ± 0.10[b]	42.0	40.6
Ethyl acetate fraction	200	2.76 ± 0.05[b]	2.49 ± 0.13[b]	18.3	22.1
Residual fraction	200	2.92 ± 0.13[c]	2.92 ± 0.18[c]	13.6	9.37
Allopurinol	10	1.22 ± 0.08[b]	1.08 ± 0.03[b]	63.9	65.9

All drugs were given orally and potassium oxonate injected intraperitoneally. Values are mean ± S.E.M. N = 6 in each group. [a]P < 0.01 vs normal control; [b]P < 0.01, [c]P > 0.05 vs hyperuricemic control.

synthesis of uric acid from purines. However, it has been observed that allopurinol induce side effects such as skin allergy, Steven- Johnson's syndrome and kidney damage (Kumar *et al.*, 1996; Wallach, 1998).

An alternative to allopurinol is the use of medicinal plants, which possess phytochemical constituents. We thus began our program to look for XO inhibitors of phytochemical origin from the various fractions of the hydromethanolic extract of the leaves of *Coccinia grandis*. The leaves of *Coccinia grandis* (Cucurbitaceae) are being frequently used in Indian traditional medicinal system for the treatment of gout and rheumatism.

All the fractions of *Coccinia grandis* inhibited XO in a concentration-dependent manner. The *in vitro* inhibition of XO by the chloroform fraction (IC_{50} 17.87 μg) is moderate when compared to allopurinol (IC_{50} 6.1 μg). However, at higher doses of the fraction, XO would be significantly inhibited. The *in vivo* experiments demonstrated that the fractions possess significant hypouricemic activity in oxonate-induced hyperuricemic mice. Potassium oxonate, an uricase inhibitor partially blocks the conversion of uric acid to allantoin and hence elevates the serum uric acid level in experimental animals. The pet-ether, chloroform and ethyl acetate fractions at a dose of 200 mg/kg restored the serum uric acid level of the hyperuricemic animals similar to the control group. The hypouricemic activity of the fractions could be explained, at least in part, by a lowering of XO/ xanthine dehydrogenase activities in the drug-treated animals. It is interesting to note that the fractions only lower the serum urate levels of the hyperuricemic mice, but not of the normal mice. In contrast, allopurinol exerts its hypouricemic activity in both normal and hyperuricemic mice. This characteristic feature of the fractions could be considered as an advantage because excessive lowering of uric acid level in the circulation beyond that of the normal range might be counter-productive.

In addition, we have previously reported the *in vitro* antioxidant activity of the fractions of the leaves of *Coccinia grandis* and the presence of high phenolic and flavonoid content in the fractions has contributed directly to the antioxidant activity by neutralising the free radicals (Umamaheswari and Chatterjee, 2008). This antioxidant property is actually an added advantage for this species towards XO inhibition. Flavonoids are a group of polyphenolic compounds, which have been reported to possess XO inhibitory activity (Costantino *et al.*, 1992). Hence, the antioxidant activity of the fractions may also contribute to its hypouricemic and XO inhibitory activities. 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? *J. Rheumatol.* **29**, 2403-2406.

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

Bowman WC, Rand MJ. (1980) Textbookof pharmacology, 2nd ed., pp.243-244. Blackwell, Sydney.

Burke A, Smyth E, FitzGerald GA. (2006) Analgesic – antipyretic agents; pharmacotherapy of gout. In: The pharmacological basis of therapeutics, edited by Brunton LL, Lazo JS, Parker KL. 11th ed., p. 706-710, McGraw–Hill Medical Publishing Division, New York.

Chang WS, Lee YJ, Lu FJ, Chaing HC. (1993) Inhibitory effects of flavonoids on xanthine oxidase. *Anticancer Res.* **13**, 2165-2170.

Costantino L, Albasini A, Rastelli G, Benvenuti S. (1992) Activity of polyphenolic crude extracts as scavengers of superoxide radicals and inhibitors of xanthine oxidase. *Planta Med.* **58**, 342-344.

Emmerson BT. (1996) The management of gout. *N.*

Engl. J. Med. **334**, 445-451.

Emmerson BT. (1998). Hyperlipidaemia in hyperuricaemia and gout. *Ann. Rheum. Dis.* **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]-1*H* pyrazole-4-carboxylic acid]: a potent xanthine oxidoreductase inhibitor with hepatic excretion. *J. Pharmacol. Exp. Ther.* **311**, 519-528.

Gonzalez AG, Bazzocchi IL, Moujir L, Ravelo AG, Correa MD, Gupta AP. (1995) Xanthineoxidase inhibitory activity of some Panamanian plants from Celastraceae and Lamiaceae. *J. Ethnopharmacol.* **46**, 25-29.

Hall IH, Scoville JP, Reynolds DJ, Simlot R, Duncan P. (1990) Substituted cyclic imides as potential anti-gout agents. *Life Sci.* **46**, 1923-1927.

Halliwell B, Gutteridge JMC. (2001) Free radicals in biology and medicine, 3rd ed. pp. 28-30, Oxford University press, Oxford.

Iio M, Moriyama A, Matsumoto Y, Takaki N, Fukumoto M. (1985) Inhibition of Xanthine oxidase by Flavonoids. *J. Agric. Biol. Chem.* **49**, 2173-2176.

Kirthikar KR, Basu BD. (1987) Indian Medicnal Plants, 2nd ed., pp. 1151-1154. International Book Distributors, Dehradun.

Kong LD, Cai Y, Huang WW, Cheng CHK, Tan RX. (2000) Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. *J. Ethnopharmacol.* **73**, 199-207.

Kong LD, Yang C, Ge F, Wang HD, Guo YS. (2004) A Chinese herbal medicine Ermia wan reduces serum uric acid level and inhibits liver xanthine dehydrogenase and xanthine oxidase in mice. *J. Ethnopharmacol.* **93**, 325-330.

Kramer HM, Curhan G. (2002) The association between gout and nephrolithiasis. *Am. J. Kidney Dis.* **40**, 37-42.

Kumar A, Edward N, White MI, Johnston PW, Catto GR. (1996) Allopurinol, erythema multiforme and renal insufficiency. *Br. Med. J.* **312**, 173-174.

Lewis HB, Doisy EA. (1918) Studies in uric acid metabolism. I. The influence of high protein diets on the endogenous uric acid elimination. *J. Biol. Chem.* **36**, 1-7.

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

Liote F. (2003). Hyperuricaemia and gout. *Curr. Rhemumatol. Rep.* **5**, 227-234.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951) Protein measurement with protein phenol reagent. *J. Biol. Chem.* **193**, 265-275.

Mazzali M, Hughes J, Kim YG, Jefferson JA, Kang DH, Gordon KL, Lan HY, Kivlighn S, Johnson RJ. (2001) Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. *Hypertension* **38**, 1101-1106.

Nakagawa T, Mazzali M, Kang D, Sanchez-Lozada LG, Herrera-Acosta J, Johnson RJ. (2006) Uric acid – a uremic toxin? *Blood Purif.* **24**, 67-70.

Owen PL, Johns T. (1999). Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. *J. Ethnopharmacol.* **64**, 149-160.

Rang HP, Dale MM, Ritter JM. (2001) Pharmacology, 4th ed., pp.239, Churchill Livingstone, London.

Stavric B, Clayman S, Gradd REA, Hebert D. (1975) Some in vivo effects in the rat induced by chlorprothixene and potassium oxonate. *Pharmacol. Res. Commun.* **7**, 117-124.

Theoduloz C, Pacheco P, Schemeda-Hirschmann G. (1991) Xanthine oxidase inhibitory activity of Chilean Myrtaceae. *J. Ethnopharmacol.* **33**, 253-255.

Umamaheswari M, Chatterjee TK. (2008). *In vitro* antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract. Afr. J. Trad. CAM. **5**, 61-73.

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

Wallach SL. (1998) The side effects of allopurinol. *Hosp. Pract.* **33**, 22.

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 Clin. Pharmacol. Toxicol.* **94**, 232-237.

Wasantwisut E, Viriyapanich T. (2003) Ivy gourd (*Coccinia grandis* Voigt, *Coccinia cardifolia, Coccinia indica*) in human nutrition and traditional applications. In: Plants in Human Health and Nutrition Policy: World Reviews of Nutrition and Dietics, edited by Simopoulous AP, Gopalan C, p. 60-66, Karger, Basel.