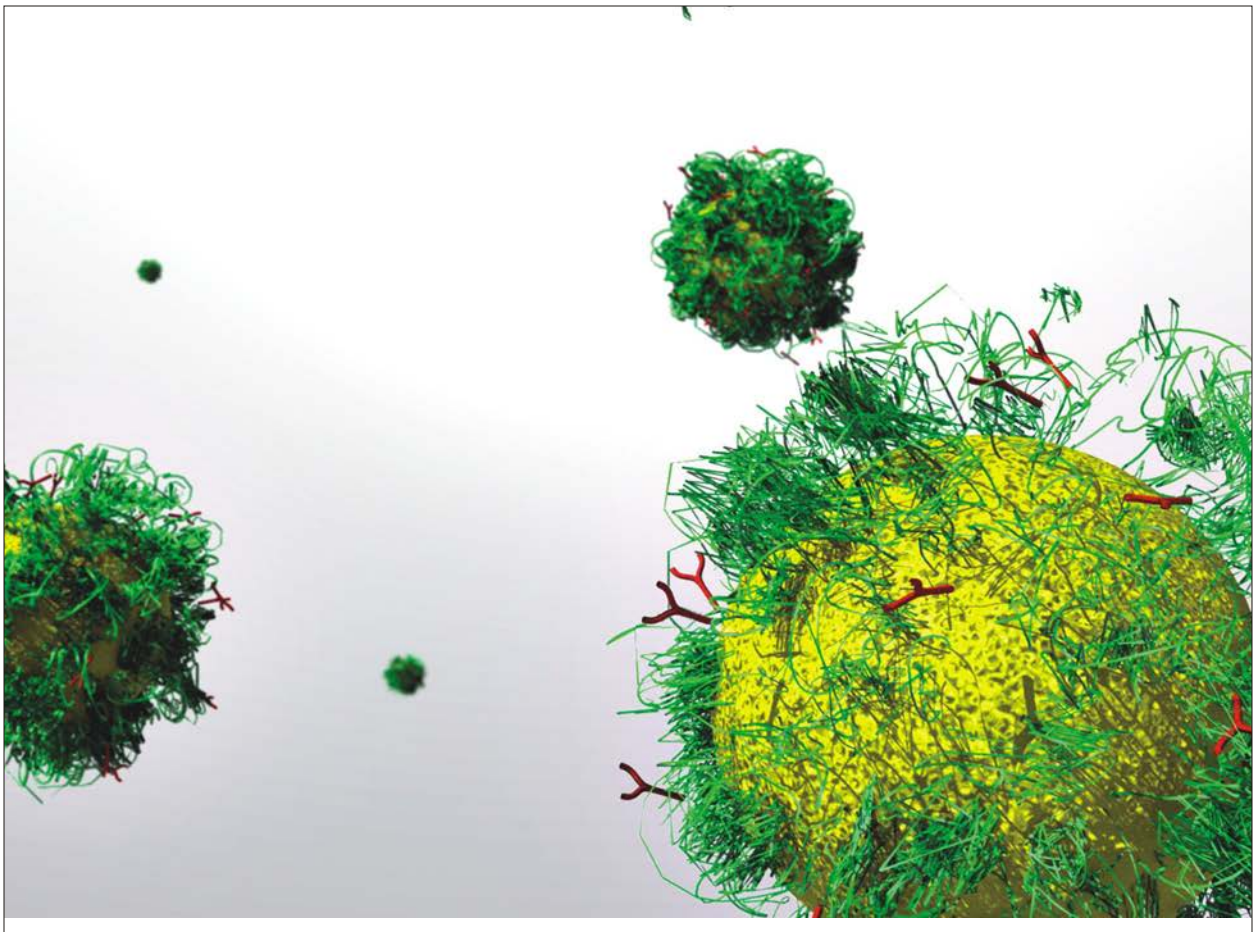

American Journal of Drug Discovery and Development

ISSN 2150-427X

Academic Journals Inc.

www.academicjournals.com

American Journal of Drug Discovery and Development 4 (2): 102-112, 2014
ISSN 2150-427x / DOI: 10.3923/ajdd.2014.102.112

Antigout and Antioxidant Activity of Stem and Root of *Origanum majorana* Linn.

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ABSTRACT

The present study was aimed at investigating the antioxidant and antigout activity of the various extracts of the stem and root of *Origanum majorana* Linn. The antioxidant activity of ethanol extracts of both stem and root of marjoram was evaluated by various in vitro antioxidant assays. The ethanol extracts of stem and root showed potent antioxidant activity in all models when compared with ascorbic acid having IC_{50} values 21.05 μg mL^{-1} and 84.98 μg mL^{-1} for DPPH (2, 2-diphenyl-picrylhydrazyl); 492.8 μg mL^{-1} and 477.6 μg mL^{-1} for H_2O_2 radical scavenging assay; 156.9 and 141.79 μg mL^{-1} for metal chelating assay, respectively. The reducing ability of root ethanol extract (absorbance 0.747±0.23) was found to be high, compared to stem ethanol extract (0.527±0.14). The *in vitro* xanthine oxidase inhibitory activity was performed on ethanol and aqueous extracts (stem and root) of the plant and activity was found to be significant with IC_{50} 59.21 and 148 μg mL^{-1} in both ethanol extracts. Further *in-vivo* antigout was studied gout induced in rats. Oral administration of stem and root ethanol extracts (200 and 400 mg kg^{-1} body weight) showed a significant decrease in uric acid, Creatinine, ESR (Erythrocyte Sedimentation Rate) and MDA (Malondialdehyde) levels in the gouty rats. A significant increase was observed in reduced glutathione. No change in protein content was noticed. In conclusion, the studied plant extracts showed significantly variable anti-gout activity associated with both antioxidant and anti-inflammatory effects which may be due to the presence of flavonoids, phenolics, saponins and triterpenoidal compounds revealed by preliminary phytochemical screening.

Key words: *Origanum majorana*, xanthine oxidase inhibitor, gout, antioxidant activity

INTRODUCTION

Gout is a multi-factorial disease affecting the flexibility of joints. It is usually characterized by re-current attacks of acute inflammatory arthritis-a red, tender, hot, swollen joints leading to bursitis. It is caused by elevated uric acid levels in the blood, resulting into deposition of urate (as monosodium urate-monohydrate) crystals, generally known as tophi crystals in joints, tendons and surrounding tissues, characterized by hyper-uricemia and in chronic stage, may lead to renal failure (Singh *et al*., 2010). These crystals cause an acute inflammatory response and can induce a permanent tissue damage which is characterised by the appearance of ulceration of the joint cartilage, marginal osteophytosis, geodic and erosive lesions and chronic inflammation of synovial membrane (Dalbeth and Haskard, 2005; Corrado *et al*., 2006).

Current treatments to gout includes (a) NSAIDS such as ibuprofen, naproxen, indomethacin, aspirin, etoricoxib (cox-2 selective inhibitors); corticosteroids such as prednisone; allopurinol, probencid, colchicines (to decrease severity of episodes). Although these agents are generally effective, they generates superoxide (Berry and Hare, 2004) and lead to several side effects such as skin allergies, fever, rash and diarrhoea progressively developing leukocytosis, eosinophilia, vasculitis, aseptic meningitis, nephritis and renal dysfunction, and hepatic dysfunction (Nguyen *et al*., 2004; Strazzullo and Puig, 2007). So, there is a need of herbal extracts with antioxidant property to nullify oxidative and inflammatory response produced by xanthine oxidase.

O*riganum majorana* Linn. is a tender perennial aromatic herb of the mint family (Labiatae), rich in phenols, flavonoids and terpenoids (Novak *et al*., 2002; Raina and Negi, 2012; Proestos and Komaitis, 2006; Janicsak *et al*., 1999). Polyphenols (Costantino *et al*., 1992), flavonoids (Chang *et al*., 1993; Selloum *et al*., 2001), coumarins (Chang and Chiang, 1995), ellagic acid, Valoneic Acid Dilactone (VAD) (Unno *et al*., 2004) have been reported as potent plant-based Xanthine Oxidase Inhibitors (XOI).

The objective of the present research was to determine the potential of stem and root of *Origanum majorana* as a potent antioxidant and antigout agent. This was accomplished through testing different extracts of stem and root of the plant as uric acid lowering (XOI), *in vitro* antioxidant and *in vivo* anti-inflammatory in experimental gout model in rats. The possible role of marjoram in reducing serum uric acid levels and its effect on biomarkers of oxidative stress was also investigated. In the present investigation XO inhibitory activity of *O. majorana* was evaluated to discover a natural substitute of plant origin, which could have a superior effect of inhibiting XO activity and can be used as an alternative to allopurinol for the treatment of gout as well as for the treatment of other inflammatory-related diseases.

MATERIAL AND METHODS

Collection and authentication of plant material: Plant was collected from Guru Jambheshwar University of Science and Technology Hisar in August, 2012. Identified by Dr. K.C Bhatt, Senior Scientist, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic Resources, New Delhi, vide reference no. NHCP/NBPGR/2012-28/. The plant has been deposited in Pharmacognosy division of Department of Pharmaceutical Sciences, GJUS&T, Hisar for further references.

Chemicals: Folin-Ciocalteu reagent, 2, 2-Diphenyl-Picrylhydrazyl (DPPH), hydrochloric acid (HCl), ferric chloride ($FeCl_3$) and gallic acid were obtained from Sigma-Aldrich, USA. Sodium carbonate (Na_2CO_3), methanol, potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), sodium hydroxide and hydrochloric acid were purchased from S D Fine Chemicals Ltd., New Delhi. Xanthine, xanthine oxidase of bovine milk origin, allopurinol (standard drug) and potassium oxonate (gout inducing drug) were obtained from Sigma Aldrich, USA. All the other chemicals used were of analytical grade, obtained from S D Fine Chemicals Ltd., New Delhi.

Animals: Swiss albino rats were procured from disease free small animal house of LLRUVS, Hisar (Haryana). The experimental protocol was approved vide letter no. IAEC/136-144 dated 10.01.13 by Institutional Animals Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Registration No. 0436). Rats were maintained at normal pellet diet in the animal house of Guru Jambheshwar University of Science and Technology, Hisar.

Preparation of the extracts: The 1000 g powdered herb (stem and root) of *Origanum majorana* was extracted successively first with 2.5 L of ethanol (95 %) by using soxhlet apparatus and then with 2.5 L of water by cold maceration for 72 h. The extracts were filtered and concentrated at 45°C using rotary vacuum evaporator. The extract obtained was vacuum dried and stored for further studies.

Preliminary phytochemical screening: The ethanol and aqueous extracts of both stem and root of were screened for the presence of alkaloids, glycosides, carbohydrates, sterols, phenolic compounds, tannins, flavonoids, saponins, proteins and free amino acids using standard procedures (Goyal *et al*., 1997; Kar, 2003; Kokate *et al*., 2005; Ahmad, 2007).

***In vitro* antioxidant activity**

DPPH radical scavenging assay: Plant extracts (stem and root ethanol) and standard ascorbic acid solution (1 mL) of different concentrations viz; 25, 50, 75, 100, 125 and 150 μg mL^{-1} were added to 3 mL of a 0.004% methanol solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 min incubation in the dark, absorbance was recorded at 517 nm and the percentage inhibition was calculated. All the tests were performed in triplicate and the graph was plotted with the average of three observations (Kumaran and Karunakaran, 2007). The percentage inhibition was calculated from:

$$\frac{A_o\text{-}A1}{A_o}\times 100$$

where, A_o is the absorbance of the control and A1 is the absorbance of the extract/standard.

Scavenging of hydrogen peroxide: A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4), different concentrations of plant extracts and standard ascorbic acid solution viz; 10, 25, 50, 100, 200 and 500 μg mL^{-1} in methanol (1 mL) were added to hydrogen peroxide solution (2 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. A separate control sample was prepared for back ground subtraction. The percentage inhibition activity was calculated with the average of three observations (Kumaran and Karunakaran, 2007; Srinivasan *et al*., 2007):

$$\text{Inhibition (\%)}(H_2O_2)=\frac{A_o\text{-}A1}{A_o}\times 100$$

where, A_o is the absorbance of the control and A1 is the absorbance of the extract/standard.

Ferric reducing power assay: Different concentrations of plant extracts and standard ascorbic acid solution viz; 10, 25, 50, 100, 200 and 500 μg mL^{-1} in 1 mL of methanol were mixed with phosphate buffer (2.5 mL, 0.2 M pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of tricholoroacetic acid (10%) was added to the mixture which was then centrifuged at 3,000 g (rpm) for 10 min at room temperature. The upper layer (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%) and the absorbance of the reaction mixture indicated increased reducing power. The absorbance was

measured at 700 nm. All the tests were performed in triplicate and the graph was plotted with the average of three observations (Kumaran and Karunakaran, 2007; Kumar *et al*., 2005).

Metal chelating complex assay: The reaction mixture containing 1 mL of different concentrations of extracts viz. 10, 20, 40, 80, 100 and 200 μg mL^{-1} in methanol were added to 0.1 mL of 2 mM ferrous chloride and 0.2 mL of 5 mM ferrozine to initiate the reaction and the mixture was shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solution was measured at 562 nm. The positive controls were those using ascorbic acid and all tests and analysis were run in triplicate (Subhashini *et al*., 2011). The percentage chelating effect of ferrozine-Fe_{2+} complex formation was calculated from:

$$\frac{A_{\circ} - A1}{A_{\circ}} \times 100$$

where, $A_{\circ}$ is the absorbance of the control and A1 is the absorbance of the extract/standard.

***In-vitro* xanthine oxidase inhibitory activity:** Xanthine oxidase inhibition assay was measured spectrophotometrically at λ 295 nm under aerobic condition (Unno *et al*., 2004; Umamaheswari *et al*., 2007). The reaction mixture contained solution of xanthine as a substrate, a solution of enzyme (xanthine oxidase) and the test solutions (50, 100, 250 and 500 μg mL^{-1}) prepared in Di-Methyl Sulfoxide (DMSO). Concentrations of allopurinol Viz., 10, 25, 50 and 100 μg mL^{-1} were prepared in phosphate buffer (ph 7.5). To each 1 mL sample, added 2.9 mL phosphate buffer and 2 mL xanthine (0.15 Mm) solution. The sample mixture was pre-incubated for 10 min at 30°C. 0.1 mL of xanthine oxidase (0.1 units mL^{-1}) was added to each test tube and incubated at 30°C for 30 min. After the incubation period, 1 mL of 1 N HCL was added to stop the reaction and the contents homogenized. After then absorbance was measured at 284 nm to see the formation of uric acid that occurs in the test solution and percentage inhibition was determined according to the following equation:

$$\text{Inhibition (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

IC_{50} values were obtained through linear regression analysis the plot of concentration against percent inhibition.

***In-vivo* antigout activity**

Experimental design: Thirty swiss rats were maintained on laboratory stock diet and divided into five groups, each group comprised of 6 rats. Group1 (Normal control), received only vehicle (0.5% carboxy methyl cellulose in water) whereas group 2 (Gouty control) received Potassium Oxonate (P.O) (250 mg kg^{-1} i.p dissolved in 0.9% saline) solution for the induction of gout along with the vehicle (Yonetani *et al*., 1987). An hour later group 3, 4, 5 (gouty rats) received root alcohol, stem alcohol and standard drug (allopurinol) at the oral dose of 200, 400, 10 mg kg^{-1} extract, respectively. The procedure was repeated for 4 days. Rats were fasted for 16 h before blood collection on fourth day. After 1 h from injection, blood samples were withdrawn from eye vein orbital and divided into two parts one mixed with sodium citrate (109 mmol L^{-1}) for determination of erythrocyte sedimentation rate (ESR) (Westergren, 1921; Gilmour and Sykes, 1951) and the

sec part mixed with heparin for the separation of plasma for the determination of uric acid (Watts, 1974; Yonetani *et al.*, 1987), malondialdehyde (MDA) as an indicator for lipid per-oxidation (Satoh, 1978), glutathione (Ellman, 1959) and creatinine content (Owen and Johns, 1999) as an indicator for renal functioning. Along with this, animals were sacrificed and total protein present in the liver tissue was determined by standard methods (Smith *et al.*, 1985).

Statistical analysis: The results obtained were expressed as the mean±SEM. Rats of the control gouty group were compared with the healthy rats. Test groups were compared with the control gouty groups. The significance of values was analysed by ANOVA followed by Dunnet's Test statistical value of $p>0.5$ was considered as significant.

RESULTS

The ethanol extract of both stem and root showed the presence of terpenoids, flavonoids and tannins whereas saponins and carbohydrates were present in stem and root aqueous extracts, respectively. Alkaloids, glycosides ad proteins were absent in both of the extracts (root and stem).

***In-vitro* antioxidant activity:** A significant decrease was observed in the concentration of DPPH radicals due to the scavenging ability of extracts of the plant. The activity was found to be dose dependent. Maximum scavenging activity was observed at different concentrations in stem and root, respectively. All the extracts demonstrated H^+-donor activity. The highest DPPH radical scavenging activity was detected in stem ethanol extract (IC_{50} 21.05 μg mL^{-1}) in comparison to root ethanol extract (84.98 μg mL^{-1}) and that of standard ascorbic acid was (IC_{50} 8.45 μg mL^{-1}).

A significant dose dependent response in the hydrogen peroxide scavenging activity aws reflected in both ethanol stem (IC_{50} 492.8 μg mL^{-1}) and root (IC_{50} 477.6 μg mL^{-1}) extracts whereas, IC_{50} value of standard was found to be 173.4 μ mL^{-1}.

The extracts of *O. majorana* interferes with the ferrous-ferrozine complex and the red colour of the complex is decreased with the increasing concentrations of the extracts. Both the extracts captured ferrous ions before ferrozine and thus have ferrous chelating ability. The root ethanol extract showed the highest ferrous ion chelating ability (IC_{50} 141.79 μg mL^{-1}) among both the extracts (stem ethanol extract: IC_{50} 156.9 μg mL^{-1}; standard: IC_{50} 15.8 μg mL^{-1}).

Figure 1 showed the reductive capabilities of different extracts of *O. majorana* when compared to the standard, ascorbic acid. The root ethanol extract showed the highest reducing ability (absorbance 0.747±0.23) compared to stem ethanol extract (0.527±0.14).

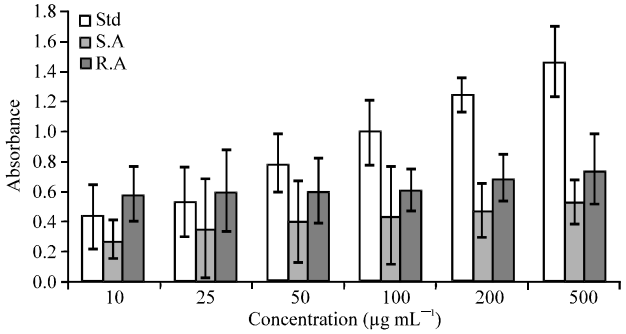

Fig. 1: Ferric reducing power assay. Absorbance of standard (Std), stem ethanol extract (S.A), root ethanol extract (R.A) at different concentrations

Table 1: Xanthine oxidase (XO) inhibitory activity of allopurinol (Std.)

Concentration (μg mL^{-1})	Inhibition (%)
10	66.30
25	69.20
50	71.08
75	72.89
100	78.00

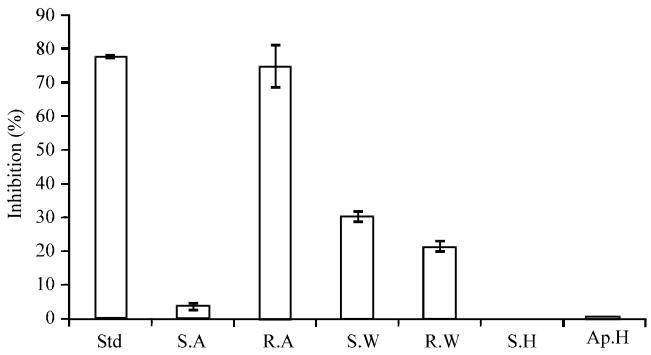

Fig. 2: Xanthine oxidase inhibitory activity. std: Standard, S.A: Sstem ethanol extract, R.A: Root ethanol extract, S.W: Stem water, RW: Root water, SH: Stem hydrosol, Ap.H: Aerial part hydrosol, showing %inhibition at concentration 100 μg mL^{-1}

***In-vitro* xanthine oxidase inhibitory activity:** The XO inhibitory activity of allopurinol and optimized different extracts of *O. majorana* at various concentrations by *in-vitro* method are represented in Table 1. The percent xanthine oxidase inhibitory activity of the assayed samples was determined through the slope of the plot of absorbance against time (sec). IC_{50} values were obtained through the slope of plot of concentrations (50, 100, 250, 500 μg m^{-1}) against percent inhibition.

Ethanol extracts of stem and root has shown significant XO inhibition with 42.4 and 75.4% inhibition at concentration of 100 μg mL^{-1} whereas aqueous extracts demonstrated non significant XO %inhibition with values of 25.62, 21.83% stem and root aqueous extract, respectively. The percentage inhibition was found to be concentration dependent and comparable to the standard (78.0%) in case of root ethanol extract as shown in Fig. 2.

The IC_{50} values of stem, root ethanol and aqueous extracts were calculated from the curve and compared with standard (allopurinol IC_{50} 1.11 μg mL^{-1}). A significant value of IC_{50} i.e.148 and 59.21 was obtained in case of stem and root ethanol extracts, whereas very high value of IC_{50} i.e., 500 and 600, respectively was determined in stem and root aqueous extracts.

***In-vivo* anti-gout activity:** *In-vivo* antigout activity was determined for the extracts that showed significant results in *in-vitro* anti-gout model and further dose of the extracts (stem ethanol extract: 400 mg kg^{-1}; root ethanol extract: 200 mg kg^{-1}) were decided according to significant results.

Xanthine oxidase (XOD) catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid and is a key enzyme in the pathogenesis of hyperuricemia. The ability of extracts of *O. majorana* to inhibit uricase was investigated in this study. Treatment with the uricase inhibitor

Table 2: Various biochemical parameters studied during *in-vivo* anti-gout activity

Paramater	Uric acid (mg dL^{-1})	ESR (mm h^{-1})	Creatinine (mg dL^{-1})	MDA (µm L^{-1})	Glutathione (µm L^{-1})	Total protein content (g dL^{-1})
Control	1.206±0.11	2.6±0.04	0.74 ±0.02	1.88±0.02	3.52±0.11	3.66±0.03
Positive control	2.796±0.09[a]	9.83±2.0[a]	1.347±0.06[a]	4.75±0.39[a]	1.04±0.15**	3.230±0.29[a]
Allopurinol (standard)	0.9520±0.07**	1.9±0.15**	0.765±0.07**	1.97±0.12**	3.19±0.10**	3.54±0.05**
Root ethanol	1.08±0.10**	2.5±0.22**	1.045±0.06**	2.05±0.15**	1.94±0.09**	3.38±0.04*
Stem ethanol	1.582±0.19**	3.83±0.30**	1.07± 0.07*	2.43±0.52**	2.13±0.011**	3.30±0.04

Values are presented as mean±S.E.M n: 6 in each group, one way ANOVA followed by Dunnet's test. [a]$p<0.01$ significant as compared to normal control, *$p<0.005$, **$p< 0.001$ as compared to gouty control

potassium oxonate significantly affected various bio-chemical parameters (uric acid, ESR, creatinine, MDA, glutathione, total protein content) on wistar rats blood (Table 2).

An increased serum urate levels compared to normal control group was observed after potassium oxonate injection. Allopurinol (10 mg kg^{-1}), significantly ($p<0.01$) reduce serum urate levels of hyperuricemic rats to values than that found in normal animals. A four day treatment with ethanol extracts (stem and root) at the dose of 200 mg kg^{-1} and 400 mg kg^{-1}, respectively reduce significantly ($p<0.01$) serum uric acid levels compared to hyperuricemic control group.

The present study also investigated the efficacy of orally administered *O. majorana* extracts and allopurinol on serum non-invasive biomarkers of oxidative stress (malondialdehyde concentration) and lipid peroxidation (reduced glutathione) levels in rats blood. As shown in Table 2 significant increase ($p<0.01$) in serum was observed in root and stem ethanol extract treated normal rats compared to positive control group in case of MDA levels, whereas a drastic decrease in glutathione levels. A similar effect was obtained in hyperuricemic animals following extracts administration compared to hyperuricemic control group.

In the present study gouty rats showed a significant increase in Erythrocyte Sedimentation Rate (ESR) and creatinine levels. The oral administration of ethanol root and stem extracts of marjoram produced the significant reduction in ESR and creatinine levels 3 hours after i.p administration of oxonate. The root and stem ethanol extract showed significant ($p<0.01$) decrease in ESR level compared to positive gouty control rats and values are comparable to the standard (allopurinol) whereas in case of reduction of creatinine levels, root extract showed more significant ($p<0.01$)reduction than stem ethanol extract ($p<0.05$).

As shown in Table 2, the amount of protein content did not vary neither with administration of oxonate nor with the oral dose of extracts. There were insignificant ($p>0.015$) results shown by both stem and root ethanol extracts compared to positive control in the total protein content in the liver tissues of rats treated with the uricase inhibitor, potassium oxonate. It was thus concluded that as per the results, there is no effect of potassium oxonate on protein content in liver tissues.

DISCUSSION AND CONCLUSION

Anti-oxidant activity: Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements. DPPH is widely used to evaluate the free radical scavenging effect of natural antioxidants. DPPH is a stable free radical at room temperature, it contains an odd electron which is responsible for visible deep purple (Sreejayan and Rao, 1996). DPPH shows a strong absorption band at 517 nm in visible spectrum. As the electron became paired in the presence of free radical scavenging the absorption

diminishes and the resulting discoloration stochiometrically coincides with the number of electrons taken up. The bleaching of DPPH absorption represents the capacity of the test drugs to scavenge free radicals independently. The bleaching of DPPH molecules can be correlated with the number of available hydroxyl groups. A significant decrease was observed in the concentration of DPPH radicals in all extracts of the plant. The highest DPPH radical scavenging activity was detected in stem ethanol extract. It can be concluded that, the good antioxidant activity of the extract may be probably due to the presence of substance with an available hydroxyl group.

Hydroxyl radicals are the major active oxygen species that cause lipid oxidation and enormous biological damage. Thus, scavenging of H_2O_2 is a measure of the antioxidant activity of the fractions. H_2O_2 can cross membranes and may oxidize a number of compounds. Both the ethanol extracts of stem and root of *O. majorana* scavenged hydrogen peroxide which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water (Haraguchi *et al.*, 2001). A dose dependent hydrogen peroxide scavenging activity in both ethanol stem and root extracts was observed with IC_{50} values 492.8 and 477.6, respectively.

Ferrozine can quantitatively form complexes with Fe^{2+} but in the presence of ion chelating agents, the complex formation is disrupted resulting in a reduction in the red colour of the complex measurement of the rate of reduction of the colour, therefore,s allows estimation of the chelating activity of the coexisting chelator (Halliwell *et al.*, 1987). The absorbance of Fe^{2+}-Ferrozine complex linearly decreased in dose dependent manner. Both the extracts (stem and root ethanol) demonstrated an effective capacity for iron binding, suggesting that its action as peroxidation protector may be related to its iron binding capacity. In this assay the extract and standard compound interfered with the formation of ferrous complex with the reagent ferrozine, suggesting that it has chelating activity and captures the ferrous ion before ferrozine. The results of our study express that the extracts have an effective capacity for iron binding, suggesting its antioxidant potential. In addition, the metal chelating ability of the fractions demonstrated that they reduce the concentration of the catalysing transition metal involved in the peroxidation of lipids. The root ethanol extract showed the highest ferrous ion chelating ability (IC_{50}; 141.79 μg mL^{-1}) among both the extracts.

The conversion of Fe^{3+} into Fe^{2+} in the presence of various fractions was calculated to determine the reducing power ability (Umamaheswari *et al.*, 2007). The reducing ability of a compound generally depends on the existence of reductones (antioxidants) which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom. The root ethanol extract showed the highest reducing ability (absorbance 0.747±0.23) compared to stem ethanol extract (0.527±0.14). The antioxidant principles present in the extracts cause reducing power ability.

Based on the results obtained, it may be concluded that both ethanol extracts (stem and root) of *O. majorana* showed strong antioxidant activity, reducing power ability, free radical scavenging activity and metal chelating ability when compared to standards such as ascorbic acid which may be due to presence of flavonoids, phenols, tannins (phenolic compounds) and triterpenoids found in the preliminary phytochemical screening. Overall, the plant extract is a source of natural antioxidant that can be important in disease prevention, health preservation and promotion of longevity.

Anti-gout activity: Gouty arthritis is characteristically acute inflammatory reaction which occur in response to articular deposits of monosodium crystals and associated with edema and erythema of joints, together with severe pain (Sabina *et al.*, 2010). Gout is an inherited metabolic disease and

results from hyperuricemia, an elevation of serum uric acid level. Control of hyperuricemia is most often achieved by reducing uric acid production with an inhibitor of XO, the enzyme catalyzing the two terminal reactions in uric acid synthesis, or less frequently, by employing uricosuric agents to increase renal clearance of uric acid (Boss and Seegmiller, 1979; Emmerson, 1996). To date, the only commercially available XO inhibitor is allopurinol, a purine analogue in clinical use for more than 30 years (Takano *et al*., 2005). However, allopurinol generates superoxide (Berry and Hare, 2004) and lead to several side effects such as skin allergies, fever, rash and diarrohea progressively developing renal dysfunction and hepatic dysfunction (Osada *et al*., 1993; Takano *et al*., 2005). The hypouricaemic activity was found to be significant with IC_{50} 59.21 and 148 in both root and stem ethanol extracts, after *in vitro* studies, hence the further *in-vivo* study was performed. The doses of extracts (root ethanol extract: 200 mg kg^{-1}; stem ethanol extract: 400 mg kg^{-1}) given to the rats were selected based on the results of *in-vitro* xanthine oxidase inhibitory activity. Serum uric acid levels were elevated to approximately two-fold in oxonate-induced hyperuricaemic rats. The groups treated with stem, root ethanol extracts and allopurinol for four days decreased the uric acid level to almost normal value.

Flavonoids are a group of polyphenolic compounds which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, antiulcer activities, etc. They are well known anti-oxidants and used as therapeutic agents for diseases mediated by free radicals (Rice-Evans *et al*., 1996). Also, the structure–activity relationship of different chemical classes of flavonoids have been reported as potential inhibitors of XO (Costantino *et al*., 1992). Since, phytochemical screening of the different extracts of *O. majorana* revealed the presence of flavonoids, phenolics, saponins and triterpenoids accounting for its antioxidant property, the present activity of the extracts may be attributed to the same.

A significant increase ($p<0.01$) in serum was observed in root and stem ethanol extract treated normal rats compared to positive control group in case of MDA levels, whereas a decrement in levels of glutathione was there. Both the extracts (root and stem ethanol) have shown significant decrease in level of MDA and increase in glutathione level, comparable to normal. Thereby revealing that serum uric acid through lipid peroxidation, might be working towards the etiopathogenesis of oxidative stress diseases and its serum level may be a deciding factor for progression of the disease. In conclusion, root and stem ethanol extracts (more percentage was found in root extract) prevented oxidative stress by enhancing total antioxidant capacity and decreasing lipid peroxidation. Moreover, the hypouricemic and XO inhibitory action of ethanol extracts of *O. majorana* has been confirmed in this study. Taking into account that marjoram is an antioxidant, non toxic and can be used safely long term, this feature makes it a possible alternative for allopurinol, or at least in combination therapy to minimise the side-effects of allopurinol. Therefore, the use of suboptimal dosages of allopurinol in combination with dietary changes may provide a safer approach for prevention and treatment of hyperuricemia.

ACKNOWLEDGMENTS

The authors are grateful to express our gratitude to Dr. K.C Bhatt, NBPGR, Pusa campus, New Delhi for identification and authentification of the plant for our project. We thank LLRUVS, Hisar (Haryana) for providing the animals for biological studies.

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