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The present study was aimed to examine the phytochemical screening, identification of bioactive compounds by GC-MS & HPLC and antibacterial activities of *Decalepis hamiltonii* root extracts. It is one of the important traditional medicinal plant, belongs to the family Apocynaceae**,**The qualitative and quantitative phytochemical analysis were carried out by using standard methods. To identify and quantify the bioactive compounds, GC-MS and HPLC analysis were done. Antibacterial activities of different solvent extracts were carried out by using agar well diffusion method. Preliminary phytochemical analysis showed the presence of phenols, tannins, alkaloids and flavonoids, proteins, reducing sugar, glycosides, amino acids, steroids, terpenoids, resins, volatile oil, emodols and coumarins. Total phenolic and flavonoid contents were higher in methanolic extracts compared to other solvent extracts. 2-hydroxy 4-methoxybenzaldehyde was identified and quantified through GC-MS and HPLC analysis.The maximum zone of inhibition was observed in the methanolic extracts of root against *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*. The results concluded that the *D. Hamiltonii* plant contains many chemical compounds and its root indicatesthe wide range of antimicrobial activity. Further studies have to be carried out to find out the method of the *in vitro* multiplication of plant to conserve this medicinal plant and to enhance the root flavouring compounds for medicinal, food and beverage industries.

Key words: Antimicrobial activity,*Decalepis hamiltonii,* methanolic extracts, phytochemicals

INTRODUCTION

Plants have been the traditional source for raw materials for drugs, since the dawn of civilization. It has been estimated that about 20,000 plant species are known to have worldwide use as drugs. The Indian sub-continent has a very rich diversity of plant species in a wide range of ecosystems. There are about 17,000 species of higher plants, of which approximately 8,000 species are considered medicinal and used by village communities, particularly tribal communities or in traditional medicinal systems. The term medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities (Hassan, 2012). The use of medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health has been widely observed by UNESCO. The medicinal plants consider as a rich resource of ingredients which can be used in drug development and synthesis. Medicinal plants frequently used as raw material for extraction of active ingredients which can be used in the synthesis of different drugs, laxatives, blood thinners, antibiotics and anti-malarial medications (Singh, 2015).

Decalepis hamiltonii (*D. hamiltonii*) is one of the important medicinal plant in ayurvedic system of medicine in India. This plant has been used in the preparation of several herbal drugs and are used in curing various diseases.*D. hamiltonii* is a monogenetic climbing shrub native of Deccan peninsula and forest areas of Western Ghats of India. Its roots has numerous medicinal importance used in wide drug preparations and pharmacognostical studies (Reddy and Murhty,2013). The roots of *D. hamiltonii* are used as a flavouring agent and appetizer and it is considered as "*Sariva Bheda*" in ayurveda which finds use as an alternative to roots of *Hemidesmus indicus* in the preparation of several herbal drugs like Amrutamalaka taila, Drakshadi churna, Shatavari rasayana and Yeshtimadhu taila.

D. hamiltonii is utilized in tribal, traditional Indian and Chinese medicine for treatment of a wide

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variety of diseases including digestive, lungs and circulatory systems. The roots of this plants are used in folk medicine and ayurvedic preparation of treating indigestion. Roots are also used to cure dysentery, cough, bronchitis, leucorrhoea, uterine haemorrhage, skin disease, fever, vomiting, chronic rheumatism, anaemia and blood related diseases. It is also used as a popular drink in the forest areas of eastern and Western Ghats, known as “Nannari” which has a cooling effect without any toxic (Rajani *et al*. 2016). This plant has numerous phytochemical compounds to cure many diseases and it act as antimicrobial, antipyretic, antiulcer, antidiabetic, antioxidant, anti-inflammatory, chemoprotective, cytoprotective, insecticidal, neuroprotective and hepatoprotective activities. The 2-hydroxy 4-methoxybenzaldehyde (2H4MB) is an isomer of vanillin, it is one of the major compound in the volatile oils of *D. hamiltonii* and *Hemidesmus indicus*.Based on the above medicinal importance, the present work is carried out on the phytochemical and antibacterial screening of the different extracts of the *D. hamiltonii* root.

MATERIALS AND METHODS

Collection and preparation of plant materials

Fresh and healthy *D. hamiltonii* roots were collected from Hindupur of Andhra Pradesh on January 2018. The fresh roots were separated from the debris and rinsed thoroughly in running tap water. Theroots were rinsed in distilled waterand cut into small pieces, the cleaned rootbits were shade dried for about 5-6 days. The dried roots were finely powdered with the help of blender and stored for further application.

Preparation of extracts

The 20 g of dried root powder was weighed and packed with Whatman no. 1 filter paper. The extraction was done by soxhlet extraction method by using methanol, petroleum ether and aqueous solvents about 08-10 h. Each extract was collected and concentrated by evaporation, stored at 4 ºC for further analysis (Samydurai and Saradha, 2016).

Preliminary phytochemical analysis

The preliminary phytochemical analysis were carried out by standard methods. Briefly the lyophilized extracts were dissolved in respective solvents and screened for the qualitative analysis of alkaloids, flavonoids, proteins and amino acids, phenols, tannins, steroids and terpenoids, glycosides, coumarins, reducing sugar, resins, volatile oils and emodols (Mohan *et al*. 2016; Kota *et al*. 2017).

Quantitative analysis
Total phenol content

The total phenol content was determined by the method of Samydurai and Thangapandian (2012).To 0.5ml of root extractwas mixed with 0.5 ml of FC reagent and allowed to stand for room temperaturefor 2-3min. Then 1 ml of 7% sodium carbonate was added and the final volume was made up to 5ml with distilled water. After 90min of incubation at room temperaturein dark, the absorbance was read at 725nm using UV visible spectrophotometer in triplicates. Gallic acid was used for calibration of standard curve. The results were expressed as mg of gallic acid equivalent (mg GAE/g) of dry weight of material.

Total flavonoid content

The total flavonoid content was determined by aluminium chloride method with slight modifications. Briefly 0.5ml of sample extract was mixed with 0.1 ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate, 1.5ml of 90% ethanol and 1.8ml of distilled water. After incubation at room temperaturefor 30min, the absorbance of the reaction mixture was measured at 415nm with spectrophotometer. The final absorbance of each sample was compared with a standard curve plotted from quercetin. The total flavonoid content was expressed in micrograms of quercetin equivalents (QEE) per mg extract (Rajendran *et al*. 2014).

Total tannin content

The total tannin content was determined by using catechin hydrate as a standard. To 0.5ml of plant extract add 3ml of 4% vanillin solution in methanol and 1.0ml of concentrated hydrochloric acid (HCl) were added. The mixture was then shaken and incubated at room temperature for 15 min. the absorbance was measured at 500nm against blank. The tannin content was expressed as mg of catechin equivalent per g of dry weight (mg CE/ g DW). After incubation for 15min at room

temperature,the absorbance was measured at 500nm spectrophotometrically (Medini *et al*. 2014).

Extraction of volatile oil

Fresh and healthy roots were collected, washed under running tap water to remove the dust and adherent rotten material and the tuberous roots were shade dried under room temperature. The dried roots (100 g) were powdered with the help of a mechanical blender, subjected to hydrodistilation using a clevenger-type apparatus for 5 h. The essential oil was collected through a funnel containing anhydrous sodium sulphate to remove the water content (Rassem *et al*. 2016).

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of methanolic root extract and root oil of *D. hamiltonii* were analysed with the help of GC-MS analyser (Mickymaray*et al*. 2016).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 × 250 ìm 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 1µ of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min); followed by 300°C at the rate of 10°C/ min; and 300°C, where it was held for 6 min. The mass detector conditions were transferred line temperature 240°C; ion source temperature 240°C; and ionization mode electron impact at 70 eV, a scan time 0.2 s and scan interval of 0.1 s. The fragments were elevated from 40 to 600 Da. The spectrums of the components were compared with the database of the spectrum of known components stored in the GC-MS NIST (2008) library.

High-performance liquid chromatography (HPLC) analysis

HPLC analysis was done according to the procedure of Zishan *et al* (2017). The methanolic extract was redissolved in methanol, filtered through a 0.45 ìm membrane filter and used for HPLC analysis. About 10µ of the volume was injected for quantitative detection of C18 analytical column. The mobile phase comprising of methanol/ water (80:20, v/v) for 2H4MB at pH 5 with the flow rate of 1 ml/min was used. The chromatograms were monitored at 280 nm. The peak identification of extract was based on the comparison of retention time with those of standard. The calibration plot was drawn by plotting the peak area against the concentration of the compound.

Antibacterial activity
Preparation of inoculum

The solvent extracts were assayed against the following organisms *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescence* and *Staphylococcus aureus*. All the stocks were obtained from Department of Microbiology and Biotechnology, Bangalore University, Bengaluru. Loopful of overnight grown bacterial culture was inoculated in known volume of nutrient broth (NB) and incubated at 37^0C for 6-8 h. The actively growing culture suspension was adjusted with NB to obtain a turbidity that could be visually comparable with 0.5 MacFarland standard. The turbidity is approximately equal to $1x10^8$ CFU/ ml.

In vitro antibacterial activity was performed by agar well diffusion method according to the protocol of Johnson *et al* (2010). To determine the inhibitory activity of different solventextracts in various concentration was prepared in DMSO. Wells were bored into nutrient agar using a sterile 8 mm diameter cork borer. Streptomycin was used as a standard. Different concentration of the solvent extracts were added into the wells using sterilized pipettes and allowed to diffuse at room temperature for 2 h. The plates were incubated at 37 ºC for 24 h. After the incubation the diameter of the zone of inhibition were recorded in millimeter and compared with the standard antibiotics. The experiments were repeated thrice in triplicates.

Statistical Analysis

The results were expressed as mean ± standard deviation and data were analysed statistically by one-way analysis of variance followed by Duncan's multiple range tests using SPSS software. $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of D. hamiltonii

The methanol, petroleum ether and aqueous extracts of root of *D. hamiltonii* showed the

presence of phenols, tannins, alkaloids, flavonoids, proteins, reducing sugar, glycosides, amino acids, steroids, terpenoids, resins, volatile oil, emodols and coumarins. Among the three solvent extracts, methanolic extract shows presence of all these phytochemicals.In petroleum ether extract, phenols, flavonoids, amino acids and coumarins are absent. In aqueous extract of *D. hamiltonii* amino acids are absent(Table 1). The results were concordance with reports of Prakash *et al.* (2014)whorevealed the presence of saponins, tannins, steroids, flavonoids, phenols, glycosidesand terpenoids rich in methanolic root extract of *D. hamiltonii*. The phytochemical studies performed by Rajani *et al.* (2016) showed the presence alkaloids, flavonoids, phenols, steroids, tannins, terpenoids, saponins and glycosides in the root extract of *D. hamiltonii.*

Quantitative estimation of total phenolic content

Phenolic content was determined by Folin Ciocalteu (FC) method using gallic acid as a standard phenolic compound (y = 0.058x + 0.0531 R^2 = 0.9978).The highest phenolic content was noticed in the methanolic extracts of roots (21.94±4.30µg/GAE), when compared to other extracts(Fig. 1).The results are concordance with the reports of Sulaiman *et al.*(2011)in some raw vegetables. Higher phenolic content might be due to its polarity and by solubility of the compound in the solvent.

Total flavonoid content

The flavonoid content was determined using regression equation of calibration curve (y = 0.047x - 0.0879 R^2 = 0.993). The highest flavonoid content (13.60±1.14µg/QE) was present in methanolic extract compared to other solvent extracts(Fig. 1).Samydurai and Thangapandian(2012) reported that the methanolic root extract showed the highest phenol and flavonoid contents when compared to other extracts in *D. hamiltonii*.

Total condensed tannins

The total condensed tannin was determined using regression equation of calibration curve (y=0.0071 x +0.0286 R^2 = 0.9947).Higher content of condensed tannin was observed in methanolic extract (78.36 ± 3.39µg/CE) compared to the other solvent extracts (Fig. 1). Similar results were reported by Zishan *et al.* (2017) stated that the methanolic extract of tuberous root of *D. arayalpathra* witness the presence of total phenolics, flavonoids and tannin content.

Hydrodistilation is one of the conventional method to isolate the essential oil. The yield of volatile oil extracted from tuberous root of *D. hamiltonii* was 0.15% (v/w)obtained by hydrodistilation. The chemical composition of oil was analysed by GC-MS and HPLC.

GC-MS analysis

GC-MS is one of the best technique toidentification of compound occurs on the basis of molecular mass, molecular structure and fragments. The mass spectrum of *D. hamiltonii* showed three and five prominent peaks in methanolic extract of root and root oil respectively (Table 2a,b). The relative amount of each compound in percentage was determined by comparing its average peak area to total areas. Analysis of *D. hamiltonii* root oil showed the presence of many major compounds such as, Benzaldehyde 2-hydroxy 4-methoxy, Pyrolo (3, 2-D) Pyrimidin–2,4(1H,3H)–Dione, 2-Adamantanol,2-(Bromomethyl), 1-Bromo-3,7-Dimethylocatne and N-Hexadecanoic acid with the retention time 12.34, 14.88, 16.04, 16.73 and 19.86min respectively (Fig. 2a).Methanolic root extracts showed the presence of compounds such as 14b-Octamethyl-Octadecahydro-2, 2r-Acetoxymethyl -1, 3, 3-Trimethyl-4t-(3 - Methyl-2-Buten - 1 - Yl) - 1t - Cyclohexanol and 9, 19-Cyclolanost - 24 - En - 3-Ol, Acetate, (3.Beta.) with the retention time 29.91, 30.31 and 30.53 respectively (Fig. 2b).

HPLC analysis

The HPLC study is to determine the amount of 2H4MB in the methanolic root extract and root oil of *D. hamiltonii* by using 2H4MB as standard. The graph represents the standard 2H4MB chromatogram, methanolic root extract and root oil of *D. Hamiltonii* showed 2H4MB(Fig 3 A-C).Many researchers reported the presence of 2-hydroxy 4-methoxybenzaledhyde in the root extracts of *D. hamiltonii.* Rajendran *et al.*(2014)studied the HPLC analysis of ethanolic rhizome extract andfound that the main components of active principle is 2H4MB.Sharma *et al.*(2014) also

Table 1: Preliminary phytochemical screening on *D. hamiltonii*

Tests	Methods	Methanol	Petroleum ether	Aqueous
Phenol's	Ferric chloride test	+	_	+
Tannin	Gelatin test	+	+	+
Alkaloid's	Dragendroff's reagent test	+	+	+
Flavonoid's	Alkaline reagent test	+	_	+
Protein	Biuret test	+	+	+
Amino acid	Ninhydrin test	+	_	_
Reducing sugar	Fehling's reagents (I and II)	+	+	+
Glycoside's	Keller killani test	+	+	+
Steroids	Glacial acetic anhydride test	+	+	+
Terpenoids	Chloroform test	+	+	+
Resin	Turbidity test	+	+	+
Volatile oil	Ethanol & $FeCl_3$	+	_	+
Emodols	Ammonia test	+	+	+
Coumarin's	Alcoholic KOH test	+	_	+

+positive,-negative.

Table 2 a: Compounds identified in methanolic root extract of *D. hamiltonii* by GC-MS

Name of the Compounds	MF	MW	RT	Peak area %
4,4,6a,6b,8a,11,11,14b-Octamethyl-Octadecahydro-2	$C_{30}H_{48}O$	424	29.91	12.87
2r-Acetoxymethyl-1,3,3-Trimethyl-4t-(3-Methyl-2-Buten-1-Yl)-1t-Cyclohexanol	$C_{17}H_{30}O_3$	282	30.31	32.54
9,19-Cyclolanost-24-En-3-Ol, Acetate, (3.Beta.)-	$C_{32}H_{52}O_2$	468	30.53	54.58

MF-Molecular Formula, MW-Molecular Weight, RT-Retention Time, % - Percentage.

Table 2 b: Compounds identified in root oil of *D. hamiltonii* by GC-MS

Name of the Compounds	MF	MW	RT	Peak area %
Benzaldehyde 2-hydroxy 4- methoxy	$C_8H_8O_3$	152	12.34	85.05
Pyrolo(3, 2- D) Pyrimidin – 2, 4 (1H,3H) - Dione	$C_6H_5O_2N_3$	151	14.88	07.30
2-Adamantanol,2-(Bromomethyl)	$C_{11}H_{17}O_{Br}$	244	16.04	04.78
1-Bromo-3,7-Dimethylocatne	$C_{10}H_{21}Br$	220	16.73	01.97
N-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	19.86	00.87

MF-Molecular Formula, MW-Molecular Weight, RT-Retention Time, % - Percentage.

reported the presence of 2H4MB in the root extract of *D. hamiltonii*.

Antibacterial activity

The antibacterial activity of different concentration of methanol, petroleum ether and aqueous extracts of the roots of *D. hamiltonii* against different bacterial pathogens represented in the Fig. 4. All the tested bacterial organisms showed growth inhibition towards the root extracts, with different sensitivity. Among the bacterial pathogens *S. aureus* is more sensitive for aqueous and methanolic root extracts when compared to the other bacteria. The highest zone of inhibition was noticed in methanolic extract against *S. aureus* (20.66± 0.57 mm) followed by *K. pneumoniae* (19.00± 0.09 mm). The highest zone of inhibition was observed in petroleum ether extract against *K. pneumoniae* (19.66± 1.15 mm) and aqueous extract against *S. aureus* (21.00 ±1.00 mm). (Fig.5). Prakash *et al.*(2014) reported similar observations in antibacterial activity of different solvent extracts of *D. hamiltonii*. Among

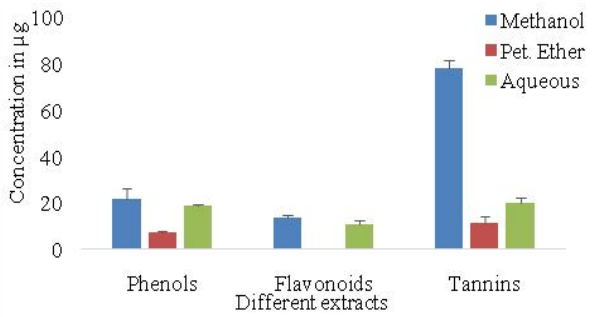

Fig. 1: Quantitative analysis of phenols, flavonoids and tannins of *D. hamiltonii.*

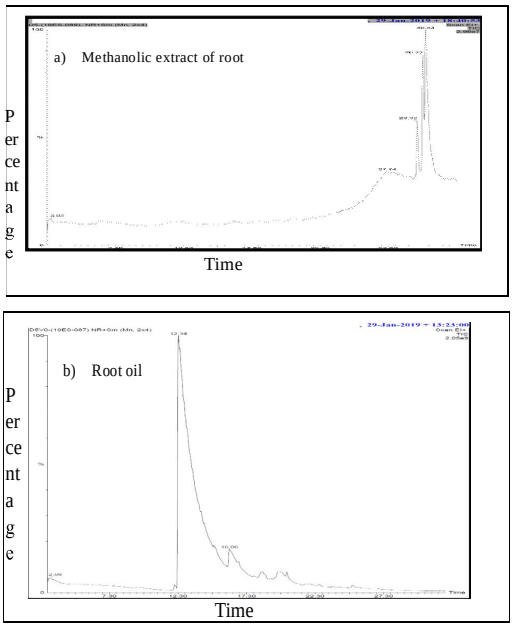

Fig. 2 : GC-MS chromatogram of a) Methanolic extract of root and b) Root oil of *D. hamiltonii*

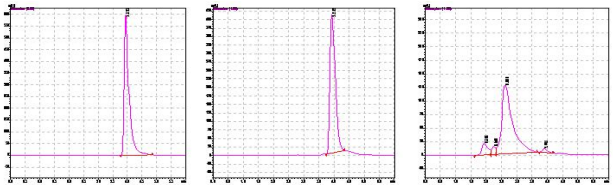

Fig. 3 : HPLC analysis report of A) Standard 2H4MB B) Root oil and C) Methanolic root extract of *D. hamiltonii.*

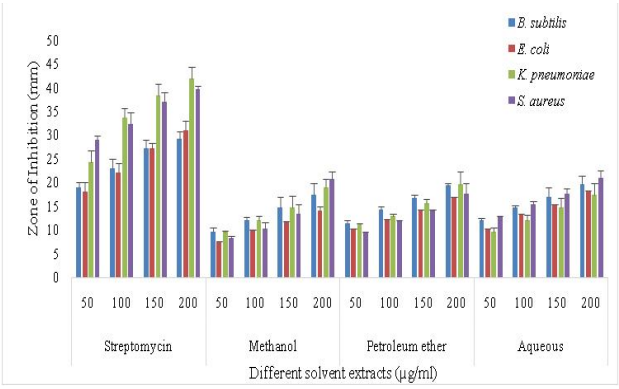

Fig. 4 : Antibacterial activity of *D. hamiltonii* against different bacterial strains.

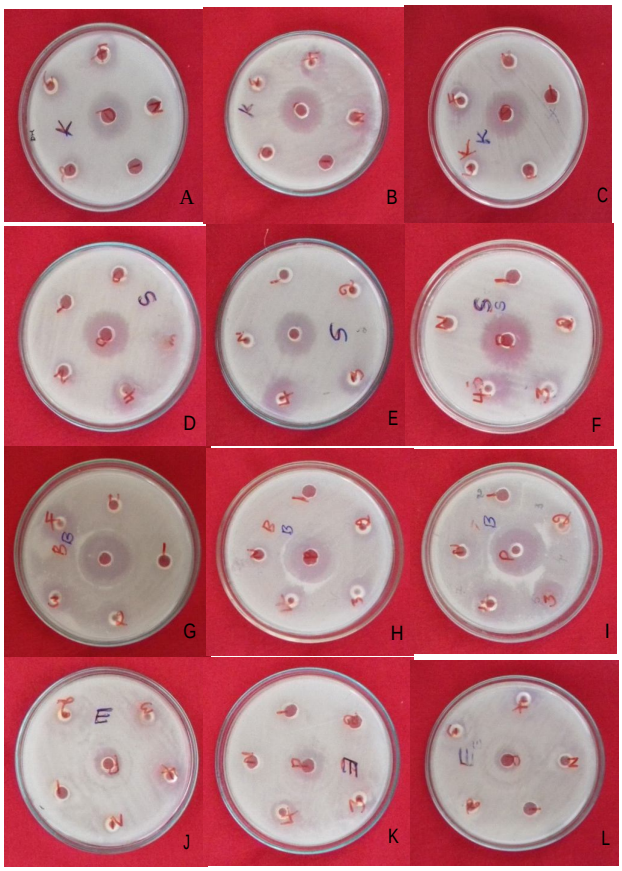

Fig. 5 : Zone of Inhibition of *D. hamiltonii* against different bacterial strains.A: Methanolic extract; B: Petroleum ether extract; C: Aqueous extract against *K.pneumoniae.* D: Methanolic extract; E: Petroleum ether extract; F: Aqueous extract against *S. aureus*. G: Methanolic extract; H: Petroleum ether extract; I: Aqueous extract against *B. subtilis*. J: Methanolic extract; K: Pet. ether extract; L: Aqueous extract against *E. coli*.
(P-positive, N- negative, 1-50 2-100, 3-150, 4-200 µg/ml)

these bacterial pathogens, *S. aureus* is more sensitive when compared to other bacteria. Prakash and Manivasagaperumal (2016) observed that the higher zone of inhibition in methanolic root extract of *D. Hamiltonii* against *Bacillus subtilis.* The extracts of higher plant can be very good sourcing of antibiotics against various bacterial pathogens (Devi and Latha, 2012).

The present study indicates that *D. hamiltonii* have many bioactive components like alkaloids, phenols, flavonoids, tannins and volatile oils etc. 2-hydroxy 4-methoxybenzaldehyde was observed in both root and root oil through GC-MS and HPLC method. The maximum content of 2H4MB was recorded in root oil, which might be helpful in the pharmaceutical and food industries.Further

investigations are needed for *in vitro* multiplication and to enhance the phytoconstituents of roots using different promoters.

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