Table 1. Chronology of flower opening and development on a branch in *G. superba*

Sl. no. of flower starting from base on a branch	Stage of flower development (days after opening of first flower)			
	I	II	III	IV
1	0	3.20	5.80	9.00
2	2.20	5.20	7.40	10.80
3	4.20	7.55	9.05	12.50
4	6.00	9.25	11.25	14.75
5	7.80	10.96	13.30	16.63
6	8.90	12.20	14.70	17.50

I, Bud-opening stage; II, Stigma-receptive stage (perianth colour is crimson at the tip, yellow in the middle and greenish at the base); III, Perianth colour is crimson up to the middle portion and yellow towards the base; IV, Perianth colour is entirely crimson with lobes starting to dry.

reported to be visiting these flowers. This limits the possibility of good cross-pollination, although wind is another factor which would be aiding in its pollination.

To overcome this problem, the species has developed the mechanism of sequential opening of its flowers. An average of six flowers develop fully on a branch and they open in a sequential manner. The first flower opens towards the base of the branch with the subsequent flowers opening away from the first flower. No two flowers on a branch were observed to be at the same stage of development at any given time (Table 1). The next flower opens only after the earlier flower has undergone pollination which is characterized by stigma losing receptivity and the perianth colour gradually changing to scarlet crimson[5].

The amount of pollen delivered by natural pollinators is likely to vary independently of the stage of flower development. However, only the pollen delivered at the stigma-receptive stage has chances of siring seeds, thereby making seed production pollen-limited. As has been reported elsewhere[5], the low seed set in the species is a consequence of pollinator limitation and the stigma-receptive stage is characterized by the perianth colour being crimson at the tip, yellow in the middle and greenish at the base. The sequential opening of flowers ensures that every flower receives adequate attention from pollinators since the number of fully opened flowers (at the stigma-receptive stage) at any given time would be considerably less compared to if all the buds on a branch would bloom simultaneously. The species seems to have made a compromise between low seed set and good quality seed (through cross-pollination). The low seed set does not affect the chances of multiplication of the species as it can still multiply through its tubers, but cross-pollinated seed ensures newer gene combinations which enable the species to colonize diverse climatic regions, from the tropics to subtemperate/sub-tropic regions. The altitudinal range of the species is up to 2100 m above mean sea level and in India it is spread from the hotter southern parts to the milder mid-hill zones of Himachal Pradesh, Jammu & Kashmir and Uttar Pradesh[1,8–10].

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Antioxidant property of the bulb of *Scilla indica*

Scilla indica (Baker), Liliaceae[1], is commonly known as 'Bhuikanda', 'jangali pyaj' (wild onion) or 'koal-kand' in Hindi and Indian squill in English. In the Ayurvedic pharmacy, a mixture of 2 species is used, *Urginea indica* Kunth and *S. indica* Baker. According to Ayurvedic literature, both the plants have similar biological property. Pharmacognostically, they have a prominent difference, i.e. *U. indica* has a tunicated bulb, whereas *S. indica* has a scaly bulb[2]. In our study we have selected samples of scaly bulb, which belong to the species *S. indica*. This plant grows wildly in the forests of Madhya Pradesh, Bundelkhand, Gwalior, Bihar, Mahabaleswar and all districts of the Tamil Nadu state, except the west coast, up to 4000 feet. It is a bulbous plant. Bulbs are ovoid, with scaly leaves of

dull white or pale colouration. Lower leaves are flat, narrow and pointed[2].

The bulb is used as anthelmentic, cardiac stimulant, digestive, diuretic, emmenagogue and expectorant. It is also used in asthma, cough and bronchitis, paralytic attacks, ailments of the heart, calculous affections, rheumatism and skin diseases[3]. Its pharmacological action is similar to digitalis, i.e. it has negative inotropic response and gives strength to the heart by reducing the rate of heart beat. Clinically, it has been observed that its response time is less than digitalis, because of its quick elimination from the body.

Fresh squill yields at least two types of glycosides–Scillarin-A, which is crystalline and Scillarin-B, which is amorphous[3]. Scillarin-A ($C_{36}H_{52}C_{13}$) is sparingly soluble in water, whereas Scillarin-B is freely soluble in water and chloroform, but insoluble in ether and alcohol. Interestingly, the natural mixture of both the glycosides, known as Scillarin, is freely soluble in water.

In this paper, we have investigated the antioxidant property of the bulb in terms of its effect on ferrous sulphate-induced lipid peroxidation and concentration of reduced glutathione in mice liver homogenate and on superoxide radical scavenging, °OH radicals and iron-chelation in a chemical model. The results show that the alcoholic extract of the bulb of *S. indica* inhibits lipid peroxidation (Figure 2). It also traps the hydroxyl radicals and chelates the free iron (Figure 3). No effect was found on superoxide radicals and the rate of glutathione oxidation (Table 2).

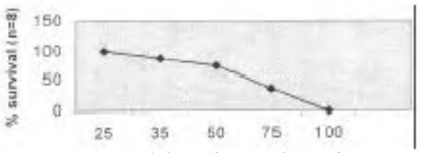

Figure 1. Toxicity of *S. indica* after 72 h of intraperitonial injection in mice.

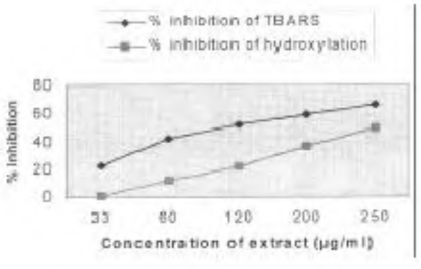

Figure 2. Effect of alcoholic extract of *S. indica* on lipid peroxidation and hydroxyl scavenging property.

Table 1. Acute toxicity of single dose of extract of *S. indica* in Swiss strain mice, observed up to 72 h of drug administration (intraperitonial injection)

Dose (mg/kg body weight)	Survival (%) (n = 8)
25	100
35	87.5
50	75
75	37.5
100	0

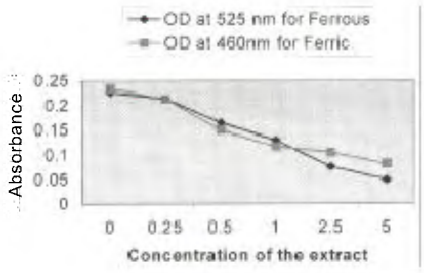

Figure 3. Effect of *S. indica* on ferrous/ ferric metal chelation.

Thiobarbituric acid (TAB), trichloroacetic acid (TCA), ferrous sulphate, ferric chloride and acetic acid were purchased from Central Drug House (Pvt) Ltd. Reduced glutathione (GSH), 5,5-dithio *bis*(2-nitro benzoic acid) (DTNB), sodium salicylate, ascorbate, riboflavin, 2,2′-bipyridyl and potassium thiocynate were obtained from Sigma Chemical Company, St. Louis, USA. All the other reagents used were of analytical grade.

Fresh bulb of *S. indica* was collected from a forest in the Madhya Pradesh region and kept in 90% alcohol for one week. 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. This extract was dissolved in double-distilled water and used for all biological experiments.

The toxicological study was done by giving intraperitonial injection of the extract, dissolved in normal saline, to the Swiss strain mice. Lipid peroxidation was assayed in the *in vitro* system in 5% liver homogenate, by estimating the thiobarbituric acid-reactive substances (TBARS) at 535 nm, according to standard method of Okawa *et al.*[4] with slight modification[5]. Lipid peroxidation was induced by the addition of 1.5 mM $FeSO_4$ to the liver homogenate. The degree of lipid peroxidation was assayed 30 min after the addition of $FeSO_4$. The results were compared with the anti-lipid peroxidative property of vitamin E and parabenzoquinone in similar conditions[6]. The ED_{50} (mg/ml) for vitamin E was 0.28 and for benzaquinone, it was 0.06. Similarly, its effect on the aerial oxidation of GSH assay was carried out by the method of Ellman[7], by using DTNB.

In chemical kinetics, its effect on superoxide anion (O_2^-) generation was assayed by monitoring the formation of blue-coloured formazan[8] at 560 nm, which is formed by the reduction of nitroblue tetrazolium (NBT)[9]. Hydroxyl radical trapping property was assayed by monitoring the hydroxylation of salicylate (2-hydroxybenzoate, 2.5 mM) by Fe^{3+} – ascorbate – H_2O_2 system[10]. The hydroxylated product (2,3-dihydroxy benzoate) was extracted with ether and determined spectro-photometrically at 510 nm. The response was compared with the standard °OH scavengers such as thiourea and mannitol[11]. ED_{50} for thiourea was 0.3 mM and for mannitol, it was 7.2 mM. Similarly its metal chelation property for ferric iron (Fe^{3+}) was estimated by using thiocynate[12], which gave an intense red-coloured compound (460 nm). Ferrous ion (Fe^{2+}) chelation was conducted by using 2,2-bipiridyl[13], which gave a pink-coloured complex (absorption maxima, 525 nm). Here different concentrations of the extract were mixed with a fixed concentration of $FeSO_4$ and $FeCl_3$ (10 µg). The mixture was incubated for 30 min and then its absorbance was recorded. The results were compared with EDTA (Table 3). In similar conditions, EDTA showed 99% chelation for both the ions. The results given here are the mean ± SD of six different experiments. Level of significance was evaluated by using Student's t test.

Alcoholic fraction of *S. indica* showed protection against the lipid peroxidation induced by ferrous sulphate in a dose-dependent manner. It showed 49% protection for hydroxyl radical-mediated hydroxylation at a concentration of 250 µg/ml. However, there was no scavenging effect on the superoxide anion radical generation. It strongly chelated Fe^{2+} (78%) and Fe^{3+} (64%) at

Table 2. Effect of the alcoholic extract of *S. indica* on different antioxidant parameters

Dose (μg/ml)	% Inhibition of TBARS value	% Inhibition of GSH oxidation	% Inhibition of hydroxylation	% Inhibition of formazan formation
33	23	0	1	0
60	42	0	12	0
120	52	0	22	0
200	59	0	36	0
250	66	0	49	0

Standard values in the control tubes without the plant extract; (1) $FeSO_4$-induced TBARS after 30 min (587.32 ± 12.81 nmol/mg); (2) Aerial oxidation of GSH after 20 min (14.32 ± 0.16); (3) Hydroxylation of salicylate after 90 min (228.97 ± 1.55 nmol); (4) Formazan formation after 4 min (OD = 0.234 ± 0.009).

Table 3. Effect of *S. indica* on Fe^{2+}/Fe^{3+} metal chelation and its comparison with EDTA

Iron: *S. indica* extract	OD at 525 nm	% chelation of Fe^{2+}	OD at 460 nm	% chelation of Fe^{3+}
1: 00	0.227 ± 0.002	0	0.234 ± 0.003	0
1: 0.25	0.213 ± 0.003	6	0.213 ± 0.002	8
1: 0.5	0.165 ± 0.002[a]	27	0.150 ± 0.001[a]	35
1: 1	0.130 ± 0.005[a]	44	0.117 ± 0.002[a]	50
1: 2.5	0.076 ± 0.004[a]	67	0.105 ± 0.004[a]	55
1: 5	0.050 ± 0.006[a]	78	0.082 ± 0.001[a]	64
EDTA (1:5)	0.002 ± 0.005[a]	98	0.007 ± 0.005[a]	97

Fe^{2+} and Fe^{3+} were quantitated by Fe^{2+}-dipyridyl complex (525 nm) and Fe^{3+}-thiocynate complex (460 nm), respectively. Values are mean ± SD of six different experiments.
Statistical comparison with control value were 0.01 mg $FeSO_4$ (group A), *P* value: $a < 0.001$.

1 : 5 ratio of iron : *S. indica*, but no response was seen on the rate of aerial oxidation of GSH, the first line of defence against free radicals. *In vivo* acute toxicity study showed that in higher doses it is highly toxic, as high mortality was noted above the dose of 50 mg/kg body weight after 72 h of intraperitonial injection.

Thus based on the above results, it could be concluded that the alcoholic extract of the bulb of *S. indica* is a potent antioxidant in a low dose range. The mechanism of action could be through the chelation of transition metals in the body and by breaking the chain reaction of lipid peroxidation by removing the hydroxyl radicals. The active fraction of this plant is highly polar, as the active fraction is soluble in water. The toxicological study clearly indicates that this plant must be used with caution, because at higher doses, it is highly toxic.

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