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Research Article

Standardization, Qualitative – Quantitative Analysis and *Invitro* Antioxidant Capacity of Methanolic Extract of *Begonia Laciniata* Roxb. Roots

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Abstract

Herbal medicines are free from side effects, adverse effects and they are economical and easily available will be beneficial for the mankind over the centuries. Numerous diseases are induced by free radicals via lipid peroxidation, protein peroxidation and DNA damage. It has been known that a variety of plant extracts have antioxidant activities to scavenge free radicals. *Begonia laciniata* Roxb. belongs to the family *Begoniaceae;* an important Indian medicinal plant being used in the folk therapy. The aim of this work was to estimate the total phenolic, flavonoid and alkaloid content and to evaluate *in vitro* antioxidant capacity of methanolic extract of *B. laciniata* Roxb. by DPPH, hydroxyl and superoxide radical scavenging assays. The radical scavenging activity was found to be concentration dependent and increased with increased concentrations and produced maximum scavenging activity at a dose of 360µg. The antioxidant capacity could be due to the presence of flavonoids, alkaloids, triterpenoids, glycosides and steroids. Further, this can be confirmed by qualitative-quantitative analysis.

1. INTRODUCTION

Since the introduction of the herbal medicines, many people were impelled to consider the importance of many herbs for treating several forms of disorders. It is no wonder, during the past decade there has been an exponential rise in the application of herbal remedies and such notable increase even continues in these days. WHO report 80% of the world population relies on the drugs which are from natural origin[1]. However, several herbal products lining in those shelves are not really standardized in terms of its effectiveness and safety.

Experimental evidence suggests that free radicals and reactive oxygen species can be involved in a higher number of diseases via lipid peroxidation, protein peroxidation and DNA damage[2,3]. It has been known that a variety of plant extracts have antioxidant activities to scavenge free radicals[4]. Pro-oxidant condition dominates either due to the increased generation of the free radicals caused by excessive oxidative stress of the current life, or due to the poor scavenging/quenching in the body caused by depletion of the dietary antioxidants[5]. So it becomes very essential to maintain balancing of oxidants in the body.

Much attention has been focused on antioxidant compounds present in edible plants, because of safety concerns associated with synthetic antioxidants. Keeping this in mind for giving scientific proof, the present work was designed and screened the *Begonia laciniata* Roxb for antioxidant capacity, which is used traditionally for treating liver disorders in Chittoor and Khammam districts of Andhra Pradesh, India[6].

2. MATERIALS AND METHODS

2.1 Materials

The roots of *Begonia laciniata* were collected from Sathupally, Kuppam and surrounding villages of Khammam and Chittoor districts of Andhra Pradesh, India and authenticated by Dr. Madhava Chetty, taxonomist and HOD of Botany, Sri Venkateswara University, Thirupathi, India (Voucher specimen No.SVU-B-12), ascorbic acid (Sigma Aldrich Chemie, Germany), Riboflavin (S.D chemicals, India), gallic acid, and catechin (Nature remedies, Bangalore, Karnataka, India). All other solvents and chemicals used were of analytical grade purchased from local source.

2.2 Preparation of Extract

Before going to extraction, the collected plant materials i.e., roots of *Begonia laciniata,* were subjected to standardization according to the guidelines of WHO for organoleptic, physiochemical, heavy metal, microbiological and pathogen analysis[7]. After collection, the plant materials were shade dried, powdered (40 mesh size) to get a coarse powder and then subjected to soxhlet extraction continued for 8 cycles (6 hrs) using methanol as a solvent. The extract was filtered and concentrated at reduced temperature on a rotary evaporator. The percentage yield was found to be 29.31% w/w and then subjected to preliminary qualitative[8-12] and quantitative (for phenolics, flavonoids and alkaloids) phytochemical analysis [Table 1].

2.3 Determination of Total Phenolic Content

The total phenolic content was estimated using the modified Folin-Ciocalteu photometric method[13]. The appropriate amount of filtered methanol extracts were oxidized with Folin-Ciocalteu's reagents and after 5 minutes was the reaction neutralized with saturated sodium carbonate. The solution was then immediately diluted to the volume of 50 ml with distilled water. The absorbance was measured at 750 nm after 90 minutes of incubation at room temperature against the blank. As the standard was used gallic acid. The total phenolic content is here expressed as g gallic acid equivalents (GAE) per 100 g of dry weight (dw) [Table 1].

2.4 Determination of Total Flavonoid Content

The total flavonoid content was measured using a modified colorimetric method[13]. The appropriate amount of extract was

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added to a test-tube together with distilled water. Then was added 5% NaNO2, after 5 minutes 10% $AlCl_3$ and after another 5 minutes 1 M NaOH followed by the addition of distilled water. The absorbance was measured against the blank at 510 nm after 15 minutes. The standard curve was prepared using different concentration of catechin. The flavonoid content was expressed as g catechin equivalents (CE) per 100 g of dry weight (dw) [Table 1].

2.5 Determination of Total Alkaloid Content

The total alkaloid content was determined according to UV-Spectrophotometer method[14]. This method is based on the reaction between alkaloid and bromocresol green. The part of the plant extract was dissolved in 2 N HCl and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of bromocresol solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was fractioned with chloroform by vigorous shaking. The fractions were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of mean ± S.E.M. [Table 1].

2.6 *In-vitro* Antioxidant Capacity

The selected plant methanolic extract was evaluated by DPPH radical scavenging assay, super oxide radical scavenging assay and hydroxyl radical scavenging assay. There is no detailed study on free radical scavenging activity on the selected plant. Hence, a detailed study was carried out.

2.6.1 Screening for 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Radical-Scavenging Activity

In this method[15], an aliquot of 3 ml of 0.004% DPPH solution in methanol and 0.1 ml of plant extract at various concentrations (40, 80, 120, 160, 200, 240, 280, 320 and 360 µg/ml) were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition activity was calculated by using the following formula [Figure 1].

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control - Absorbance of plant extract/ascorbic acid}}{\text{Absorbance of control}} \times 100$$

2.6.2 Screening for Superoxide Radical Scavenging Activity

Riboflavin photo reduction method[16] was used to screen the selected plant methanolic extract. 0.1 ml of different concentrations (40, 80, 120, 160, 200, 240, 280, 320 and 360 µg/ml) of plant extract and 0.1 ml of 6 µM ethylene diamine tetraacetic acid (EDTA) containing NaCN, 0.1 ml of 50 µM nitroblue tetrazolium, 0.05 ml of 2 µM riboflavin were transferred to a test tube, and final volume was made up to 3 ml using phosphate buffer. Then the assay tubes were uniformly illuminated with an incandescent light (40 Watt) for 15 minutes and thereafter the optical densities were measured at 560 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes [Figure 2].

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control - Absorbance of plant extract/ascorbic acid}}{\text{Absorbance of control}} \times 100$$

2.6.3 Screening for Hydroxyl Radical Scavenging Activity

Deoxyribose degradation method[17] was used to screen the different concentrations (40, 80, 120, 160, 200, 240, 280, 320 and 360 µg/ml) of selected plant extract. Fenton reaction mixture consisting of 200 µl of 10 mM ferrous sulphate ($FeSO_4$. $7H_2O$), 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 H_2O_2 was added before the incubation at 37^{o}C for 4 h. Then 1 ml of this Fenton reaction mixture was treated with 0.2 ml of 8.1% sodium dodecyl sulphate, 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^{0}C for 1 hour. After the mixture had been cooled, 5 ml of 15:1 v/v butanol-pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer containing the thiobarbituric acid reactive substances was measured at 532 nm. A control was prepared using 0.1 ml of vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of hydroxyl radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes as calculated for hydroxyl radical assay [Figure 3].

2.6.4 Calculation of 50% Inhibition Concentration

IC_{50} value was calculated for extract and positive control and obtained by plotting a graph by taking concentration on X-axis and %inhibition on Y-axis. The graph was extrapolated to find the concentration needed for 50% inhibition [Table 2, Figure 4].

3. RESULTS AND DISCUSSION

For thousands of years, natural products have played an important role throughout the world in treating and preventing human diseases. Naturopathy, the medical system indigenous to India recommends the use of plant derived products or extracts for testing various disorders apart from times immemorial in humans without preclinical evidence, which is essential to make the systems popular and scientific. The claimed usefulness of medicinal plants in several disorders might be due to their antioxidant activity. To support the use of the selected plant extract in traditional use in Ayurveda and naturopathy, the antioxidant potential of the methanolic extract of roots of *Begonia laciniata* was investigated for antioxidant property in comparison with the known antioxidant ascorbic acid. The quantities of the extract required for the *in vitro* inhibition of radical such as DPPH, superoxide and hydroxyl were compared to the known antioxidant ascorbic acid.

The DPPH radicals are widely used to investigate the scavenging activity of some natural compounds. In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to the yellow colored diphenyl-picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction. The method helps to determine the antiradical power of an antioxidant by measuring of a decrease in the absorbance of DPPH at 517 nm.

Superoxide radical is considered to play an important role in the peroxidation of lipids[18]. At low pH value superoxide will protonate to form the perhydroxyl radical ($HO_2^{\cdot}$), a more reactive oxidizing species but at physiological pH less than 1%will be in protonated form[19]. The reactive oxygen radicals are unstable and react readily with other groups or substances in the body, resulting in cell damage and hence human diseases[20]. Among the oxygen radicals specifically, the hydroxyl radical is the most reactive. It severely damages adjacent biomolecules such as all proteins, DNA, PUFA, nucleic acid, and almost any biological molecule it touches. This damage causes ageing, cancer and several diseases. Therefore, the removal of hydroxyl radical is probably one of the most effective defenses of a living body against various diseases[21].

The selected plant methanolic extract showed dose dependent scavenging activity. The standard drug ascorbic acid also showed similar dose dependent activity and produced maximum scavenging activity at a dose of 360µg [Figures 1-3]. Qualitative phytochemical studies on the selected plant revealed flavonoids, alkaloids, triterpenoids, glycosides and steroids. The presence of above said constituents in selected plant extract alone or in combination might be responsible for the observed antioxidant potential. Further, this was supported by quantitative estimation of phytoconstituents. The total phenolic, flavonoid and alkaloid contents were found to be 2.23±0.12, 0.92±1.12 and 32.24±0.54 respectively [Table 1].

4. CONCLUSION

All these scientific observations support the traditional use of *Begonia laciniata* for treating liver disorders could be due to generation of free radicals. The free radical scavenging and antioxidant properties of phytoconstituents may be the possible mechanism.

Table 1: Standardization and qualitative-quantitative analysis of roots of *Begonia laciniata*

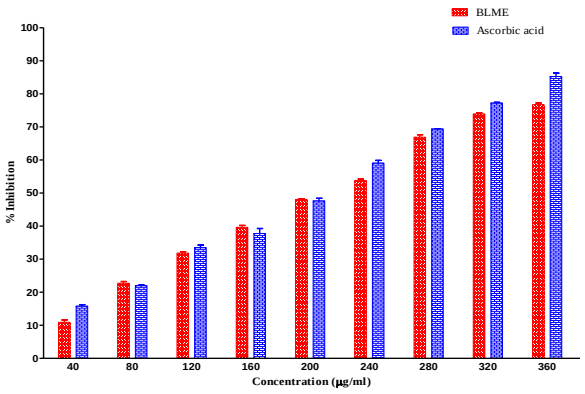
S. No.	Parameter	*Begonia laciniata* Roxb.	
1.	**Organoleptic characters**		
	Colour	Pale brownish white	
	Odour	Characteristic	
	Taste	Characteristic	
	Physical appearance	Free flowing powder	
2.	**Physiochemical characters**		
	Water soluble extractive	57.13%	
	Alcohol soluble extractive	79.27%	
	PH 1% w/v solution	5.21	
	Loss on drying	4.32%	
	Ash content	4.02%	
	Acid insoluble ash	0.63%	
	Moisture content by K.F	3.41%	
	Foreign organic matter	1.0%	
3.	**Heavy metals**		
	Lead	5 ppm	
	Arsenic	1 ppm	
	Cadmium	0.2 ppm	
	Mercury	1 ppm	
4.	**Microbiological analysis**		
	Total aerobic count	280 CFU/g	
	Yeast & mould	25 CFU/g	
5.	**Pathogen analysis**		
	E. Coli	Absent	
	Salmonella	Absent	
	Pseudomonas aeruginosa	Absent	
	Staphylococcus aureus		
6.	**Qualitative preliminary phytochemical analysis**		
	Alkaloids	**+**	
	Carbohydrates	**-**	
	Flavonoids	**+**	
	Glycosides	**+**	
	Phytosterols	**+**	
	Proteins & amino acids	**-**	
	Saponins	**-**	
	Tannins	**-**	
	Triterpenoids	**+**	
7.	**Quantitative phytochemical analysis**		
	Phenolic content (g GAE/100 g dw)	**Flavonoid content (g CE/100 g dw)**	**Alkaloid content (mg/100 g plant material)**
	2.23±0.12*	0.92±1.12*	32.24±0.54*

'+'Present, '-' Absent
*Values are means of triplicate determination ± Standard deviation

Table 2: IC_{50} values (µg/ml) of methanolic extract of *Begonia laciniata* and ascorbic acid

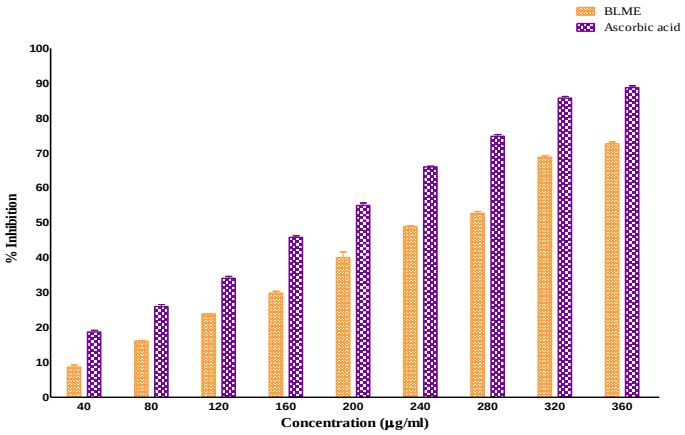
Extract/Positive control	DPPH	Superoxide	Hydroxyl
Begonia laciniata	214.33	246.97	199.36
Ascorbic acid (positive control)	201.75	176.95	211.61

Figure 1: Bar diagram of concentration-dependent percentage inhibition of DPPH radical scavenging activity



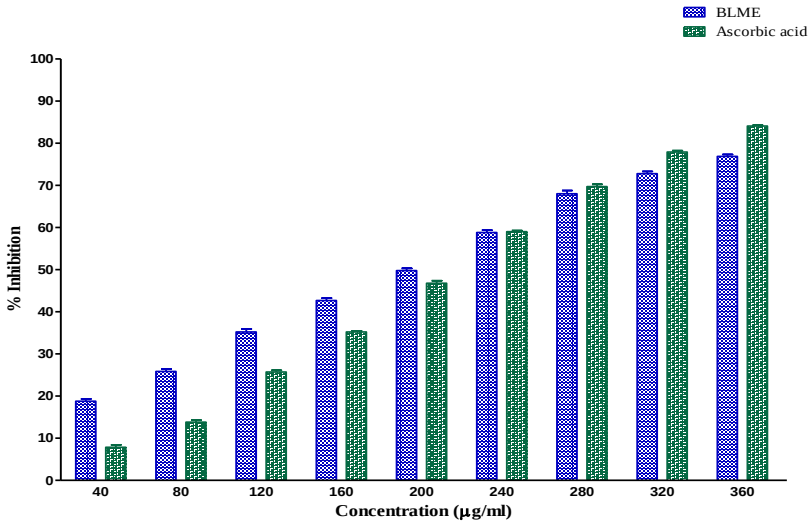
BLME: Methanolic extract of *Begonia laciniata*

Figure 2: Bar diagram of concentration-dependent percentage inhibition of superoxide radical scavenging activity



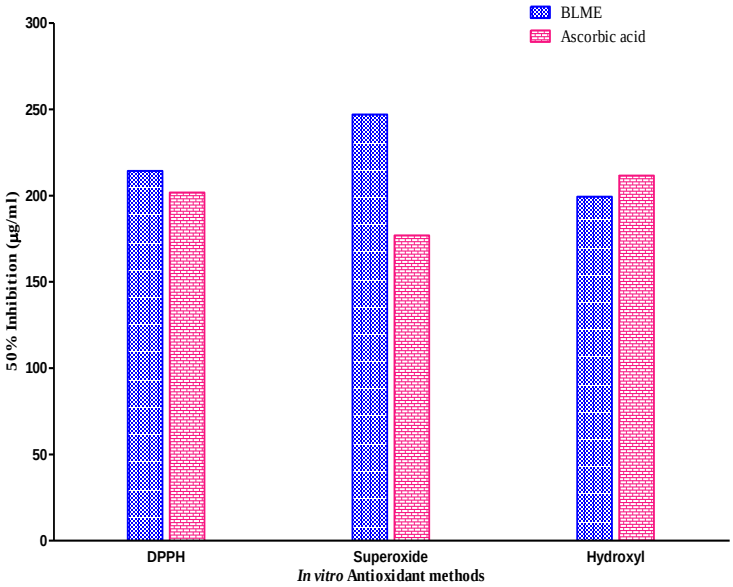
BLME: Methanolic extract of *Begonia laciniata*

Figure 3: Bar diagram of concentration-dependent percentage inhibition of hydroxyl radical scavenging activity



BLME: Methanolic extract of *Begonia laciniata*

Figure 4: Bar diagram of IC_{50} values (µg/ml) of methanolic extract of *Begonia laciniata* and ascorbic acid



BLME: Methanolic extract of *Begonia laciniata*

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