ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.4.L64-72

International Journal of Life science and Pharma Research

Research Article

Botany for Medicinal science

Phytochemical Screening, GC-MS & HPLC Analysis of Decalepis arayalpathra (Joseph & Chandras) an Endangered Medicinal Plant

[*1]Ashalatha K S and [2]Raveesha H R

[1] *Faculty (Guest faculty), Department of Botany, Jnanabharathi Campus, Bangalore University, Bengaluru - 560 056 Karnataka, India*

[2]*Professor, Department of Botany, Jnanabharathi Campus, Bangalore University, Bengaluru - 560 056 Karnataka, India*

Abstract: *Decalepis arayalpathra* is one of the important medicinal plants with milky latex, which grows on the exposed rocky slopes at an 800-1200 m elevation. It is endemic to the southern region of the Western Ghats chiefly in the Agasthyamalai region of Tamil Nadu. The plant has been used by traditional people to treat various diseases such as peptic ulcers, cancer like affliction, skin diseases etc. The moniliform tuberous roots have special fragrance like vanilla, because the roots contain different compounds such as α-amyrin acetate, ß-sitosterol, naringenin, kaempferol and aromadendrin especially in 2-hydroxy 4-methoxy benzaldehyde (2H4MB). The present study was aimed to examine the phytochemical screening and identification of bioactive compounds of *Decalepis arayalpathra* by GC-MS & HPLC analysis. The phytochemical analysis was carried out by using standard methods. Preliminary phytochemical screening showed major bioactive compounds such as alkaloids, flavonoids, proteins, steroids, terpenoids, quinones, resins, phenols, tannins, volatile oils and other phytocompounds. FTIR analysis identified various bioactive components and those were confirmed through GC-MS analysis. 2-hydroxy 4-methoxybenzaldehyde (2H4MB) and ß-sitosterol were quantified by HPLC analysis. Further studies have to be carried out in the *in-vitro* multiplication, isolation and characterization of compounds to enhance the root flavouring complexes for medicinal, food and beverage industries.

Keywords: *Decalepis arayalpathra,* methanolic extracts, phytochemicals, vanillin, ß-sitosterol, HPLC.

***Corresponding Author**

Ashalatha K S , Department of Botany, Jnanabharathi Campus, Bangalore University, Bengaluru - 560 056 Karnataka, India

Recieved On 24 February 2020
Revised On 06 April 2020
Accepted On 11 June 2020
Published On 05 October 2020

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Ashalatha K S and Raveesha H R , Phytochemical Screening, GC-MS & HPLC Analysis of Decalepis Arayalpathra (Joseph & Chandras) an Endangered Medicinal Plant.(2020).Int. J. Life Sci. Pharma Res.10(4), L64-72 http://dx.doi.org/10.22376/ijpbs/lpr.2020.10.4.L64-72

1. INTRODUCTION

Folk medicine is the mother of all other systems of modern medicines, it displays the significant role in rural sectors and it is the only source of health care[1]. *Decalepis arayalpathra* (Joseph and Chandras) is one of the important traditional medicinal plants, it is a perennial woody laticiferous shrub, belongs to the family Apocynaceae [2,3]. The plant was first described by Joseph and Chandrasekharan, Botanical Survey of India in 1978. It grows in clumps without any firm holding and is always exposed to heavy wind velocity, high temperature and moderately good rain fall almost throughout the year[4]. Ayurvedic literature indicates that the plant may be the divine drug named variously as Mritha Sanjeevini (the drug that can revive unconscious or dead) or Sanjeevini, Thampra Rasayani in the Oushadha Nighantu (Ayurvedic Oushadhi Nighandu 1906)[5]. The moniliform tuberous roots of *Decalepis arayalpathra* (*D. arayalpathra*) are highly aromatic and are consumed as pickles and used as a popular cool drink known as Nannari[6-8]. Tuberous root oil of this plant contains higher content of 2-hydroxy 4-methoxybenzaldehyde, an important flavour compound [4]. This compound possesses antimicrobial and antioxidant activities against seed borne fungal pathogens[9]. It is used as a flavouring agent in the preparation of soft drink and bakery products[10]. Based on the phytochemical investigations, it contains aromadendrin 3-hydroxy 4-methoxybenzaldehyde (3H4MB), 4- hydroxy-3 methoxy benzaldehyde (4H3MB), α-amyrinacetate, 4-methoxy-salicylaldehyde, magnificol, ß-sitosterol, naringenin, kaempferol, chlorogenic acid, benzoic acid, inositol and squalene[11]. Due to their high demand, the destructive harvesting of the roots leads to extinction of the species. Therefore, plant tissue culture techniques offer a powerful tool for mass multiplication of many plant species and this necessitates other alternative methods for propagation and conservation. Fourier Transform Infrared Spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption of solid, liquid or gaseous samples. It is a technique to identify the functional groups of chemical constituents and widely used in identification, quality control and manufacturing process in pharmaceutical drugs. Components of complex bio molecular mixtures (proteins, lipids, carbohydrates etc.) have separable IR spectra therefore they can be analysed simultaneously[12]. FTIR spectrometry was used for the rapid, direct measurement of acetylsalicylic acid (ASA), ascorbic acid (vitamin C), biotin (vitamin H) and bucillamine in different pharmaceutical products. Conventional KBr spectra were compared for the best determination of active substances in drug preparations [13]. Gas Chromatography-Mass Spectrometry (GC-MS) is the synergistic combination of most universal analytical techniques for the identification and quantitation of organic substances such as fatty acids, esters, alcohols, aldehydes and terpenes etc. The GC separates the components of a mixture in time, and the MS provides information that aids in the structural identification of each component [14]. GC-MS is exclusively used in bio-analysis of blood, urine for the presence of barbiturates, narcotics, alcohols, residual solvents, drugs like anesthetics, anticonvulsant, antihistamine, anti-epileptic drug, presence of free steroids, blood pollutants, metabolites in serum etc [15]. High Performance Liquid Chromatography (HPLC) is a chromatographic technique used in the fields of analytical chemistry, biochemistry and industrial. The applicability of HPLC for the analysis, separation and determination of alkaloids, antibiotics, nitrogen-containing compounds (nucleic acids), steroids, sulphur containing compounds, antioxidants, carcinogens and preservatives in pharmaceutical analysis [16] HPLC has many applications in both laboratory and clinical science. It is a common technique used in pharmaceutical development, as it is a dependable way to obtain and ensure product purity. This technique is obviously useful in observing multiple species in collected samples, as well, but requires the use of standard solutions when information about species identity [17]. Therefore, the present study was aimed to investigate the phytochemical compounds through FTIR, GC-MS and quantification of 2H4MB & ß-sitosterol through HPLC analysis.

2. MATERIALS AND METHODS

2.1 Collection of plant material

D. arayalpathra seeds and seedlings were collected around Thiruvananthapuram, Western Ghats, India. The plants were maintained in the greenhouse conditions at the Department of Botany. The plant material was authenticated in the Botanical Survey of India, Western Regional Centre, Pune and the voucher specimen (No.BSI/WRC/100-1/IDEN. CER./018/76) was deposited in the Department of Botany, Bangalore University, Bengaluru.

2.2 Callus induction

Young and healthy leaves (1 cm^2) were cultured on Murashige & Skoog (MS) medium fortified with different concentration of auxins (2, 4-D, NAA, IAA and IBA) and cytokinins (BAP, KIN and Zeatin) individually and in combination for induction of callus [18].

2.3 Preparation of plant extracts for preliminary phytochemical analysis

2 g of powdered samples (root, stem, leaf and callus) were soaked in methanol for 3 to 4 days. The solvent extract was filtered through Whatman No. 1 filter paper. The procedure was repeated for another two cycles to ensure complete extraction of phytochemical compounds [19].

2.4 Quantitative estimation

2.4.1 Preparation of plant extracts

10 g of powder was extracted with 200 ml of methanol in soxhlet apparatus. The solvent extraction was carried out for 08-10 h. Then the residues were collected and concentrated by evaporation and stored in an airtight container at 4 °C for further analysis [20].

2.4.2 Total proteins by modified Folin Ciocalteu(FC) method

Reagents

Reagent-**A:** 2 % Na_2CO_3 in 0.1N NaOH solution.
Reagent-**B**: 0.5 % $CuSO_4.5H_2O$ in 1 % Na-K-tartarate.
Reagent-**C**: 50 ml of reagent A and 1ml of reagent B were mixed to prepare reagent C.
Reagent-**D**: FC reagent.

0.5 g of plant samples (root, stem, leaf and callus) were weighed and grinded in 5-10 ml of phosphate buffer solution. The homogenate was centrifuged at 1500 rpm for 10 min. and the supernatant was collected in the test tubes. The solution was shaken well and allowed to stand for 10 min. Different aliquots of 0.2-1.0 ml of standard protein solution (BSA) pipetted out and the volume was made up to 1.0 ml of distilled water. 5 ml of reagent C (alkaline copper solution) was added in all the test tubes including 0.1 ml of plant samples and 1.0 ml of distilled water (blank), mixed well and tubes were incubated at room temperature for 10 min. 0.5 ml of reagent D was added and the tubes were incubated for 30 min in dark and the absorbance was recorded to 660 nm. [21].

2.4.3 Total Carbohydrates by Anthrone Method

10 mg of dried powder samples (root, stem, leaf and callus) were weighed and hydrolysed by boiling water bath for 3 h with 5 ml of 2.5 N HCl and cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence stops. The volume was made upto 10 ml and centrifuged at 1500 rpm for 10 min. 0.1 ml of supernatant was taken, without sample it serves as blank. The volume was made up to 1 ml in all the tubes including samples by adding distilled water. Add 4 ml of anthrone reagent and incubated in a boiling water bath for 8 min. Then it was cool rapidly and the green to dark green color was recorded at 630 nm [22].

Anthrone reagent: 200 mg of anthrone was dissolved in 100 ml of ice cold conc. H_2SO_4, prepared freshly before use.

Standard glucose stock: 100 mg of glucose was dissolved in 100 ml of distilled water (1mg/L). Working standard - 10 ml of stock diluted with 100 ml of d.w (0.1 mg/L)

2.4.4 Extraction of Oil

Fresh and healthy roots were collected from the greenhouse, washed in running tap water to remove the dust and the adherent rotten material. The tuberous roots were shade dried for 4 to 5 days. The dried roots (100 g) were powdered and subjected to hydro distillation using a Clevenger apparatus for 5 h. The essential oil was collected through a funnel containing anhydrous sodium sulphate to remove the water content [23].

2.4.5 FTIR Spectroscopic analysis

10 mg of the dried extract powder (root, stem, leaf & callus) was encapsulated in 100 mg of KBr pellet, in order to prepare a translucent sample disc by using a pelletizer. The powdered sample of each plant specimen was loaded in FTIR spectroscopy (Shimadzu, IR Affinity1, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.4.6 GC-MS analysis

Methanolic extract of different samples were analysed with the help of GC-MS analyser. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260 °C during the chromatographic run. The 1µl of extract sample injected into the instrument and the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min^{-1}; and 300 °C, where it was held for 6 min. The mass detector conditions were transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library [20].

2.4.7 HPLC analysis

2.4.7.1 Preparation of standard solutions

A stock solution of 100 µg/ml was prepared by dissolving 10 mg of 2H4MB and ß-Sitosterol in 100 ml of HPLC grade methanol in each.

2.4.7.2 Quantification of standards

Methanolic extracts were redissolved in methanol, filtered through 0.45 µm membrane filter and used for HPLC analysis [24]. 10 µl sample was injected for quantitative detection of 2H4MB C_{18} analytical columns. The mobile phase consisting of methanol/water (80:20, v/v) for 2H4MB with the flow rate of 1 ml/min were used. The chromatogram was monitored at 280 nm. Oven temperature 35 °C with total running time of 6 min. For ß-Sitosterol the mobile phase consisting of isocratic methanol with the flow rate of 1.5 ml/min was used. The chromatogram was monitored at 206 nm. Oven temperature 30 °C with total running time of 20 min. The peak identification of extract was based on the comparison of retention time with standard of total running time. Three distinct calibration levels were used to set the calibration plot. The calibration plot was drawn by plotting the peak area against the concentration of the compound. Concentration of standards were determined using the following formula

$$\text{Standard concentration} = \frac{\text{Sample area}}{\text{Standard area}} \times \text{concentration of standard} \times \text{dilution factor}$$

The analytical HPLC experiments were performed with an Agilent Technologies with variable wavelength detector operating at 280 and 206 nm. Separation was carried out with C_{18} (5 µm) column with respective mobile phases and flow rate of 1 and 1.5 ml/min respectively.

3. RESULTS AND DISCUSSION

3.1 In vitro callus induction from leaf explants

The initiation of callus was observed within two weeks of Inoculation, maximum callus induction was observed after eight weeks of culture on MS media supplemented with 2, 4-D (2 mg/L) and BAP (1 mg/L) individually. Whereas, combination of BAP (0.5 mg/L) +2, 4-D (1 mg/L) and BAP (1 mg/L) + NAA (0.5 mg/L) also showed higher callus induction. The morphology of the callus was varied with different growth regulators. Creamish compact callus was observed in

2, 4-D. While, in combination, resulted in the formation of green friable and profuse callus. Similar results were observed in *Decalepis hamiltonii,* where in maximum callus induction was observed in combination of BAP and NAA from cotyledonary explants [25]. Studies by Ahmad *et al.* [26] reported higher callus induction from leaf explants of *Ruta graveolens* on MS media fortified with individual growth regulators as well as combination.

3.2 Phytochemical studies

Phytochemicals play an important role and it provides information about the presence of primary and secondary metabolites in the plant extracts. The methanolic extract of the root, stem, leaf and callus were qualitatively screened for phytochemicals. All the plant samples indicated the presence of alkaloids, proteins, phenols, tannins, flavonoids, triterpenoids, phytosterols, glycosides, quinone, carbohydrates, resins, emodels, phlobatannins and volatile oils as shown in Table 1. In the present study methanolic extracts of root and stem showed major phytochemicals. Least accumulation of phytochemicals were noticed in the callus with respect to other parts of the plant. The results were concordance with the reports of Arumugam *et al.*[27], wherein methanolic leaf and callus extracts of *Centella asiatica* revealed the presence of alkaloids, glycosides, terpenoids, flavonoids, reducing sugars etc. Several studies have reported the presence of major phytochemicals in the methanolic root extract of *D. hamiltonii* compared to other solvent extracts[28,29]. Johnson *et al.*[30] also reported higher secondary metabolites in methanolic extracts of root, stem and leaf of *D. hamiltonii*.

Table 1. Preliminary phytochemical analysis of *D. arayalpathra*

Sl. No.	Phytocompounds	Tests	Root	Stem	Leaf	Callus
01	Alkaloids	Mayer's	+	+	+	+
		Dragendorff's	+	+	+	+
02	Betacyanin	Sodium hydroxide	+	+	+	-
03	Carbohydrates	Molisch's	+	+	+	+
04	Emodels	Ammonia	+	+	-	-
05	Flavonoids	Ferric chloride	+	+	+	-
		Alkaline reagent	+	+	+	+
		Lead acetate	+	+	+	+
06	Glycosides	Keller killani	+	+	+	+
07	Gums and Mucilage	Ruthenium red	+	+	+	-
08	Phenols	Ferric chloride	+	+	+	-
		Lead acetate	+	+	+	+
09	Phytosterols	Salkowski	+	+	+	+
		Liebermann Burchard	+	+	-	+
10	Proteins	Biuret test	+	+	+	+
11	Quinone	Sulphuric acid	+	+	+	+
12	Resins	Acetone	+	+	+	-
13	Tannins	Ferric chloride	+	+	+	-
		Lead acetate	+	+	+	+
		Gelatin	+	+	+	+
14	Terpenoids	Salkowski	+	+	+	+
15	Volatile oils	Ferric chloride	+	+	+	+

+ = Positive, - = Negative

3.3 Estimation of total protein and carbohydrate content of D. arayalpathra

The total protein content was determined by the FC method using Bovine Serum albumin as a standard phenolic compound ($y = 0.7388x + 0.0523$ $R^2 = 0.9984$). Higher protein content was observed in root (0.63 ± 0.06 mg/g) and leaf (0.58 ± 0.03 mg/g) compared to stem (0.38 ± 0.02 mg/g) and leaf derived callus (0.27 ± 0.02 mg/g). The total carbohydrate content was determined by an anthrone method using glucose (G) as a standard ($y = 0.904x + 0.1224$ $R2 = 0.9852$). Root sample exhibits the more carbohydrate content (0.14 ± 0.01 mg/g) than the leaf (0.11 ± 0.02 mg/g), stem (0.07 ± 0.01 mg/g) and callus (0.03 ± 0.00 mg/g) samples (Table 2).

Table 2. Total Protein and Carbohydrate content of *D. arayalpathra*

Plant Samples	Total Protein (mg/g)	Total Carbohydrate (mg/g)
Root	0.63 ± 0.06^{c}	0.14 ± 0.01^{c}
Stem	0.38 ± 0.02^{b}	0.07 ± 0.01^{b}
Leaf	0.58 ± 0.03^{c}	0.11 ± 0.02^{c}
Callus	0.27 ± 0.02^{a}	0.03 ± 0.00^{a}

Values were represented as Mean ± SD in triplicates (n=3). Means with the different letters in columns indicate significant differences at 5% level. Analyzed by Duncan's multiple range tests using SPSS software. Probability values P<0.05 were considered significant.

3.4 *Extraction of Volatile oil*

Plant volatile oils are generally isolated from non woody plant material by distillation method, usually steam or hydro distillation. In this study, 0.09 % of volatile oil was extracted from roots of *D. arayalpathra* by hydro distillation method. The obtained oil was taken to GC-MS and HPLC analysis. The plant volatile oils and their constituents from a wide variety of plants contains antimicrobial and food preservative properties [31]. The yield of oil was better compared to the reports of Verma *et al.* [4] Nagarajan *et al.* and Thangadurai *et al.* [32,23] reported 0.68 & 0.33 % of volatile oil through steam and hydro distillation process, in the tuberous root of *D. hamiltonii*.

3.5 *FTIR analysis*

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. The results of FTIR analysis confirmed the presence of alcohol, alkanes, aromatic carboxylic acid and halogen compounds. The obtained absorption bands and wave number (cm^{-1}) of the prominent peaks were represented in the Table 3 and Figure 1. Methanolic extracts of root, stem, leaf and callus showed the peaks at 3465, 3399, 3436 and 3433 cm^{-1} revealed the presence of Poly hydroxy compounds (O-H stretch). Leaf methanolic extract showed the presence of Lipids (2922 cm^{-1}) and Proteins (2851cm^{-1}). A peak of 2427 cm^{-1} indicated the presence of a carboxylic acid group (O-H stretch). The peaks of 1615, 1603 and 1613 cm^{-1} represent the ketone group (C=O stretching). The phenol groups (O-H bend) were identified at 1381, 1383 and 1385 cm^{-1}. The peaks of 1108, 1110 and 1111 cm^{-1} revealed the cyclic ether group (C-O stretch). Phosphate compounds (phosphate ion) were detected corresponding to the peaks of 1067, 1068 and 1069 cm^{-1}. Peaks of 923, 921, 926 and 922 cm^{-1} indicate the presence of aromatic phosphates (P-O-C stretch). Aliphatic chloro compounds (C-Cl stretch) were detected in root, stem and callus extracts at 721 cm^{-1}. The peaks at 614, 619 and 615 cm^{-1} shows the existence of aliphatic bromo compounds (C-Br stretch).The functional groups of the compounds were separated based on its peak ratio and creating a molecular fingerprint of the sample. Many studies revealed the FTIR spectrum as an active tool for classifying and differentiating the closely related plants [33].

Table 3. FTIR spectra interpretation of *D. arayalpathra*

Sl. No.	Wave number (cm^{-1})	Functional groups	Phyto components	Wave number (cm^{-1})			
				Root	Stem	Leaf	Callus
1	3570-3200	O-H stretch	Poly Hydroxy Compounds	3465	3399	3436	3433
2	2935-2915	-CH (CH_2) Asymmetric	Lipids, Proteins	---	---	2922	---
3	2865-2845	-CH (CH_2) Symmetric	Lipids, Proteins	---	---	2851	---
4	3000-2400	O-H stretch Acidic	Carboxylic acid	2427	2427	2427	2427
5	1650-1600	C=O stretching	Ketone group	1615	1615	1603	1613
6	1410-1310	O-H bend	Phenol	1381	1383	1385	1383
7	1140-1070	C-O stretch	Cyclic ether	1110	1108	1111	1111
8	1100-1000	Phosphate ion	Phosphate compound	1069	1068	1067	1068
9	995-850	P-O-C stretch	Aromatic phosphates	923	921	926	922
10	800-700	C-Cl stretch	Aliphatic Chloro compounds	721	721	---	721
11	700-600	C-Br stretch	Aliphatic Bromo compounds	614	614	619	615

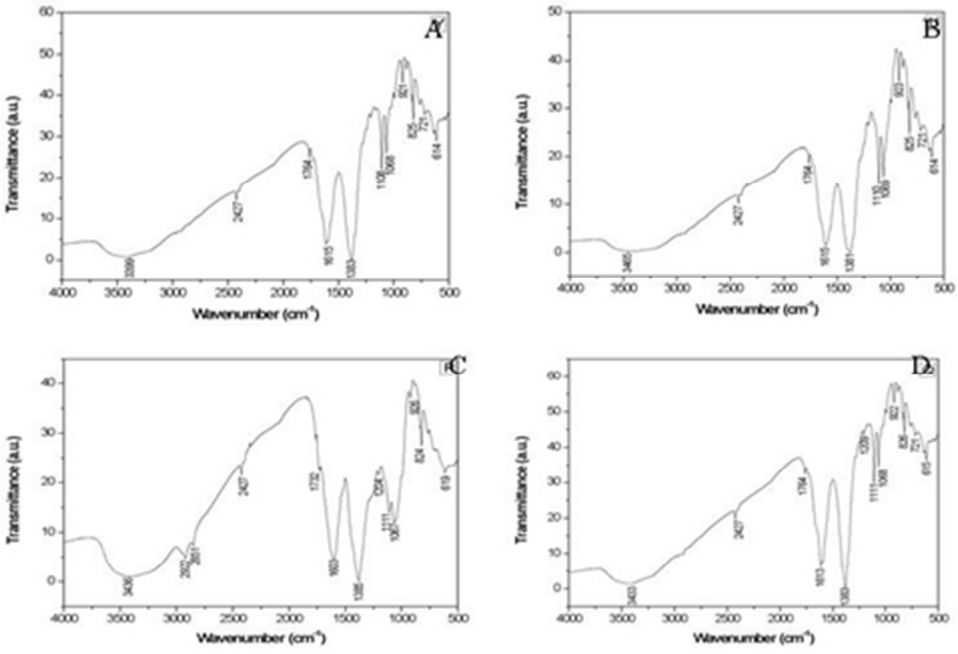

Fig 1. FTIR spectrum of methanolic extracts of A) root B) stem C) leaf and D) callus

3.6 GC-MS analysis

GC-MS is the most ubiquitous analytical technique, identification of compounds based on the molecular mass, structure and fragments. Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of unknown components was compared with the library version NIST-2008. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and peak area percentage (%) were represented in Table 4. Analysis of *D. arayalpathra* root oil revealed the presence of various compounds such as Vanillin, Benzaldehyde 2-hydroxy 4-methoxy, Benzaldehyde 2-hydroxy 4- methoxy and Ethanone, 1 – (2-hydroxy 4- methoxy Phenyl). Vanillin (68.42 %) and Benzaldehyde 2-hydroxy 4- methoxy (11.92 %) were found to be the major compounds. The GC-MS analysis of methanolic extracts of root, stem, leaf and callus showed ten different compounds that are eluted as a function of retention time. 4, 6-O-Ethylidene-alpha-D-Glucose, 14B Octamethyl, 2, 3,-bis [(trimethylsilyl) oxy] Propyl ester and URS-12-EN-24 Oic acid, 3-Oxo, Methyl ester (+) with the peak area percentage 10.99, 12.85 and 42.15 respectively, and were identified in the root against their retention time. Whereas in stem, 2R - Acetoxymethyl-1, 3, 3 - Trimethyl - 4T - (3-Methyl-2-Buten-1-yl) - 1T Cyclohexanol and 3-O-Acetyl-6-Methoxy-Cycloartenol were identified as the major compounds with retention time 30.21 and 30.32 min. Leaf extracts represents the presence of three compounds namely 2-T-Butyl-4-Methyl-5-Oxo-(1,3)Dioxolane-4-Carboxylic acid, Lupeol and 9,19-Cyclolanost-24-En-3-Ol,Acetate, (3,Beta) with retention time 5.99, 15.36 and 61.90 respectively. However in callus 1, 1, 1, 3, 5, 5, 5 – Hepta methyl trisiloxane were identified as the major compound followed by Silicic acid and diethyl bis (Trimethylsilyl) ester. GC-MS analysis of the methanolic extract of root oil was perform on the structure of major phyto constituents. The mass spectrum of *D. arayalpathra* root oil revealed 2H4MB as a major phyto constituent in the extract. GCMS analysis carried out in *D. arayalpathra* by Zishan *et al.* [24], revealed the presence of various phyto constituents. Several studies also reported 2H4MB as a major compound present in the root extract of *D. hamiltonii* [34, 35].

Table 4. Compounds identified of *D. arayalpathra* by GCMS

Samples	Name of the Compound	MF	MW	RT	Peak area %
Root oil	Benzaldehyde 2-hydroxy 4- methoxy	$C_8H_8O_3$	152	13.748	11.920
	Benzaldehyde 2-hydroxy 4- methoxy	$C_8H_8O_3$	152	13.813	07.132
	Thieno (2, 3- B) Pyridine – N – Oxide	C_7H_5ONS	151	13.923	04.537
	Vanillin	$C_8H_8O_3$	152	13.983	68.426
	Ethanone, 1 – (2-hydroxy 4- methoxy Phenyl)	$C_9H_{10}O_3$	166	15.889	5.543
	2 – Adamantanol – 2- (Bromomethyl)	$C_{11}H_{17}OBr$	244	17.674	01.215
Root	4,6-O-Ethylidene-alpha-D-Glucose	$C_8H_{14}O_6$	206	19.65	10.994
	14B Octamethyl,2,3,-bis[(trimethylsilyl) oxy] Propyl ester	$C_{30}H_{48}O$	424	29.85	12.851
	URS-12-EN-24 Oic acid, 3-Oxo, Methyl ester (+)	$C_{31}H_{48}O_3$	468	30.54	42.154
Stem	2R-Acetoxymethyl-1,3,3-Trimethyl-4T-(3-Methyl-2-Buten-1-yl)-1T Cyclohexanol	$C_{17}H_{30}O_3$	282	30.21	15.578
	3-O-Acetyl-6-Methoxy-Cycloartenol	$C_{33}H_{54}O_3$	498	30.32	66.734
Leaf	2-T-Butyl-4-Methyl-5-Oxo-(1,3)Dioxolane-4-Carboxylic acid	$C_9H_{14}O_5$	202	21.75	5.997
	Lupeol	$C_{30}H_{50}O$	426	30.17	15.361
	9,19-Cyclolanost-24-En-3-Ol,Acetate, (3,Beta)	$C_{32}H_{52}O_2$	468	30.29	61.902
Callus	1,1,1,3,5,5,5-Heptamethyltrisiloxane	$C_7H_{22}O_2Si_3$	222	26.38	67.171
	Silicic acid, Diethyl bis (Trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296	30.41	21.370

RT- Retention Time, MF- Molecular Formula, MW- Molecular Weight

3.7 HPLC analysis

The root oil, root, stem, leaf and callus were subjected to HPLC studies. The chromatogram of root and root oil of 2H4MB and root oil of ß - sitosterol showed varying peaks with corresponding retention time (Figure 2). The results were compared using the standards 2H4MB and ß - sitosterol with the retention time of 3.93 and 12.46 respectively. The percentage of 2H4MB and ß - sitosterol of plant samples were calculated. The methanolic extract of root and root oil (hydro distilled) exhibited 0.187 % and 23.369 % of 2H4MB content. Whereas 2.40 % of ß - sitosterol was recorded in root oil. There were no peaks observed in stem, leaf and callus extracts (Table 5). The root oil exhibited maximum content of 2H4MB and ß - sitosterol, however a considerable amount of 2H4MB was recorded in methanolic root extract. The results were concordance with the reportsof Zishan *et al.* [24], wherein methanolic root extract of *D. arayalpathra* identified the compound 2H4MB at a retention time of 3.631 min. Nagat *et al.* [36] also identified that ethanolic root extract of *Hemidesmus indicus* contains 2H4MB and 2-hydroxy 4-methoxy benzoic acid at the retention time 11.8 and 13.3 min respectively. Kakade and Magdum, [37] reported the ß-sitosterol in various vegetable oil (wheat germ oil, cotton seed oil, soya bean and peanut oil) with the retention time 36.91, 36.21, 36.47 and 36.12 min. Similar results were also reported by Pednekar *et al.*[38] in methanolic leaf extract of *Ampelocissus latifolia* with the retention time 2.74 min.

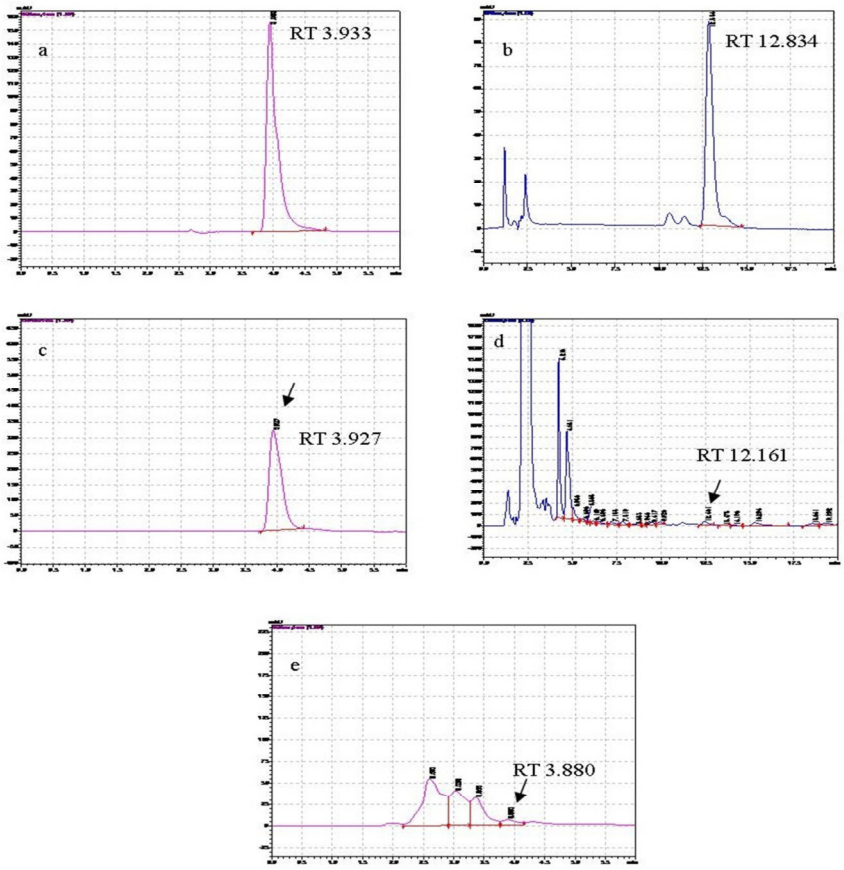

Fig 2. HPLC analysis of standard a) 2H4MB b) ß-sitosterol c) 2H4MB content in root oil, d) ß-sitosterol content in root oil and e) 2H4MB content in methanolic root extracts of *D. arayalpathra*.

Table 5: HPLC analysis detonation of 2H4MB and ß-sitosterol

Samples	RT	Area	Conc.	% of 2H4MB
Root	3.880	102112	0.00046	0.187
Root oil	3.927	4242539	0.02618	23.369
				% of ß-sitosterol
Root oil	12.461	650306	0.08852	2.408

RT- Retention Time, % - Percentage, Conc. – Concentrations

4. CONCLUSION

Limited studies are available on *in vitro* regeneration of *D. arayalpathra.* Hence it needs large scale production and conservation of these important medicinal plants. The phytochemical screening of the methanolic extract showed the presence of different compounds such as alkaloids, betacyanin, flavonoids, reducing sugars, glycosides, resins, volatile oils and terpenoids etc. FTIR analyses of the methanolic extract of root showed the presence of major bioactive compounds with various functional groups. Through GC-MS analysis, different compounds were identified and quantified phyto compounds based on their retention time and peak area. HPLC analysis of root extract and root oil showed the presence of 2H4MB and while β-sitosterol was detected only in root oil. In future appropriate strategies have to design for enhanced production of secondary metabolites exhibiting antioxidants and antimicrobial properties. It has great commercial applications and to fulfil the demands of pharmaceutical and nutraceuticals industries.

5. ACKNOWLEDGMENT

The authors are thankful to the Department of Horticulture, GKVK, Bengaluru for timely help and valuable suggestions.

6. AUTHORS CONTRIBUTION STATEMENT

Ashalatha K.S has done the phytochemical work and manuscript writing. H.R Raveesha coordinated the work and helped to draft the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

8. REFERENCES

1. Tapan R. Role of folk medicine in primary health care: A case study on West Bengal. India. International Journal of Interdisciplinary and Multidisciplinary Studies. 2014;1(2):13-8.
2. Sudha CG, Seeni S. Establishment and analysis of fast growing normal root culture of *Decalepis arayalpathra,* a rare endemic medicinal plant. Curr Sci. 2001;81(4):371-4.
3. Gangaprasad A, Decruse SW, Seeni S, Nair GM. Micropropagation and ecorestoration of *Decalepis arayalpathra* (Joseph & Chandra.) Venter – an endemic and endangered ethnomedicinal plant of Western Ghats. Indian J Biotechnol. 2005;4:265-70.
4. Verma RS, Mishra P, Kumar A, Chauhan A, Padalia RC, Sundaresan V. Chemical composition of root aroma of *Decalepis arayalpathra* (J. Joseph and V. Chandras.) Venter, an endemic and endangered ethnomedicinal plant from Western Ghats, India. Nat Prod Res. 2014:28(15):1202-5.
   Doi: 10.1080/14786419.2014.918127
5. Krishnan TK. Ayurvedic Oushadhi Nighandu. New Delhi: Central Council for Ayurvedic Research. 1966:p.47-48.
6. Vedavathy S. Decalepis hamiltonii Wight & Arn. – an endangered source of indigenous health drink. Nat Prod Radiance. 2004;3(1):22-3.
7. Vijayakumar V, Pullaiah T. An ethno-medico-botanical study of Prakasam district, Andra Pradesh, India. Fitoterpia. 1998;69:483-9.
8. Harish R, Divakar S, Srivastava A, Shivanandappa T. Isolation of antioxidant compounds from the methanolic extract of the roots of *Decalepis hamiltonii* (Wight and Arn.). J Agric Food Chem. 2005;53(20):7709-14.
   doi: 10.1021/jf051047c, PMID 16190621.
9. Wang J, Liu H, Zhao J, Gao H, Zhou L, Liu Z, Chen Y, Sui P. Antimicrobial and antioxidant activities of the root bark essential oil of Periploca sepium and its main component 2-Hydroxy-4-methoxybenzaldehyde. Molecules. 2010;15(8):5807-17.
   doi: 10.3390/molecules15085807, PMID 20736908.
10. Patnaik J, Debata BK. Micropropagation of *Hemidesmus indicus* (L.) R. Br. through axillary bud culture. Plant Cell Rep. 1996;15(6):427-30.
    doi: 10.1007/BF00232069, PMID 24178423.
11. Chacko S, Sethuraman MG, George V. Phytochemical investigation of the roots of *Janakia arayalpathra* Joseph and Chandrasekharan. Herba Pol. 2000;46:212-19.
12. Griffiths P, Hasseth JA. Fourier transform infrared spectrometry. 2nd ed. Wiley-Blackwell; 2007.
13. Bunaciu AA, Aboul-enein HY, Fleschin S. Application of Fourier Transform infrared spectrophotometry in pharmaceutical drugs analysis. Appl Spectrosc Rev. 2010;45(3):206-19. doi: 10.1080/00387011003601044.
14. Sparkman OD, Penton ZE, Kitson FG. Academic gas chromatography and mass spectrometry. 2nd ed. Press; 2011. p. 2-13.
15. Chauhan A, Goyal MK. GC-MS Technique and its analytical applications in Science and Technology. J Anal Bioanal Tech. 2014;5(6):1-5.
    doi: 10.4172/2155-9872.1000222.
16. Bailey F. Applications of high-performance liquid chromatography in the pharmaceutical industry. J Chromatogr. 1976;122:73-84.
    doi: 10.1016/s0021-9673(00)82237-2, PMID 777018.
17. Gerber F, Krummen M, Potgeter H, Roth A, Siffrin C, Spoendlin C. Practical aspects of fast reversed-phase high-performance liquid chromatography using 3 microm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. J Chromatogr A. 2004;1036(2):127-33.
    doi: 10.1016/j.chroma.2004.02.056, PMID 15146913.
18. Koroch A, Juliani HR, Kapteyn J, Simon JE. *In vitro* regeneration of Echinacea purpurea from leaf explants. Plant Cell Tissue Organ Cult. 2002;69(1):79-83.
    doi: 10.1023/A:1015042032091.
19. Dhanapal ACT, Ming TW, Aung HP, Hao SJ. Preliminary screening of *Artemisia argyi* for antioxidant potentials. Int J Pharmacogn Phytochem Res. 2016;8:347-55.
20. Mickymaray S, Al Aboody MS, Rath PK, Annamalai P, Nooruddin T. Screening and antibacterial efficacy of selected Indian medicinal plants. Asian Pac J Trop Biomed. 2016;6(3):185-91.
    doi: 10.1016/j.apjtb.2015.12.005.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75. PMID 14907713.
22. Mahadevan A, Sridhar R. Methods in physiological plant pathology; 1986. p. 141-6.
23. Thangadurai D, Anitha S, Pullaiah T, Reddy PN, Ramachandraiah OS. Essential oil constituents and in vitro antimicrobial activity of *Decalepis hamiltonii* roots against food borne pathogens. J Agric Food Chem. 2002;50(11):3147-9.
    doi: 10.1021/jf011541q, PMID 12009977.
24. Zishan A, Anwar S, Shiwali S. Evaluation of in vitro antioxidant activity, HPLC and GC–MS analysis along with chemo profiling of *Decalepis arayalpathra*: a critically endangered plant of Western Ghats, India. Rend Fis Acc Lincei. 2017;28(4):711-20.
    doi: 10.1007/s12210-017-0645-8.
25. Samydurai P, Saradha M, Ramakrishnan R, Santosh Kumar S, Thangapandian V. Micropropagation perspective of cotyledonary explant of *Decalepis hamiltonii* Wight & Arn. an endangered edible species. Indian J Biotechnol. 2016;15:256-60.
26. Ahmad N, Faisal M, Anis M, Aref IM. In vitro callus induction and plant regeneration from leaf explants of *Ruta graveolens* L S Afr J Bot. 2010;76(3):597-600.
    doi: 10.1016/j.sajb.2010.03.008.
27. Arumugam T, Ayyanar M, Koli Pillai YJ, Sekar T. Phytochemical screening and antibacterial activity of leaf and callus extracts of *Centella asiatica*. Bangladesh J Pharmacol. 2011;6:55-60.
28. Devi M, Latha P. Antibacterial and phytochemical studies of various extracts of roots of *Decalepis hamiltonii* Wight and Arn. Int J Pharm Pharm Sci. 2012;4(2):738-40.
29. Manickam D, Preetha D. Phytochemical analysis and in vitro anticancer activity of methanol extract of *Decalepis hamiltonii* root against hepatic cancer cell lines (HepG2). World J Pharm Res. 2016;5(3):1180-6.
30. Johnson M, Wesely EG, Zahir Hussain MI, Selvan N. *In vivo* and *in vitro* phytochemical and antibacterial efficacy of *Baliospermum montanum* (Willd.) Muell. Arg. Asian

Pac J Trop Med. 2010;3(11):894-7. doi: 10.1016/S1995-7645(10)60215-5.
31. Lis-Balchin M, Deans SG. Bioactivity of selected plant essential oils against Listeria monocytogenes. J Appl Microbiol. 1997;82(6):759-62. doi: 10.1046/j.1365-2672.1997.00153.x, PMID 9202441.
32. Nagarajan S, Jagan Mohan Rao LJ, Gurudutt KN. Chemical composition of the volatiles of *Decalepis hamiltonii* (Wight & Arn). Flavour Fragr J. 2001;16(1):27-9. doi: 10.1002/1099-1026(200101/02)16:1<27::AID-FFJ937>3.0.CO;2-F.
33. Maitera ON, Chukkol IB. Phytochemical and Fourier Transform infrared Spectroscopy analysis of *Faidherbia albida* (Del) as a preservative agent. World J Res Rev. 2016;3(3):25-9.
34. Giridhar P, Rajasekaran T, Ravishankar GA. Improvement of growth and root specific flavour compound 2-hydroxy-4-methoxy benzaldehyde of micropropagated plants of *Decalepis hamiltonii* Wight & Arn., under triacontanol treatment. Sci Hortic. 2005;106(2):228-36. doi: 10.1016/j.scienta.2005.02.024.
35. Mohan B, Nayak JB, Kumar RS, Shiva Kumari LP, Mohan Ch, Rajani B. Phytochemical screening, GC-MS analysis of *Decalepis hamiltonii* Wight & Arn. an endangered medicinal plant. J Pharmacogn Phytochem. 2016;5(5):10-6.
36. Nagat M, Barka E, Lawrence R, Saani M. Phytochemical screening, antioxidant and antibacterial activity of active compounds from *Hemidesmus indicus.* Int J Curr Pharm Res. 2016;8(2):24-7.
37. Kakade AN, Magdum. HPLC analysis of β-sitosterol in herbal medicine and vegetable oils. Int J Pharm Life Sci. 2012;3(5):1666-9.
38. Pednekar PA, Kulkarni V, Raman B. High performance liquid chromatographic method development and validation for the simultaneous determination of gallic acid and beta sitosterol in *Ampelocissus latifolia* (Roxb.) planch. Asian J Pharm Clin Res. 2014;7(2):86-9.