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Evaluation of Antioxidant Activity of *Decalepis Hamiltonii* Tuberous Roots at Different Growth Stages and Determination of Antioxidant Substances

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Abstract
An attempt has been made to evaluate the antioxidant activity in tuberous root extracts of *Decalepis hamiltonii* taken at different growth stages. Seeds of *Decalepis hamiltonii* were sown in pots and grown over a period of two years. Tuberous roots at different growth levels were collected and evaluated for antioxidant property with DPPH (2,2-diphenyl picrylhydrazyl). There is a significant difference in antioxidant activity up to 270 days of growth and thereafter there is slight decrease in antioxidant levels. There is difference between the aqueous and methanolic extracts in antioxidant activity, aqueous extracts showed high levels of antioxidant activity when compared to methanolic extracts. Both these extracts revealed high antioxidant activity within one year of tuberous root growth when compared to later growth period. Active antioxidant substances such as phenols, flavonoids, selenium, Zinc etc were present which favored the antioxidant activity. *Decalepis hamiltonii* is found to be potently active during the entire span of life but the initial one year of growth showed high antioxidant activity.

Keywords
Decalepis hamiltonii, Antioxidant, 2H4MB and DPPH.

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INTRODUCTION:
Decalepis hamiltonii is a monogeneric climber commonly known as maredu kommulu belonging to Asclepiadaceae family which is endemic to the Deccan peninsula and Western Ghats of India. *Decalepis* is locally called as *nannari* or *sugandhapala* or *sarasaparilla.* The roots are characterized by a sarsaparilla like taste accompanied by a tengling sensation on the tongue as described in Wealth of India. Roots of *Decalepis hamiltonii* have traditionally been used as demulcent, diaphoretic, diuretic and tonic as tuberous roots contains ellagic acid [7], a volatile oil which contains 2-hydroxy-4-methoxy benzaldehyde. The drink prepared from tuberous roots is medicinal, which cools the body system gives good appetite and acts as blood purifier. The $2H_4MB$ is a major constituent responsible for antioxidant activity [5] apart from other active compounds. An antioxidant is any substance that retards or prevents deterioration or damage or destruction by oxidation of free radicals. And are heterogeneous groups of substances comprising vitamin C and E, β-carotenes, selenium and certain specific phytochemicals and minerals. Another novel antioxidant compound named decalpoline extracted from ethanolic extracts of *D.hamiltonii* roots [9]. The main characteristic of

an antioxidant is its ability to trap free radicals. The most dangerous free radicals are the atomic and molecular varieties of oxygen which is known as Reactive Oxygen Species (ROS). These free radicals may oxidize nucleic acids, proteins, lipids and can initiate degenerative diseases. Antioxidants scavenge the free radicals such as peroxide, hydro-peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms. Plant-based antioxidants derived from fruits, nuts, oils and vegetables are currently generating a lot of interest and a great deal of attention from researchers. The present study attempts to assess the antioxidant activity in tuberous roots of *Decalepis hamiltonii* grown and collected over a period of two years. Apart from antioxidant activity, determination of antioxidant compounds such as phenols, flavonoids, minerals, like selenium, zinc etc were also analyzed.

MATERIAL AND METHODS:

Decalepis hamiltonii fruit pods were collected from the Garden of Aromatic Plant Board, Andhra Pradesh for the study. Collected pods were shade dried; seeds were sown in pots in three replicates for each for germination and growth for a period of two years. Tuberous roots were collected starting from third month up to two years with a time interval of 30 days each. All chemicals and solvents used were of Analytical grade.

Preparation of the Extract

The roots of *Decalepis hamiltonii* were excised and collected from the third month of growth of the plants. The tubers were cleaned off extraneous matter and soil with tap water and later with distilled water thrice. They were then surface sterilized with 60% alcohol followed by washing with distilled water, blotted with sterile blotting paper and dried at room temperature. The ground material was soaked in methanol and water for 48 hour with stirring every ½ h using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1.

Determination of Total Polyphenols and active mineral constituents

The total phenolic content of the tuberous root extract was determined with the Folin-Ciocalteau assay [15]. The data for the total phenolic contents were expressed as milligrams of Gallic acid equivalents (GAE) per 100 grams dry mass (mg QE/100g DW).

The total flavonoid content was measured with an alumimum chloride colorimetric assay [6]. The data for the total flavonoid contents were expressed as milligrams of Quercetin equivalents (QE) per 100 grams dry mass (mg GAE/100g DW).

Mineral elements such as Selenium, Cobalt, Zinc, Copper, Manganese and Chromium were analyzed. The method employed for the digestion of the dry tuberous root tissue is dry digestion [2]. Weighed sample placed in digestion vessel, acid added and mixture heated at 80°C for several hours. After digestion, samples were diluted to specific volume and analyzed directly using Atomic Absorption Spectrophotometer (A Analyst 700).

DPPH (2,2-diphenyl-1-picryl hydrazyl) Radical Scavenging Assay

The aqueous and methanolic extract of *Decalepis hamiltonii* tuberous roots were studied for antioxidant potential using DPPH radical scavenging assay [13]. The aqueous and methanolic extracts of tuberous roots collected from 150 days, 180 days, 210 days, 240 days, and 300 days up to 2 years were analyzed with DPPH assay after preparing the extracts. Reaction mixture was prepared and incubated in the dark for 15min, there after the optical density recorded at 517nm against the blank after 30 mins., of incubation in dark. The degree of discoloration indicates the scavenging potentials of the extracts. The antioxidants react with DPPH which is a stable free radical and convert 1,1 dihydroxy 2-picryhydrazine. The decrease in OD on addition of test samples in relation to the control was used to calculate the antioxidants activity as percentage inhibition (%) of DPPH radical. The capability of scavenging DPPH radical was calculated the following equation.

$$\text{DPPH Scavenged (\%)} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Where "A control" is the absorbance of the control reaction and "A test" is the absorbance of the sample of the extracts. Further IC_{50} value is calculated. Lower the IC_{50} value indicates higher antioxidant activity.IC_{50} value denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

RESULTS AND DISCUSSIONS:

Tuberous root showed high antioxidant activity measured as scavenging of DPPH for methanolic and aqueous extracts. Both the extracts showed high antioxidant activity. Similar observation was reported earlier by Anup Srivastava, 2006. Even Ponnuswamy Samydurai and Vellaichamay Thangapandian, 2012 and Palanisamy Prakash and Rengaranjan Manivasagaperumal, 2017 also showed that methanolic root extract showed higher percentage of antioxidant activity in *Decalepis hamiltonii*. In comparison, aqueous extract showed higher antioxidant activity than methanolic extract

[Figure: I] Antioxidant activity is due to the presence of specific phenolic compounds and other active ingredients. Total phenolic and flavonoids content found to be 0.73±0.40 and 0.81±0.071[Table: I] which were correlating with the results of Ponnusamy samydurai *et al*., 2012 and according to Chandhini Rajendran, 2014 phytochemical analysis revealed the presence of active ingredients in roots. The other active minerals compounds responsible for antioxidant activity presented in the table [Table: II]. The results of the DPPH assay were expressed in percentage (%) of inhibition of DPPH free scavenging activity of aqueous and methanolic tuberous root extracts for a period of two years [Table: III]. The extent of decrease in the absorbance of DPPH in the presence of antioxidants correlates with the free radical scavenging potential of the antioxidant. These scavenging activities might be due to the presence of different contents. The study indicates that, there is a significant difference in the tuberous roots extracts which were analyzed at different stages of its growth. Tuberous roots analyzed within one year where showing higher antioxidant activity (Methonolic extact IC_{50} 43.84±0.25) than after one year (Methanolic extract IC_{50} 68.33±0.42) of growth period. *Decalepis hamiltonii* is found to be potently very active during first 270 days of the growth and after that there is a decrease in antioxidant activity levels. Similar studies were carried out by Boominathan *et al*., 2018, Umesh, 2014 and Nayaka *et al*., 2010 in *Decalepis hamiltonii* tuberous root extracts in evaluating the antioxidant activity.

Figure: [I] DPPH scavenging activity of aqueous and methanolic extract of *Decalepis hamiltonii* in comparison to Ascorbic acid.

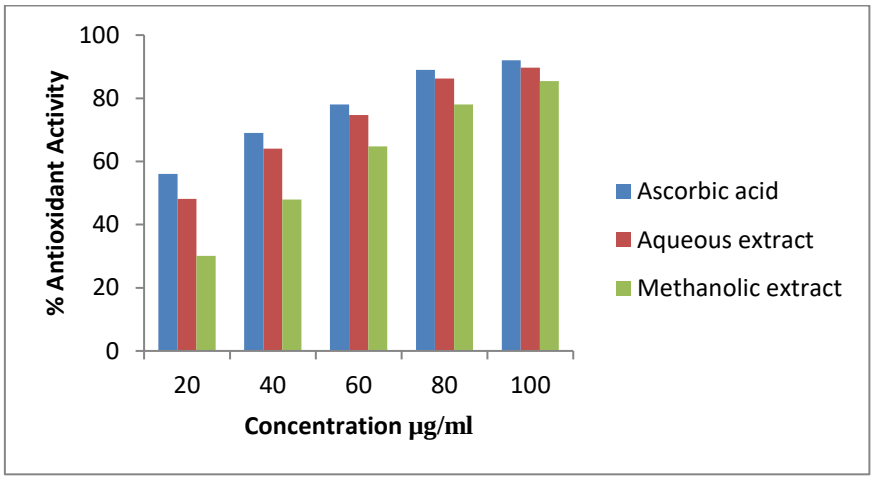

Table: [I]. Total flavonoid and phenolic contents of *Decalepis hamiltonii* tuberous root extract

S.No.	Sample	Total phenol content (mg GAE/100gm DW)	Total flavonoid content(mg QE/100gm DW)
1.	Standard	0.7616±0.392	0.896±0.100
2.	*Decalepis hamiltonii*	0.73±0.40	0.81±0.071

*Results were presented as mean ± SD (n=3)

Table: [II]. Quantitative analysis of minerals expressed in mean ± SD of *Decalepis hamiltonii*

S.No.	Mineral	Sample mg/L
1.	Copper	0.048±0.006
2.	Cobalt	0.0119±0.007
3.	Chromium	0.129±0.038
4.	Selenium	1.225±0.145
5.	Manganese	0.886±0.292
6.	Zinc	0.247±0.083

Table: [III] IC_{50} values of Methanolic and Water extracts of tuberous roots taken at different intervals of growth period.

S.No.	No. of Days of tuberous root growth	IC_{50} value µg/ml Methanol extract	IC_{50} value µg/ml water extract
1	150	44.01	44.14
2	180	43.33	44.14
3	210	43.29	47.34
4	240	44.74	48.45
5	270	43.84	47.31
6	300	68.33	51.91
7	330	68.98	51.62
8	360	69.41	53.57
9	390	70.01	55.01
10	Ascorbic acid	**41.52**	**40.71**

CONCLUSIONS:

Natural antioxidants are thought to prevent or slow down free radical induced oxidative stress and therefore possess health promoting potential. The phytochemical analysis of the crude extracts of *Decalepis hamiltonii* tuberous roots indicated the presence of major phytochemicals responsible for antioxidant activity. The antioxidant activity of the tuberous roots analyzed showed a significant difference with the increasing growth. Active ingredients such as flavonoids, Selenium, Magnesium are thought to contribute to the antioxidant potential of the tuberous roots. Still a detail and in depth study is necessary to find the active ingredients with regards to the mineral constituents present in *Decalepis hamiltonii*.

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