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Study of Bioactive components in Decalepis hamiltonii invitro

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ABSTRACT : *Decalepis hamiltonii is a climbing shrub with aromatic tuberous roots distributed in Southern parts of Peninsular India. Its tuberous roots are widely used as a health drink and are well known for its medicinal properties. D. hamiltonii is one of the important plants in Ayurvedic system of medicine in India and are used in curing various diseases like stomach disorders, gastric ulcers and to stimulate appetite. It is used as a food and health drinks, phytochemistry, pharmacology and conservation is required. The tubers have reported antimicrobial, antipyretic, antiulcer, antidiabetic, antioxidant, anti-inflammatory, chemoprotective, cytoprotective, insecticidal, neuroprotective and hepatoprotective activities. Natural seed germination is very low in this species, that is, 6% because of hard seed coat, less seed dormancy period and due to self-incompatibility. In vivo and in vitro conservation methods have been standardized to this endangered plant by developing rapid micro propagation techniques. Among different plant growth regulators tested, 6-benzylaminopurine (BAP) played a significant role in shoot multiplication, whereas, indole-3- butyric acid (IBA) and silver nitrate (AgNO3) influence rooting efficiently.*

In the present study, Antioxidant activity, total phenol and flavonoids estimation of this Tuber part of Decalepis hamiltonii was evaluated for free radical scavenging activity using 2,2-diphenylpicryl-1-picrylhydrazyl (DPPH),The FT-IR spectra results shows that ***quercitin*** *is present in this plant. Hence this tissue culture plant has rich source of antioxidant properties. If this plant started growing by micro propagation, it could be commercially viable as a nutritive and therapeutic source.*

KEYWORDS - *Decalepis hamiltonii, Micro propagation, total flavanoid content (TFC), Total Polyphenol content, Antioxidant activity, Quercitin, FTIR*

I. INTRODUCTION

Decalepis hamiltonii (Wight & Arn) is the sole species of plant in the genus Decalepis. It is endemic and endangered species of Peninsular India. It grows in between the rocks and places where there is thick vegetation. Milky latex is present in the entire plant. Morphologically as well as chemically the plant resembles African liane called Mondia whitei (Hook f.) Skeels. Both have similar ethnobotanical uses and presence of 2 - hydroxy-4-methoxybenzaldehyde an isomer of vanillin is reported from the plants [1].

It prefers to grow along rocky slopes, big rock boulders and rocky crevices and small mounds where there is thick vegetation at an altitude from 300 to 1200 m. It has good medicinal importance and used in wide drug preparations. Pharmacognostical study of roots of *D. hamiltonii* was investigated for proper identification during drug preparation [2]. The pharmacognosy, phytochemistry and pharmacology of *D. hamiltonii* were reviewed [3].

People procure and habitually carry the roots with them and chew the same whenever the digestion may seek relief. Be-sides treating indigestion the roots have been used locally to stimulate the appetite and to relieve flatulence and act as a general tonic [4]. The natural antioxidants may have free-radical scavengers, reducing agents, potential complexes of peroxidant metals, quenchers of singlet oxygen [5]. The antioxidants can interfere with the oxidation process by reacting with free radicals [6]. Recently interest has been in-creased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity [7].

A variety of analytical tools is available to study protein conformation. Of these methods, Fourier Transform Infrared Spectrometry (FTIR) has proven to be the most versatile [8]. It allows analysis of protein conformation in a diverse range of environments, e.g. upon binding to membranes [9], at the air-water interface [10], in organic solvents [11], or in the dehydrated state [12 -13]. The versatility of FTIR is based on the long wavelength of the radiation, which minimizes scattering problems. Fourier transform infrared (FTIR) analysis was carried out to determine the involvement of the type of functional groups [14].

II. MATERIALS AND METHODS

2.1 Preparation of explants

Healthy plants of *Decalepis hamiltonii* Wight & Arn, were collected from Shivamogga, Karnataka state, India, subjected for micro propagation. Axillary buds of *D. hamiltonii* were washed under running tap water to remove soil and other superficial contamination. Single bud explants (1 cm each) with upper portion were washed with Tween 20 (5% v/v) for 5 min followed by thorough washing under running tap water for 15 min. The explants were surface sterilized with 0.15% (w/v) mercuric chloride for 3 to 5 min and later rinsed 4 or 5 times with sterile distilled water [15].

For this experiment shoot induction stage MS basal medium with 3% of sucrose (w/v) was used.Explants were cultured in conical flasks (150 ml) covered with non-absorbent cotton plugs and the medium was subsequently autoclaved at a temperature of 121°C for 15 min. The growth regulators, N^6-benzyladenine (BA; 1.1 µ*M*), gibberellic acid (GA_3; 5.8 µ*M*) were added to MS basal medium and then phloroglucinol (PG; 80-1600 µ*M*) tried individually and in combination to obtain the most suitable level for proliferation of shoots in established explants [16].

The cultures were kept at an incubation temperature of $25^0 \pm 2^0$C and light for 16 hr/day using fluorescent lights for 30 days. Single shoot explant (2 cm) with a node or nodal explants was kept in flask. Rooted plantlets after 45days growth were removed from the medium, freed of agar by washing in running tap water and planted in sand: compost mixture (1:2) for hardening for 30 days at about 80% RH under polyethylene hoods in the greenhouse and then transplanted in the field [17].

The Tuber parts collected and they were surface sterilized first with 200 ml of 70% (v/v) alcohol for 5 sec, by washing with sterile water thrice, and then blotted with sterilized blotting paper. About 100 g each plant part were grounded into paste in mixer and used for extraction in dichloromethane. Dichloromethane was utilized for extraction since it is permitted for extraction of oleoresins and other food constituents. Extracts were pooled and were separated with water in separating funnel. This extract was dried in vacuum and resuspended in ethanol (1 mg mL-1) and used for further experiment at desired concentration. The dichloromethane extract dissolved in ethanol was subjected to antioxidant activity / Antiradical activity test [18].

2.2 Antiradical activity test

The antiradical activity of the extracts was estimated as 0.3mM solution of DPPH radical solution in ethanol 90% was prepared and then 1 ml of this solution was mixed with 2.5 ml of different concentrations of each extract (sample). After 30 min incubation in dark and at room temperature, absorbance (A) was measured at 518 nm in a SHIMADZU Multispect-1501 spectrophotometer [19].
The percentage of the radical scavenging activity (RSA) was calculated by the following equation:

$$RSA\% = \frac{[A\ control - (A\ sample - A\ blank)]}{A\ control} \times 100$$

Ethanol 90% (1 ml) plus each sample solution (2.5 ml) was used as a blank. DPPH solution (1 ml) plus ethanol 90% (2.5 ml) was used as a negative control. Rutin Solution (at the concentrations of 100, 50, 25, 10, 5, 2.5 µg ml-1) was used as a positive control. The IC50 value for each sample, defined as the concentration of the test sample leading to 50% reduction of the initial DPPH concentration, was calculated from the non linear regression curve of Log concentration of the test extract (µg ml-1) against the mean percentage of the radical scavenging activity.

2.3 Total Polyphenol content

The total phenol content was determined [20], with Aliquots (250 µl) of each extracts were taken in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5ml of Folin-Ciocalteu phenol reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added sequentially in each tube. Rapidly after vortexing the reaction mixture, the test tubes were placed in dark for 40 minutes and the absorbance was

recorded at 725nm against reagent blank. All determinations were carried out in triplicate. The total phenolic compound in the extract in gallic acid equivalent (GAE) was calculated [21].

2.4 Amount of total flavanoid content

The determination of the total flavanoid content (TFC) [19], with 2.5 ml of each extract solution was mixed with 2.5 ml AlCl3 reagent in ethanol 90% and allowed to stand for 40 min at room temperature. After that, the absorbance of the mixture at 415 nm was measured with a SHIMADZU Multispect-1501 spectrophotometer.Ethanol 90% (2.5 ml) plus sample solution (2.5 ml) was used as a blank. Rutin was used as a reference compound. The TFC for each extract [as µg rutin equivalents (RE) / mg of extract] was determined on the basis of the linear calibration curve of rutin (absorbance versus rutin concentration) [21].

2.5 FT-IR Spectra Analysis

Standard quercitin and compound obtained were compared with using FT-IR spectra for identification and comparing purity. The FT-IR spectrum was recorded on a Cary 640 in the form of KBr discs. The resolution was in 2 cm^{-1} and the scanning range was 4000-500 cm^{-1}.

III. RESULTS

The extract was found to contain flavor compound 2-hydroxy-4-methoxy benzaldehyde (2H4MB), which was identified by TLC [22]. The extracts were analyzed for 2H4MB content by spotting the root extracts on **TLC plate** along with standard. **Rf (0.47)** of sample coinciding with that of standard 2H4MB was eluted in solvent and UV spectrum was measured in spectrophotometer UV-160.

3.1 Total flavonoid /phenolic content and Anti oxidant activities

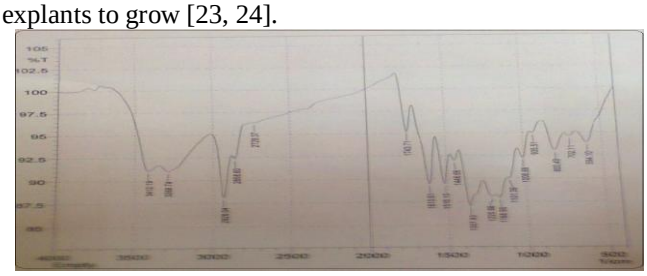
Total flavonoid content	$26.02 \pm 0.60^{\#}$
Total Phenolic content	$70.24 \pm 0.16^{@}$
Anti-oxidant activities	$62.54 \pm 0.32^{*}$
IC_{50} by DPPH method (µg/ml)	46.00^{+}

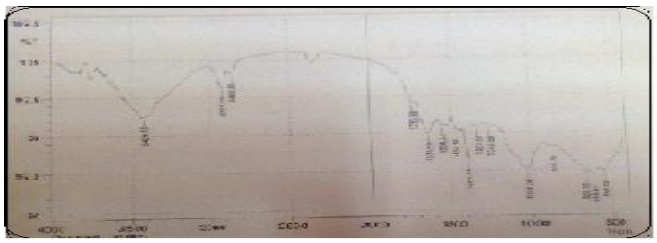
***Note:** The IC_{50} value of the positive control, rutin, was measured as 35.66 (34.21-36.68) µg ml-1. $P < 0.005$

1. The IC50 values are presented with their respective 95% confidence limits.
2. The TFC /TPC values are mean ± SEM of three determinations.
3. $P < 0.001$*#+@ represents homogenous subsets (one way ANOVA followed by Tuckey's post test).

3.2 FT-IR SPECTRA ANALYSIS

Standard quercitin and compound obtained were compared with using FT-IR spectra for identification and comparing purity. The FT-IR spectra result shows that **quercitin** is present in the *Decalepis hamiltonii* (*invitro).* These results are promissable, since it happens to be due to some of the ingredients of media provided for explants to grow [23, 24].





Quercitin Standard

Quercetin	OH	3412, 3288
	C=O Stretch	**1610,1743**
	Ar-O Stretch	**1357**

Decalepis hamiltonii* roots (*invitro)

Decalepis (invitro)	OH	3429.55
	C-H	2924
	C-O	1024
	C=O	**1639**

IV. DISCUSSION

Decalepis hamiltonii roots (*invitro)* exhibited higher total antioxidant activity of 62.54 µmol/g. The total Phenolic content is 70.24, Total flavonoid content is 26.02 and Anti oxidant activity IC_{50} by DPPH method 46.00 µg/ml, this implies that the plant extract may be useful for treating radical related pathological damage especially at higher concentration [25]. Polyphenols are the major plant compounds with antioxidant activity, although they are not the only ones. The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties [26, 27], which can play an important role in adsorbing and neutralizing free radicals.In this present study the FT-IR spectra result shows that **quercitin** is present in the *Decalepis hamiltonii* (*invitro)* C=O Stretch.

In *Decalepis hamiltonii* the tuberous root extract contain the flavor compound 2-Hydroxy 4-methoxybenzaldehyde as a major compound (97%) which is extractable by steam distillation method followed by using dichloro methane [28]. Even in this root extract also analyzed for 2H4MB content by spotting the root extracts on **TLC plate** along with standard. **Rf (0.47)**

In one of the study, the antitumor effect of methanolic extract of *Decalepis hamiltonii* was assessed by elevating tumor weight and hematological parameters of DLA tumor bearing mice. The antitumor activity of methanolic extract of *Decalepis hamiltonii* is probably due to its flavanoid content [29].

The roots of *D. hamiltonii* is a novel bioactive molecule as evident from the spectroscopic characterization and shows insecticidal activity against stored product insects by contact bioassay. Since the compound derived from an edible source with a long history of human use, it appears to be safe to mammals (Shereen, 2005). Hence, the compound from *D. hamiltonii* could belong to a new class of bioinsecticide and may serve as a promising grain protectant of natural origin [30].

The extract was found to contain HMB, medullary portion was found to contain high amount of HMB followed by peal which also showed significant antioxidant activity, but low when compared with the standard pure HMB. In *D. hamiltonii* among all the solvent extracts tested the methanolic and aqueous extracts showed high antioxidant activity measured as scavenging of DPPH [31]. Isolation of new anti oxidant compound ellagic acid from the aqueous extract of roots of *D. hamiltonii* and its antioxidant and cytoprotective effect were reported [32]. The aqueous extract of *D. hamiltonii* has boosted the antioxidant status in rat brain and liver [33].

The cytoprotective and antioxidant activity of free, conjugated and insoluble-bound phenolic acids of *D. hamiltonii* was investigated. A total phenol content of 20.72, 7.97 and 11.52 mg gallic acid equivalents (GAE)/g for free, conjugated and insoluble bound phenolic acid extracts, respectively were identified. SRCP also showed higher reducing power and DNA protection property [34]. Superior antioxidant activity of *Decalepis* rhizome of all the selected medicinal plants were reported [35].

In one of the study shows that poly-phenols content in the aqueous root extracts of *Decalepis hamiltonii* is high and these extracts exhibit strong antioxidant activities compared to that of the standard compounds such as α-Tocoperol, Rutin and Butylated hydroxytoluene (BHT) [36]. Hence, this investigation suggested that the plant naturally having rich source of antioxidants could be used in the prevention of free radical diseases and general health tonic.

V. CONCLUSION

To conclude from the present study, Antioxidant activity, total phenol and flavonoids estimation of this Tuber part of *Decalepis hamiltonii - invitro* was evaluated by free radical scavenging activity using 2, 2-diphenylpicryl-1-picrylhydrazyl (DPPH), The FT-IR spectra results shows that **quercitin** is present in this plant. Hence this tissue culture plant has rich source of antioxidant properties. If this plant started growing by micro propagation, it could be commercially viable as a nutritive and therapeutic source.

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