

COMPARATIVE EVALUATION OF PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES IN GREEN AND PURPLE VARIETIES OF ZALEYA DECANDRA

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ABSTRACT

Phytochemical antioxidant properties in green and red varieties of *Zaleya decandra*, collected from local area of Hyderabad city were estimated. Concentration of secondary metabolite such as flavonoids (88.5), flavonols (176.3), anthocyanins (176.3), total carotenoids (7.43) was recorded higher in green as compared to red variety (73.25), (139.46), (4.31) (4.17) $\mu\text{g/g}$ Dt.wt. However proanthocyanidins, phenols was recorded maximum in red varieties (67.91), (230.66), as compared to green variety (63.58), (135.66) $\mu\text{g/g}$ Dt.wt. β carotene 6.13, 6.06 mg/g Fr.wt in green and red varieties respectively, ascorbic acid content was same in both varieties. Enzyme peroxidase, polyphenol oxidase, glutathione reductase showed higher activity in

green variety with (731.05), (329), (12.11) units as compared to (539.5), (271.66), (9.05) units in red variety respectively. However catalase was recorded maximum in red variety (15.27) as compared to (11.06) units in green variety. ABTS and FRAP inhibition % between the two varieties did not differ much, however DPPH inhibition % was slightly higher in red variety.

KEY WORDS: Antioxidants, secondary metabolites, antioxidant assays, phytochemicals, *Zaleya decandra*, red and green varieties.

INTRODUCTION

The plant *Zaleya decandra*, is commonly used as leafy vegetable, occurring in open places, road sides, is also cultivated, commonly called as purslane (English), gadabani (Hindi) and vellai sharuni (Tamil) belongs to family aizoaceae. It is an annual branched, succulent herb in

India^[1]. Complete morphological and taxonomical description of the *Zaleya decandra* plant given by^[2]. It is distributed in the tropical and sub-tropical regions of the world, and also found abundantly in India. In the traditional systems of medicine such as Ayurveda and Unani, *Zaleya decandra* and its species are used for antimicrobial properties antidiabetic^[3] and skin disorders, anti-inflammatory, anti-hyperglycaemic, hepatoprotective and antioxidant treatment of oedema and diuretic^[4]. The plant, historically valued as a green vegetable by poor people on the Indian sub continent because of the high vitamin C content in the leaves It also has saponins^[5]. Very little work has been done on photochemical, antioxidant and enzymatic properties study in *Zaleya decandra* therefore this experiment is taken up to find out the differences in phytochemical and antioxidant properties between two coloured varieties of *Zaleya decandra*.

MATERIALS AND METHODS

Chemicals required: TPTZ, NADP, ABTS, (2,2'-azinobis(3-ethylbenzthiazoline-sulphonate)), DPPH (2,2-Diphenyl-1-picrylhydrazyl) obtained from Sigma-Aldrich and E Merck only.

The green and purple varieties of *Zaleya decandra* plants were collected from local area of Hyderabad city and washed with water, sun dried for 7 days, pulverized in mill and sieved and stored in an airtight container for further use. Ten grams of dried leaf powder were extracted by maceration in 100ml of methanol at 30°C overnight, followed by extracting and stirring with 100ml of distilled water overnight, centrifuged at 5000 rpm for 15 to 20 min and the supernatant were pooled and made up to 100ml.

Phenolic contents in the extracts were determined by the modified Folin ciocalteu method^[6]. 2ml of the leaf extract was mixed with 5ml Foline ciocalteu reagent (diluted with water 1:10v/v) and 4ml (75g/l) of sodium carbonate. The tubes were vortexed for 15sec and allowed to stand for 30min at 40°C for colour development. Absorbance was then measured at 765nm using Shimadzu 160A UV-VIS spectrophotometer. Total phenolic contents were expressed as mg/g Dry Wt Gallic acid equivalent.

Aluminium chloride colorimetric method was used for the determination of flavonoids^[7]. 0.5ml of leaf extract in methanol were separately mixed with 1.5ml of methanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. Kept at

room temperature for 30 minutes. The absorbance of the reaction mixture was recorded at 415nm with a Shimadzu 160 A UV/ Visible spectrometer .The calibration curve was prepared by preparing quercetin concentration 12.5 to 100µg/ml. Total flavonols in the plant extracts were estimated using the method of [⁸].To 2.0ml of the sample ,2.0ml of 2% AlCl₃, ethanol and 3.0ml(50g/l) sodium acetate solution were added .The absorption was read at 440nm by Shimadzu 160A UV-VIS spectrophotometer after 2.5h at 20⁰ C. The total flavonols content was calculated as quercetin (mg/g) using the following equation based on the calibration curve.

$Y=0.0255x$, $R^2 =0.9812$, where x is the absorbance and is quercetin equivalent.

Determination of proanthocyanidins was based on the procedure reported by [9]. A volume of 0.5 ml of the extract solution was mixed with 3ml of 4% vanillin-methanol solution and 1.5 ml of HCL ,the mixture was allowed to stand for 15min , the absorbance was measured with Shimadzu 160A UV-VIS Spectrophotometer at 500nm .Total proanthocyanidin content were expressed as catechin equivalent(mg/g). The total anthocyanins content was determined according to pH differential method by [10]. The leaf extract was dissolved in potassium chloride –hydrochloride acid buffer solution pH1.0 and sodium acetate trihydrate (CH₃COONa.3H₂O) buffer solution pH4.5.Methanolic extract with 3.6ml of the corresponding buffers and read against water as a blank at 510 and 700nm.Absorbance was calculated using the formula $A=[(a_{510}-A_{700})-(A_{510}-A_{700})]$.Results are expressed in mg/g Dry wt.

Carotenoids were estimated by the method of [11] One gram fresh sample was crushed with methanol and centrifuged. Residue was discarded and supernatant was concentrated to dryness. The dried extract was dissolved in 10ml of ether, 5ml of 10% methonolic KOH and the mixture was kept for one hour at room temperature in dark. The ether layer was washed with 1ml of 3% NaCl (in distilled water) for 3 times to remove alkaline methanol and dried over sodium sulphate for one hour .The absorbance of ether extract was measured at 450nm by using UV-Vis 160A double beam spectrometer ,and expressed as mg/g Fr wt. Extraction of β-carotene:10g sample (leaf) were rinsed with distilled water to remove sand, cut into pieces and lyophilized to remove the moisture content .Resulting dried samples were powdered using blender. These ground samples were extracted twice with a total volume of 100ml of 70% aqueous methanol .The mixture was shaken on an orbital shaker for 75min at

2500rpm and then filtered through Whatman No1 filter paper. The combined methanolic extract was then evaporated at 55⁰ C using water bath and dried to powder in a lyophilizer.

β Carotene was determined according to the method of [12]. The dried methanolic extract (100mg) was vigorously shaken with 10ml of acetone and hexane mixture (4:6) for 1 min. The absorbance of the filtrate was measured at λ=453,505,645 and 663nm by Shimadzu 160 A UV-VIS Spectrometer. Contents of β-Carotene were calculated according to the following equations:

$$\text{-Carotene mg/g Fr wt} = 0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453}.$$

Where A=absorbance recorded at specific wavelengths.

Extraction of enzymes: One gram of fresh plant materials was taken and placed in a pre cooled mortar and ground with 10ml of cold 0.005M Tri-HCl buffer (pH 7.0). The extract was passed through cheese cloth and centrifuged at 1000rpm for 20mins. the supernatant was used as crude enzyme for the activities of catalase, peroxidase, and polyphenol oxidase. Polyphenol oxidase and peroxidase was estimated as per the method of [13], the assay mixture contained, 2ml, of 0.1M (pH7.0) Tri-HCl buffer, 1ml of pyrogallol (0.01M) and 1ml of enzyme extract. The assay mixture was incubated for 5 minutes at 25⁰ C. The reaction was stopped by adding 1ml of 2.5NH₂SO₄. The absorbance at 425nm was recorded using Shimadzu 160 A UV-visible double beam spectrophotometer, enzyme activity was expressed as units/g Fr wt.

Peroxidase activity: The assay mixture consisted of 2ml of 0.1M (pH-7.0) Tri-HCl buffer, 1ml of H₂O₂ (0.05M) and 1ml of enzyme. The reaction mixture was incubated at 25⁰C for 5 minutes. The reaction was stopped by adding 1 ml of 2.5N H₂SO₄. The absorbance was recorded at 425nm in Shimadzu 160A UV-Visible double beam spectrophotometer. The activity was expressed as change in absorbance. Catalase activity was estimated as per the method of [14]. The reaction mixture consists of 1ml of enzyme, 2ml of hydrogen peroxide and 3ml of 0.05M Tri-HCl buffer (pH 7.0). The reaction was stopped by 1ml of 2.5 N H₂SO₄. After 5 minutes of incubation at 20⁰ C, the residual H₂O₂ was titrated with 0.05M, KMNO₄. A blank was prepared by adding 1 ml of 2.5 N H₂SO₄ initially to the reaction mixture at zero time. Catalase enzyme activity was expressed as units/g Fr wt.

The enzyme units were calculated by using the following formula; Catalase activity = $25/2 \times 0.85 \times V/W$

Where, V=difference in the titre value between control and treatment, W=Fresh weight of the sample in grams, $0.85\text{mg of H}_2\text{O}_2=1\text{ml of (0.05M). KMnO}_4$

Glutathione reductase enzyme extraction : The leaf material was weighed separately and ground in water at a concentration of 1g/5ml. The extraction were centrifuged at 1000rpm for 10 minutes and the supernatants were kept under refrigerated conditions and used for enzyme estimations. Glutathione reductase activity was determined according to the method of ^[15]. 0.2ml of sample, 1.5ml of 0.3 M phosphate buffer, pH6.8, 0.5ml of 25mM EDTA, 0.2ml of 12.5mM oxidised glutathione and 0.1 ml of 3mM NADPH was added. Decrease in absorbance was measured against that of blank at 340nm. The enzyme activity is calculated and expressed a Units/mg Fr wt.

Ferric Reducing Antioxidant Power (FRAP) assay: Soxhlet extraction method was employed for the preparation of 50% alcoholic extracts of the leaf powdered, sample was extracted for 6 hours. The collected solvent extract was evaporated, dried and stored at 4⁰ C. The FRAP reagent was prepared from 300mM of sodium acetate buffer, (pH3.6), 10mM of TPTZ solution, 40mM HCl as a solvent, 20mM (Fe^{+3}) chloride solution in a volume of ratio of 10:1:1, respectively ^[16]. The FRAP reagent was prepared freshly and warmed to 37⁰C in a water bath before use. 100 μ L of the diluted sample was added to 3ml of the FRAP reagent, The absorbance of the reaction mixture was recorded at 593nm by using Shimadzu 160 A UV-VIS double beam spectrophotometer, after 4 min in room temperature. The standard curve was constructed by using FeSO_4 solution and the results were expressed in percentage. Extraction for ABTS and DPPH Assays: The leaf materials were collected dehydrated in a chamber below 40⁰ C for 48h, powdered with a mechanical grinder and stored in an air-tight container. The methanolic leaf extracts were prepared by adding 1g of dry powders the leaf materials in 100ml of methanol, further stirring at 150rpm at ambient temperature for 3 hours. Insoluble residues from the solutions were removed by centrifugation at 8,000g for 10min (cooling centrifuge) and the clear supernatants were used for analysis. The extracts were stored at 4⁰C in plastic vials, till further use. All the estimations were performed in triplicates. The ABTS cation radical scavenging activity of the extracts was determined according to the modified method of ^[17]. A stock solution of ABTS was produced by mixing 7mM aqueous solution of ABTS with potassium persulphate (2.45mM) in the dark at ambient temperature for 12-16h before use. The radical cation solution was further diluted until the initial absorbance value of 0.7 ± 0.005 at 734 nm was reached. For assaying test samples, 0.98mL

ABTS solution was mixed with 0.02mL of the plant extracts. The decrease in absorbance was recorded at 0 min and after 6 min. Scavenging ability relative to the reaction control (without plant extracts as 100%) was calculated by using the formula:

ABTS* radical Scavenging activity (%) = [(Initial reading –final reading)/Initial reading] x100, where initial reading is absorbance at 0 min. And final reading absorbance for 6min. The DPPH radical scavenging activity was estimated by measuring the decrease in the absorbance of methanolic solution of DPPH by ^[18]. In brief , to 5mL of DPPH solution (3.3mg of DPPH in 100mL methanol), 1mL of each plant extracts were added , incubated for 30min in the dark and the absorbance (A^1) was read at 517 nm. The absorbance (A^0) of a reaction control (methanol instead of plant extract) was also recorded at the same wavelength .Ascorbic acid (5-50µg/mL was used as a standard .Scavenging ability (%) was calculated by using the formula: DPPH radical scavenging activity (%) = [(A^0 - A^1)/ A^0] \times 100, where A^0 was the absorbance of reaction control and A^1 was the absorbance of extracts or standards.

Statistical and Discussion: All results are expressed as mean \pm standard deviation .All results are means of three replicates. The data were correlation coefficient at $p < 0.05$. SPSS 15 Version was used for the statistical analysis.

RESULT AND DISCUSSION

Concentrations of flavonoids (88.5), flavonols (76.3), anthocyanins (176.3) ,total carotenoids(7.43) was recorded higher in green as compared to (73.25),(139.46),(4.31) (4.17) µg/g Dt.wt in red variety. However proanthocyanidins, phenols was recorded maximum with 67.91, 230.66(red variety) as compared to 63.58, 135.66 µg/g Dt.wt in green variety. β carotene is 6.13,6.06 mg/g Fr.wt in green and red varieties respectively, ascorbic acid content was same in both varieties. (table 1, figure1).

Table 1: Showing the content of antioxidant secondary metabolites (µg/g dry wt) in green and purple varieties of *Zaleya decandra* except total carotenoids and β carotene are expressed as mg/g fr.wt

Secondary metabolites	Green mean \pm sd	Red mean \pm sd
Flavonoids	88.5 \pm 44.04	73.25 \pm 36.46
Flavonols	176.3 \pm 87.75	139.46 \pm 69.40
Proanthocyanidins	63.58 \pm 31.65	67.91 \pm 33.77
Phenols	135.66 \pm 67.69	230.66 \pm 115.09
Anthocyanins	6.91 \pm 2.94	4.31 \pm 1.77
Total carotenoids	7.43 \pm 3.35	4.17 \pm 1.98
β carotene	6.13 \pm 3.03	6.06 \pm 3.00
Ascorbic acid	5.0 \pm 2.05	5.0 \pm 3.30

Enzyme peroxidase, polyphenol oxidase, glutathione reductase showed higher activity with (731.05), (329), (12.11) units in green variety as compared to (539.5), (271.66), (9.05) units in red variety respectively. However catalase was recorded maximum in red variety (15.27) as compared to (11.06) units in green variety. (table 2,figure 2)

Table-2: Showing Antioxidant Enzyme Activities (Units/g Fr.wt) In Green and Red Varieties of *Zaleya decandra*.

Enzymes	Green mean \pm sd	Red mean \pm sd
Peroxidase	731.05 \pm 365.35	593.5 \pm 296.61
Polyphenol oxidase	329 \pm 164.33	271.66 \pm 135.49
Catalase	11.06 \pm 5.28	15.27 \pm 7.33
Glutathione reductase	12.11 \pm 5.94	9.05 \pm 4.47

Radical scavenging activity ABTS assay inhibition % has showed very little difference between green in red variety, however in DPPH and FRAP inhibition % showed maximum (72.27),(94.27) activity in red variety as compared to (60.16) and(92.25 %) activity in green variety respectively. (table 3, figure 3)

Table 3: Showing the Antioxidant Assays Activity (Inhibition %) In Green and Red Varieties of *Zaleya decandra*.

Parameters	Green mean \pm sd	Red mean \pm sd
ABTS	98.73 \pm 49.35	98.13 \pm 49.05
DPPH	60.16 \pm 29.84	72.27 \pm 35.95
FRAP	92.25 \pm 46.04	94.27 \pm 47.08

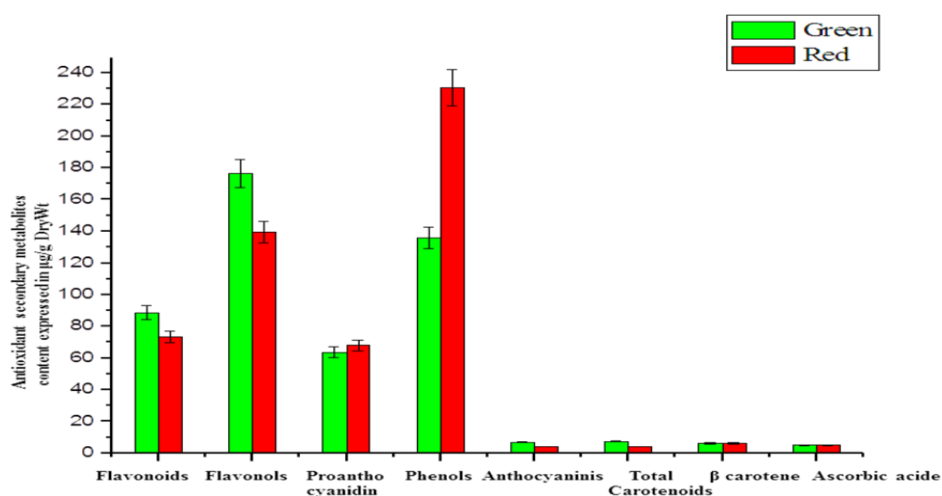


Figure 1: Showing the antioxidant secondary metabolites contents expressed in $\mu\text{g/g}$ Dry wt , except total carotenoids and β carotene expressed as mg/g Fr Wt) in green and purple varieties of *Zaleya decandra*.

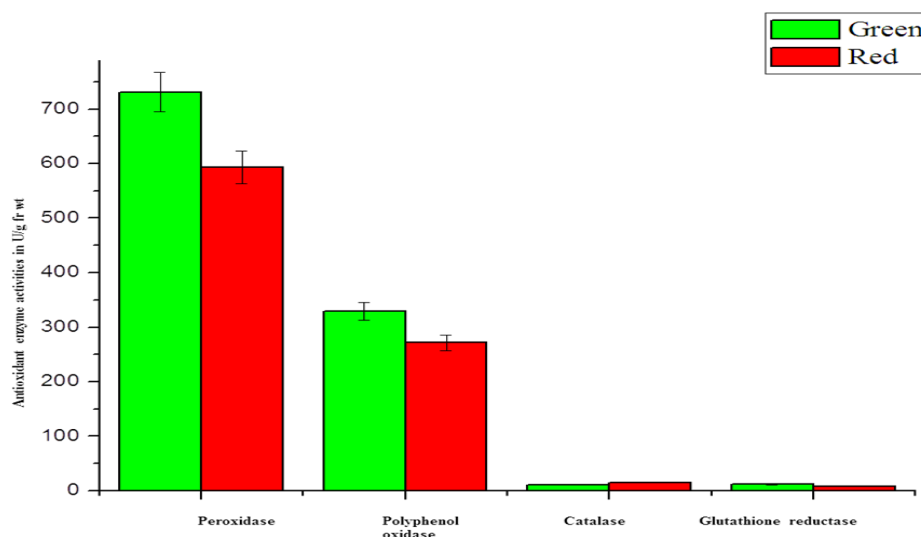


Figure 2: Showing antioxidant enzyme activities (Units /Fr wt)) in green and purple varieties of *Zaleya decandra*.

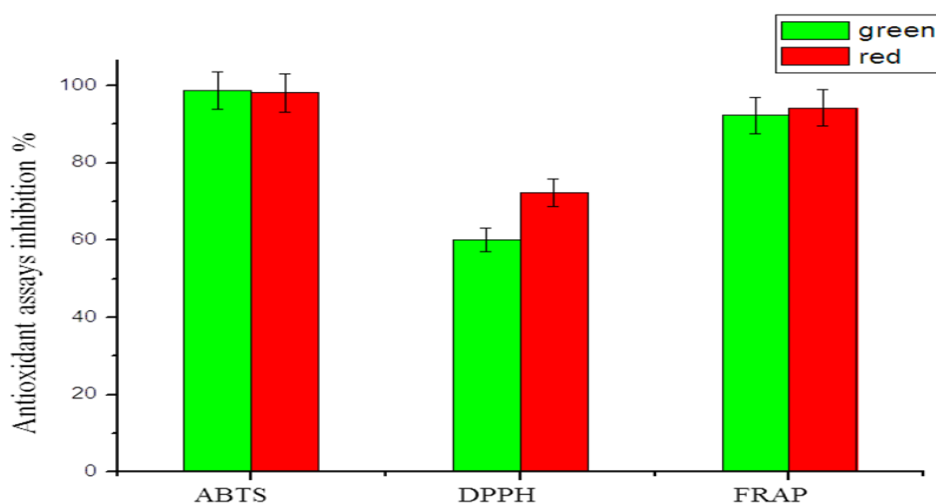


Figure 3: Showing antioxidant assays (inhibition %)) in green and purple varieties of *Zaleya decandra*.

Phytochemical and antioxidant properties were reported in *Basella alba*^[20]

CONCLUSION

Comparison of the results gave indications that flavonoids, flavonols, anthocyanins, total carotenoids were recorded higher in green as compared to red variety. However proanthocyanidins, phenols was recorded maximum in red varieties as compared to green variety. Enzyme peroxidase, polyphenol oxidase, glutathione reductase showed higher activity in green variety as compared to red variety respectively and catalase was recorded maximum in red variety as compared to green variety. FRAP and DPPH gave high inhibition percentage than ABTS in red variety.

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