

Phytochemical and Pharmacological Screening of *Begonia grandis* Dryand

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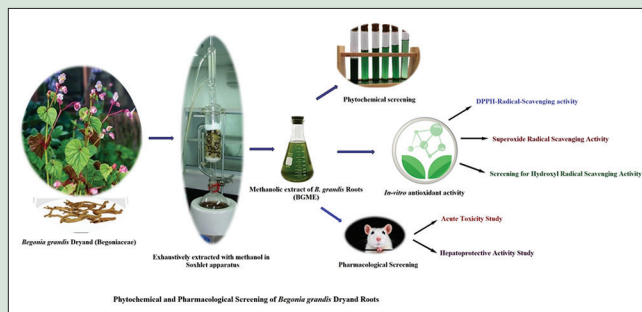
ABSTRACT

Begonia grandis Dryand (Family: *Begoniaceae*) was screened for its phytoconstituents and evaluated for its ethnomedicinal potential as an antioxidant and hepatoprotective agent. The preliminary phytochemical studies of the *B. grandis* revealed the presence of alkaloids, flavonoids, glycosides, triterpenoids, and steroids. Antioxidant effect of the methanolic extract was assessed by DPPH, superoxide, hydroxyl radical scavenging activity, and hepatoprotective effect by CCl₄-induced hepatotoxicity. The extract showed significant antioxidant and hepatoprotective effects at a dose of 400 mg/kg similar to that observed in ascorbic acid and silymarin.

Key words: Antioxidant, *Begonia grandis*, *Begoniaceae*, ethnomedicinal, hepatoprotective, silymarin

SUMMARY

- The present study provides experimental evidence for the hepatoprotective effect of *Begonia grandis* against CCl₄-induced hepatotoxicity in rats appears to be related to its antioxidant property of its phytoconstituents present in the root. Hence, the present study justified the traditional use in the treatment of liver diseases. However, additional investigations are required to determine the active constituents responsible for hepatoprotective activity.



Abbreviations Used: SGOT: Serum glutamic-oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; SALP: Serum alkaline phosphatase; T. BILL: Total bilirubin; BGME: Methanolic extract of *Begonia grandis*; mg: Milligram; kg: Kilogram; µg: Microgram; ml: Milliliter.

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INTRODUCTION

Plant medicine has been utilized successfully for thousands of years and is of great value in the field of treatment and cure of disease. In recent times, demand for herbal drugs is increasing throughout the world. According to the World Health Organization, for their primary health-care needs, 80% of the population of the developing countries depend on conventional medicines. It is necessary to investigate the rationality of folklore use of medicinal plants for various ailments in the modern scientific method in spite of sound traditional information available in the ancient literature.^[1,2]

Free radicals are highly reactive and have a wide variety of adverse effects such as DNA damage, carcinogenesis, and various degenerative disorders such as cardiovascular diseases, aging, and neurodegenerative diseases.^[3] There should be a dynamic balance in the amount of free radicals generated in the body and antioxidants to quench them and protect the body against the harmful effects. Many plant extracts and plant products are known to have significant antioxidant activity to scavenge free radicals.^[4]

Begonia grandis Dryand belongs to the family; *Begoniaceae* is a small, herbaceous, 1–4-leaved plants; root subtuberous stems are usually red, smooth slender. The root decoction is given for liver diseases and fever.^[5,6] However, the literature survey indicated no published reports on the antioxidant and hepatoprotective activities of the *Begonia*

grandis root. In view of no scientific reports on the selected plant, the authors have carried out *in vitro* antioxidant activity by DPPH, superoxide hydroxyl radical scavenging assay and hepatoprotective activity by CCl₄-induced liver toxicity model.

MATERIALS AND METHODS

Plant material

The roots of *Begonia grandis* (*Begoniaceae*) were collected from North Karnataka and were authenticated by V Chelladurai, Research Officer (Retired)-Botany, Central Council for Research in Ayurveda and Siddha, Government of India.

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Preparation of the extract

Freshly collected roots were shade-dried, coarsely powdered, and exhaustively extracted with methanol in Soxhlet apparatus for 48 h. The extracts were concentrated under controlled temperature to dryness in rotary flasks evaporator, and the percentage of yield of methanolic extract of *Begonia grandis* (BGME) was found to be 29.31% w/w.

Drugs and chemicals

Ascorbic acid (Sigma Aldrich Chemie, Germany), Riboflavin (S.D chemicals, India), silymarin (Himalaya Drug Company), serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), and bilirubin-estimated kits were purchased from Span Diagnostics, Surat, India. All others reagents and chemicals used in this study were of analytical grade purchased from local source.

Phytochemical analysis

Phytochemical screening was carried out by qualitative test for the presence of alkaloids, flavonoids, glycosides, phytosterols, tannins and triterpenoids, carbohydrates, proteins, and amino acids.^[7,8]

In vitro antioxidant activity

The BGME root was screened for antioxidant activity against DPPH, superoxide radical, and hydroxyl radicals. The percentage inhibition and 50% inhibition concentrations (IC₅₀) were calculated.

$$\% \text{ Inhibition} = \frac{[Ac - As]}{Ac} \times 100$$

DPPH radical scavenging activity

The evaluation of the DPPH radical scavenging activity was performed according to methodology described by Alessandra *et al.*^[9] BGME at various concentrations (40, 80, 120, 160, 200, 240, 280, 320, and 360 µg/ml) was reacted with 3 ml of 0.004% DPPH solution in methanol. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. The change in color from dark blue to yellow was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of vehicle in the place of BGME/ascorbic acid.

Superoxide radical scavenging activity:

Riboflavin photoreduction method was used to measure Superoxide radical scavenging activity of the extract.^[10] A mixture of EDTA (6 µM); NaCN (3 µg); riboflavin (2 µM); NBT (50 µM); KH₂PO₄ Na₂HPO₄ buffer (67 µM, PH 7.8) and 0.1 ml of different concentrations (40, 80, 120, 160, 200, 240, 280, 320 and 360 µg/ml) of BGME in a final volume of 3 ml of phosphate buffer were taken into assay tube, and the optical densities were measured at 560 nm after uniformly illuminating assay tubes with an incandescent light (40 Watt) for 15 min. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental samples.

Screening for hydroxyl radical scavenging activity

Deoxyribose degradation method is used to screen BGME's hydroxyl radical scavenging activity.^[11] Hydroxyl radicals generated from Fe³⁺-ascorbate-EDTA-H₂O₂ system (Fenton reaction) was estimated by its degradation of deoxyribose that resulted in thiobarbituric acid reactive substance (TBARS). Fenton reaction mixture consists of 200 µl of 10 mM ferrous sulfate, 200 µl of 10 mM EDTA, and 200 µl of 10 mM 2-deoxyribose and was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) and 200 µl of plant extract. Thereafter, 200 µl of 10 mM H₂O₂ was added before the incubation at 37°C for 4 h. Then, 1 ml of

this Fenton reaction mixture was treated with 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 0.8% thiobarbituric acid, and 1.5 ml of 20% acetic acid. The total volume was then made to 5 ml by adding distilled water and kept in an oil bath at 100°C for 1 hr. After cooling, 5 ml of 15:1 v/v butanol-pyridine mixture was added. The tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the TBARS was measured at 532 nm. The percentage inhibition of hydroxyl radicals by the extract was determined by comparing the absorbance values of the control and the experimental samples.

Animals and treatment

Wistar albino rats of either sex (200–250 g) which were housed under standard environmental conditions (temperature of 22°C ± 1°C with an alternating 12 h light/dark cycle and relative humidity of 60% ± 5%) were used for study. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC/ABMRCP/2012-2013/25).

Acute toxicity study

The acute toxicity study was conducted for methanol extract of the *Begonia grandis* root as per Organization for Economic Co-operation and Development (OECD) guidelines 423 (OECD 2001),^[12] using female albino rats. Mortality rate was observed at the dose levels of 5 mg, 50 mg, and 300 mg to 2 g/kg.

Hepatoprotective activity

The animals were fed with standard diet and water *ad libitum* for two weeks before and during the experimental period. The animals were divided into 6 groups containing 6 animals each. Group I served as positive control received 5% gum acacia suspension. Group II served as negative control received CCl₄ (1 ml/kg p.o.). Groups III-VI were treated with silymarin (50 mg/kg) and methanol extract of *Begonia grandis* root with 100, 200, and 400 mg/kg, respectively, for 5 days. On the 6th day, CCl₄ was administered to all groups except group-I one hour after the administration of drug. On the 7th day, blood samples were collected from retro orbital puncture and serum was separated by centrifugation for estimation of biochemical markers (SGOT, SGPT, SALP, and total bilirubin [T. BILI]) using an autoanalyzer. Then, the animals were anesthetized using ether, and liver was collected for histopathological analysis.

Histopathological studies

Liver tissue section was fixed in 10% buffered neutral formalin for 24 h. Hepatic tissues were stained with hematoxylin and were examined for histopathological studies.

Statistical analysis

All data were represented as mean ± standard error of the mean (SEM). The results were analyzed by one-way ANOVA followed by Dunnet's test. *P* < 0.05 was considered statistically significant.

RESULTS

Phytochemical analysis of methanol extract of *Begonia grandis* root

The preliminary phytochemical tests revealed the presence of alkaloids, flavonoids, glycosides, triterpenoids, and steroids [Table 1].

Effect of *Begonia grandis* extract against DPPH radicals

The free radical scavenging activity of *Begonia grandis* extract against DPPH radicals was shown in Figure 1. *Begonia grandis* extract and

Table 1: Phytochemical analysis of methanol extract of *Begonia grandis* root

Phytoconstituents	Methanol extract
Alkaloids	+
Carbohydrates	-
Flavonoids	+
Glycosides	+
Phytosterols	+
Proteins and amino acids	-
Saponins	-
Tannins	-
Triterpenoids	+

+: Present; -: Absent

ascorbic acid showed antioxidant activity in a dose-dependent manner in the range of 40–360 µg/ml and produced maximum scavenging activity at a dose of 360 µg. The IC_{50} values for *Begonia grandis* and ascorbic acid were 214.33 and 201.75 µg/ml, respectively.

Effect of *Begonia grandis* extract on the superoxide scavenging activity

The free radical scavenging activity of *Begonia grandis* extract against superoxide radical is shown in Figure 2. *Begonia grandis* extract and ascorbic acid standard showed antioxidant activity in a dose dependent manner (40–360 µg/ml) and showed significant scavenging activity at a dose of 360 µg. The IC_{50} values for extract and ascorbic acid were 246.97 and 176.95 µg/ml, respectively.

Effect of *B. grandis* extract against the hydroxyl radicals

The hydroxyl radical scavenging activity of *Begonia grandis* extract (BGME) against superoxide radical is shown in Figure 3. *Begonia grandis* extract and ascorbic acid showed antioxidant activity in a dose dependent manner in the range of 40–360 µg/ml. The IC_{50} values for *Begonia grandis* extract and ascorbic acid were 199.36 and 211.61 µg/ml, respectively.

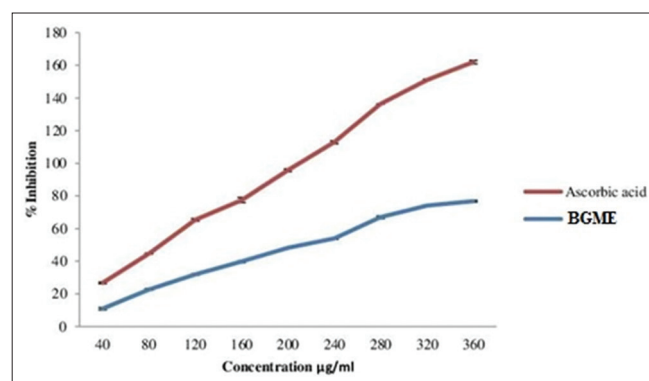
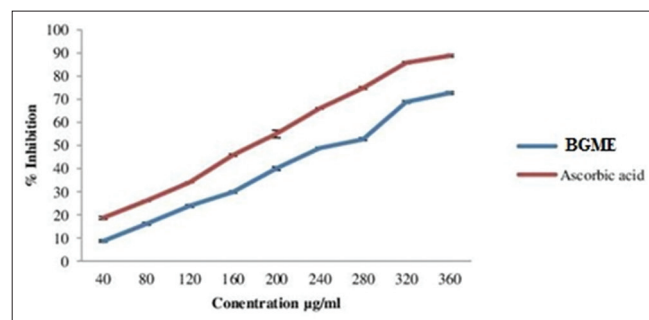
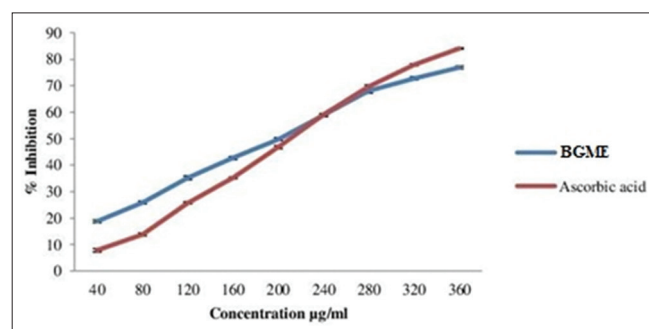
Biochemical parameters

Serum enzymes, namely SGOT, SGPT, ALP, and T. BILLI, were significantly ($P < 0.01$) increased in CCl_4 -treated control group when compared with normal group. However, levels of serum enzymes, were significantly ($P < 0.001$) decreased in extract-treated groups and silymarin when compared with CCl_4 -treated rats [Table 2].

Effect of *Begonia grandis* extract on histopathological studies

The results of light microscopy examination of the liver section of normal control, CCl_4 treated, silymarin, and extract-treated groups are shown in Figure 4a-f. Figure 4a represents control group liver cells. From that image, it can be observed that the liver cells are normal architecture with distinct hepatic cells, sinusoidal spaces, and clear central vein, a well-preserved cytoplasm with prominent nucleus. Overall, a healthy set of cells can be observed. Figure 4b shows the liver section of CCl_4 -intoxicated rats; it indicated the disarrangement of normal hepatic cells with intense centrilobular necrosis across the cells. The liver sections of these rats indicate vacuolization, fatty changes, sinusoidal hemorrhages, and dilation. Figure 4c shows liver section of silymarin treated rats, showing a normal hepatic architecture, absence of necrosis, and less visible changes as compared to control group.

The histopathological examinations of rats treated with methanolic extract of *Begonia grandis* roots at the doses of 100 mg/kg, 200 mg/kg,

**Figure 1:** DPPH radical scavenging activity**Figure 2:** Super oxide radical scavenging activity**Figure 3:** Hydroxyl radical scavenging activity

and 400 mg/kg were shown in Figures 4d, 4e, 4f, respectively, which demonstrated recovery from CCl_4 induced liver damage as evident from normal hepatocytes. The higher dose of 400 mg/kg of plant extract showed significant attenuation of inflammatory and necrotic changes, and cellular architecture of rat liver was preserved, indicating a marked protective activity similar to that observed in silymarin treated rat liver sections, and the effect was found to be dose dependant.

DISCUSSION

DPPH assay was based on reducing alcoholic DPPH solution to the nonradical form, diphenyl picryl hydrazine in the presence of a hydrogen donating antioxidant. This method, thus, helps determine the hydrogen-donating capacity of an antioxidant by measuring the reduction in the absorbance of DPPH.

Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins and

Table 2: Effect of methanol extract of *Begonia grandis* root against CCl₄-induced hepatotoxicity in albino rats

Treatment group	Serum biochemical parameters			
	SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	Total bilirubin (mg/dl)
Control (5% gum acacia 1 ml/kg p.o.)	78.99±0.45	65.77±0.47	144.84±0.45	0.47±0.06
Hepatotoxin-CCl ₄ (1 ml/kg p. o.)	526.06±0.70**	412.85±0.60***	719.11±0.66***	4.80±0.44***
Standard-silymarin (50 mg/kg)	118.15±0.52***	90.20±0.37***	200.91±0.34***	1.21±0.01**
BGME (100 mg/kg)	331.76±0.37***	203.87±0.72***	412.79±0.18***	3.85±0.26***
BGME (200 mg/kg)	197.57±0.45***	123.79±0.77***	317.97±0.56***	2.46±0.04**
BGME (400 mg/kg)	128.68±0.43***	98.91±0.80***	221.74±0.50***	1.63±0.02**

**The data was analyzed by one way ANOVA followed by Dunnet's test. n = 6 values were expressed as mean ± SEM *P < 0.05, **P < 0.001 **P < 0.001 BGME: Methanolic extract of *Begonia grandis*; SGOT: Serum glutamic-oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; SALP: Serum alkaline phosphatase

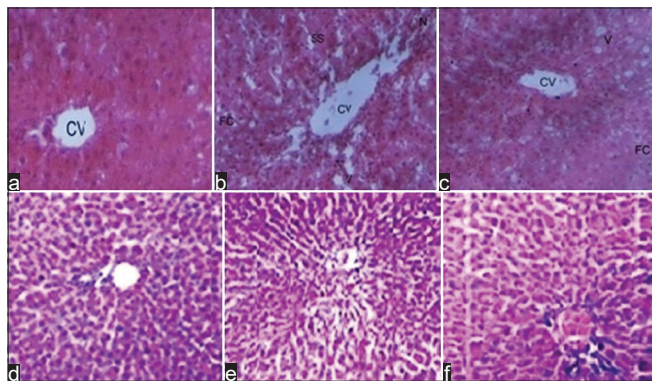


Figure 4: (a-f) Effects of methanol extract of *Begonia grandis* against CCl₄-induced liver histopathological changes in mice

DNA.^[13] Therefore, studying the scavenging activity of plant extracts on superoxide radical is most important. At low pH value, superoxide will protonate to form the perhydroxyl radical (HO₂), a more reactive oxidizing species but at physiological pH <1% will be in protonated form.^[14] The reactive oxygen radicals are unstable and likely to elicit many of the tissue damages and human diseases.^[15] The hydroxyl radicals attack adjoining biological macromolecules and cause oxidative damage to DNA, lipids, and proteins, resulting in oxidative stress-originated diseases.

The data was analyzed by one way ANOVA followed by Dunnet's test. n = 6 values were expressed as mean ± SEM *P < 0.05, **P < 0.001 **P < 0.001.

Consequently, the removal of hydroxyl radical is most likely to be one of the most effective defenses of a living body against various diseases.^[16] The hydroxylscavenging' activity of *Begonia grandis* was assessed by its ability to compete with deoxyribose for hydroxyl radical generated from the Fe²⁺/EDTA/H₂O₂ system.

Carbon tetrachloride is a hepatotoxin most widely used for the production of experimental liver toxicity. Trichloromethyl and trichloromethyl peroxy radicals are generated from CCl₄ by the mixed-malfunctions of the cytochrome P 450 oxygenase system capable of binding with proteins/lipids or abstracting a hydrogen atom from an unsaturated lipid, thus initiating lipid peroxidation. This process of lipid peroxidation can significantly damage hepatic plasma membranes. SGOT, SGPT, SALP, and T. BILI are increased during liver injury. The *Begonia grandis* extract reduced the injurious effects on hepatic tissue and physiological mechanisms that have been altered by a hepatotoxin, which is the manifestation of its protective effect.^[17]

Phytoconstituents such as flavonoids, glycosides, steroids, triterpenoids, and alkaloids were reported to exhibit hepatoprotective activity.^[18-21] The

preliminary phytochemical studies of the *Begonia grandis* revealed the presence of alkaloids, flavonoids, glycosides, triterpenoids, and steroids in the extract. The presence of these active constituents in *Begonia grandis* extract were responsible for antioxidant and hepatoprotective activity.

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Conflicts of interest

There are no conflicts of interest.

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