Antioxidant property of *Hypericum perforatum* (L.) of Indian origin and its comparison with established *Medhya rasayanas* of ayurvedic medicine

Hypericum perforatum (L.) (family Clusiaceae), commonly known as the St. John's wort, is the most popular drug in USA. It is used as an antidepressant, and its origin is considered to be from the Chinese system of medicine. About 288 species of *Hypericum* have been reported[1] in the literature. Though this plant is not described in the ayurvedic system of medicine, it grows widely in the Himalayan regions and in the hills of the central part of India[2]. Although some biological properties of this plant have been reported[3,4], this wildly-grown plant has been in use by tribals of this country for ailments such as fever, joint pain, general weakness, sleep disorders, delayed wound healing, etc. The local names of this plant are *dendhu patta*, *basant* (in Hindi), and *balsana* (in Urdu)[5]. In the ayurvedic system of medicine, there are different drugs which are used for the management of anxiety, depression, and mental disorders, grouped under *medhya rasayana*[6]. *H. perforatum* growing in Madhya Pradesh has also shown antidepressant property[7], but has not been commercially exploited to a significant extent in this country.

This plant is a perennial shrub with an erect, branched stem. The plant has the following characteristic morphological features: (i) a tap root system, (ii) leaves that are 1.5 cm long, sessile, opposite, glabrous, and elliptical with pellucid glands and possess dark spots (red brown) on their margins, (iii) yellow flowers that have black dots on their petals, and (iv) the powder of the shoot is greenish to yellowish brown, with typical woody smell. The chemical constituents of this plant are: naptho-di-anthrones (hypercine and pseudohypercine), various flavonoids[8], and procyanidine. The important flavonoids are hypercine, quercitine, routine, etc. This plant needs to be well irrigated because under water-stress conditions, there is a significant decrease in the concentration of the flavonoids. The phenolic compounds are highest during March, which is the blossoming time of this plant[9]. The plant powder should be kept in dark because its phytochemicals are sensitive to light[10]. Since this plant is of great market value and not described in the ayurvedic text, it becomes important to investigate its various properties and to compare it with some prominent well-documented medicines such as *Bacopa monniera*[11] and *Nardostachys jatamansi*[12], belonging to the *medhya rasayana* group.

In this paper, for the first time, we have investigated its potential effect on superoxide radical-scavenging, –OH radical-trapping, iron-chelation, reduced glutathione- and ferrous sulphate ($FeSO_4$)-induced lipid peroxidation. The results have shown that this plant could be used as an antioxidant in medicine. Therefore, further studies on this plant using some more clinical parameters are required before this plant could be exported. Its export would help the economic status of the tribals of this region. For the experiments, various chemicals such as thiobarbituric acid (TAB), trichloroacetic acid (TCA), ferrous sulphate, and acetic acid were purchased from Central Drug House (Pvt) Ltd. Cumene hydroperoxide (CHP), 1,1,3,3-tetra ethoxy propane (TEP), reduced glutathione (GSH), 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB), sodium salicylate, ascorbate, riboflavin, 2,2′-bipyridil, and potassium thiocynate were obtained from Sigma, USA. All the other reagents used were of analytical grade.

The study was carried out in *in vitro* conditions. Liver homogenate, 10%, was added to 35 mm petri plates. To this homogenate were added the plant extract and other pharmacological tools directly, according to the protocol given in the respective tables, for the estimation of lipid peroxidation and glutathione content. For studying the effect of this plant extract on superoxide-scavenging, and hydroxyl radical-trapping, the extract of the plant was directly added to the reaction system to evaluate its chemical reactivity against these species of free radicals.

The details of the experimental design are as follows: Normal albino rats (100–150 g body wt) of Charles Foster strain were randomly selected and kept on fast overnight. The following day, these rats were anaesthetized by pentobarbitone (3 mg/100 g body wt, ip). Liver was exposed and perfused with phosphate buffer saline, pH 7.4 (20 ml, through hepatic portal vein). Blood-free liver was taken out and homogenized in a glass teflon homogenizer (10%, w/v).

Fresh *H. perforatum* shoots were collected from Madhya Pradesh forest and dried under shade for experimental use. Its authenticity was checked using the pharmacognostical parameters as described in the text[1]. It was powdered and extracted with ethanol for 30 h in a soxhlet apparatus. The extract was evaporated under low pressure by using Buchi type rotary evaporator. The concentrated extract was kept in a vacuum desiccator till the constant weight of solvent-free extract was attained. The extracts of other medicines were also prepared using a similar method, mixed with tween-80, and diluted with distilled water to get the final ratio of water : tween-80 as 9 : 1. The same solution was used as vehicle for drugs in the control plates as well.

For estimation of lipid peroxidation in the liver, the homogenate was assayed by estimating thiobarbituric acid-reactive substances (TBARS) using a standard method[13] with slight modification[14]. Lipid peroxidation was induced by adding 1.5 mM $FeSO_4$ to the liver homogenate, and the degree of lipid peroxidation was assayed after 30 min on addition of $FeSO_4$. The assay was based on the reaction of thiobarbituric acid with malondialdehyde (MDA), which yielded a pink-coloured complex, with an absorption maxima at 535 nm. The results were compared with the antilipidperoxidative property of vitamin E, and parabenzoquinone, as described earlier[12]. These have proved to be strong antilipidperoxidatives using similar experimental condition, by the previous workers. The ED_{50} value for vitamin E was 58 µg, and for parabenzoquinone it was 35 µg. These reagents and plant extracts were added to different experimental plates just before adding the $FeSO_4$ (ref. 4). Liver homogenate was not pretreated with these reagents. It was 30 min, same as $FeSO_4$.

For the superoxide anion ($O^{\cdot}_2$)-scavenging, the assay was quantitated by

reduction of nitro blue tetrazolium (NBT) to a blue-coloured formazan[15], exhibiting absorption maxima at 560 nm. This assay is fast and sensitive and was originally reported by Beuchamp and Fridovich[16]. The results were also compared with some known superoxide radical scavengers like $CuSO_4$ and $MnCl_2$ (ref. 17) which showed the protection in the order of 87% for 0.01 mM $CuSO_4$ and 67% for $MnCl_2$ under similar conditions.

For the hydroxyl radical trapping, the assay was carried out by monitoring the hydroxylation of salicylate by Fe^{3+}–ascorbate–H_2O_2 system[18]. In this study, salicylate (2-hydroxybenzoate) at a concentration of 2.5 mM was added to the reaction mixture, and the hydroxylated product (2,3-dihydroxy benzoate) was extracted with ether and assayed spectrophotometrically at 510 nm. The –OH radical scavenging property of the alcoholic extract of *H. perforatum* was compared with known trappers of –OH radical, e.g. thiourea and mannitol[17] which had significant protection against the hydroxyl generation in similar conditions. ED_{50} for thiourea was 0.3 mM and for mannitol 7.2 mM.

The estimation of ferric ion (Fe^{3+}) by thiocyanate gave an intensely red coloured compound (max-460 nm) which remained in true solution. Estimation of ferrous ion (Fe^{2+}) was conducted with 2,2′-bipyridil, which gave a pink-coloured complex (absorption maxima 525 nm) (ref. 17). Here different concentrations of the extract were mixed with fixed concentration of $FeSO_4$ and $FeCl_3$ (10 µg). The mixture was incubated for 30 min and then its absorbance was recorded.

The reduced glutathione assay was carried out by the method of Ellman[19] based on the reaction of GSH with 5′-dithiobis (2-nitrobenzoic acid) (DTNB), to yield a chromophoric product, viz. 2-nitro-5-thiobenzoic acid (maximum absorption at 412 nm).

The results given here are the mean ± SD of 6 separate experiments. Level of significance has been evaluated by using Student's *t* test.

Alcoholic fraction of *H. perforatum* (ED_{50} 150 µg) showed the reduction in lipid peroxidation induced by $FeSO_4$ in a dose-dependant manner. It showed 13.41% of –OH radical inhibition at a concentration of 1 mg/ml. The alcoholic extract of *H. perforatum* inhibited superoxide anion radical up to 81.50% at 1 mg/ml (Table 1). This fraction of *H. perforatum* failed to maintain the aerial oxidation of reduced glutathione. It slightly chelated Fe^{2+} (22.45%), but the interaction with Fe^{3+} at 1 : 10 ratio of iron : extract was still less. The effect of alcoholic extract of *H. perforatum* on all these antioxidant parameters was compared with the alcoholic extracts of *Bacopa monniera* and *Nardostachys jatamansi* under similar conditions and concentrations (Figure 1).

Taking all the parameters into consideration, it appears that the alcoholic fraction of *H. perforatum* is antilipidperoxidative, and the mechanism of its action is perhaps due to scavenging of free radicals, and the disturbance of Fe^{2+}/Fe^{3+} ratio due to chelation, which is essential for the initiation of lipid peroxidation[17]. Its insignificant action on the protection of reduced GSH level and minor effect on –OH radical trapping (13%) support the above-proposed mechanism of action. We have found in other medicinal plants, like *R. cordifolia*, which significantly protect the GSH level[20] and in *S. nuxvomica* which significantly chelates the Fe^{2+} iron but does not protect the oxidation of GSH[21].

Table 1. Effect of alcoholic fraction of *Hypericum perforatum* on different antioxidant parameters

Dose (µg/ml)	% inhibition of TBARS value (nmole/mg)	% inhibition of GSH value (nmole/mg)	% inhibition of hydroxylation	% inhibition of formazan formation
0.00	0.00	0.00	0.00	0.00
25	9.48	2.86	0.39	4.45
50	16.23	2.97	0.61	15.59
100	31.07	3.22	1.66	48.48
500	55.26	4.67	7.38	75.31
1000	75.46	7.47	13.41	81.50

Standard values in the control tubes without the plant extract: 1. $FeSO_4$-induced TBARS after 30 min (441.34 ± 4.85 nmole/mg); 2. Aerial oxidation of GSH at 20 min (29.39 ± 2.14 nmole/mg); 3. Hydroxylation of salicylate after 90 min (203.99 ± 4.54 nmoles); 4. Formazan formation after 4 min (absorbance – 0.34 ± 0.008).

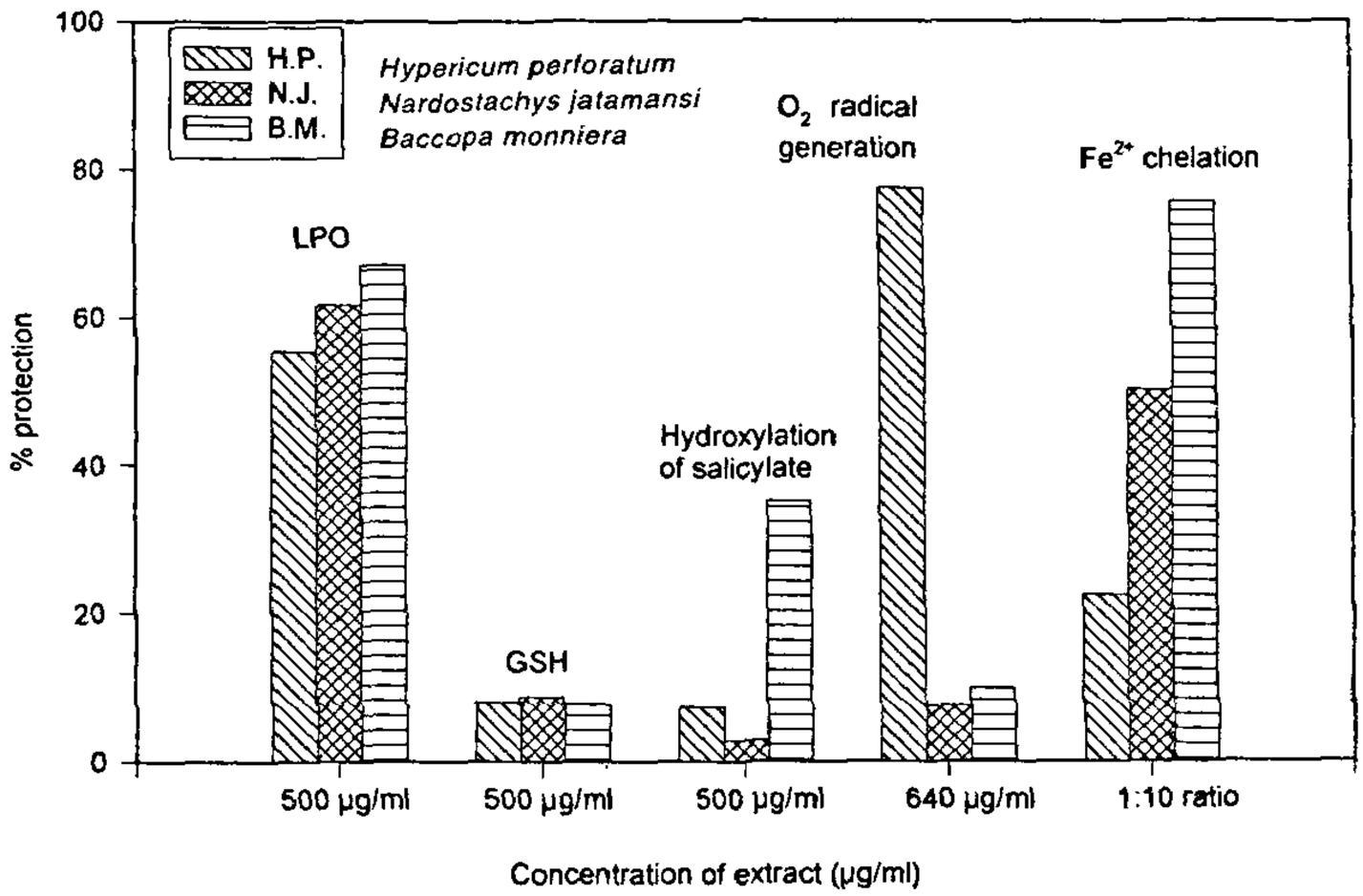

Figure 1. Effect of alcoholic extract of *Hypericum perforatum* on different antioxidant parameters, and its comparison with *B. monniera* and *N. jatamansi*: LPO–lipid peroxidation expressed as TBARS nmole/100 mg protein (control value – 441.34 ± 4.85); GSH-reduced glutathion level expressed as GSH nmole/mg protein (control value – 29.39 ± 2.14); Hydroxylation of salicylate expressed in terms of nmoles of 2,3-dihydroxybenzoate (control value – 203.99 ± 4.54); Superoxide anion radical generation expressed in terms of absorbance of formazan formation at 560 nm (control value 0.34 ± 0.008); Fe^{2+} chelation expressed in terms of absorbance of 2,2′-bipyridyl iron complex at 525 nm (control value – 0.19 ± 0.003).

Once these biological properties and its safety are proved, this wildly growing plant (St. John's wort of Indian origin) could compete in international markets.

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Nitrate content in wheat leaf blades

To boost the yield of crop plants, excessive doses of nitrogen fertilizers are applied. This results in accumulation of nitrate in the vegetative parts. The content of accumulated nitrogen varies with the environmental conditions, cultivar, and age of the plant. Further, the accumulation of nitrate is likely to be particularly high under low-light conditions, and in the winter-grown crops. Presence of higher nitrate content in leafy vegetables and forage/fodder plants is of concern, as these are consumed by human beings as well as animals[1,2]. Nitrate though is hardly toxic as such, its reduction to nitrite by bacteria in the stomach poses threat to human health, as the nitrite causes methaemoglobinemia, particularly in infants. Further, in combination with amines, nitrate also acts as a source of the carcinogenic nitrosamines[2].

To reduce the nitrate content in the leaves, the various strategies include:

(i) Looking for genotypic differences in the level of nitrate accumulation for the same level of applied nitrogen. Such differences have been reported for several crops[3,4].

(ii) Steingrover *et al.*[5], on the basis of their studies in spinach, suggested that harvesting should be carried out in the afternoon when the nitrate content in the leaf blades is low. It was further suggested that the petioles should be removed as they have four- to five-old higher nitrate content than the leaf blades.

(iii) Considering that the enzyme nitrate reductase (NR) catalyses the rate limiting step in the sequence of reactions involved

Table 1. Nitrate content (μmol g^{-1} dry weight) of leaf blades at different stages of growth in high and low NR wheat cultivars

	Days after sowing								
Variety	16	23	30	39	49	60	70	79	88
Low NR	380.3	218.0	245.8	151 8	14.8	101.2	13.2	14.4	18.3
High NR	267.7 (29.6)	151.5 (30.5)	167.9 (31.7)	139.1 (8.4)	8.2 (44.6)	52.3 (48.3)	7.2 (45.4)	8.9 (38.2)	7.9 (56.8)
LSD ($P = 0.05$)	45.5	18.3	21.3	27.4	6.6	15.0	3.8	2.4	3.2

Figures in parentheses indicate per cent decrease in nitrate accumulation in the high NR cultivar over that of the low NR cultivar.