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Research Article

# Study on phytochemical screening and antimicrobial activity of Roots of *Decalepsis hamiltonii Wight & Arn.*

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**Abstract**

*Decalepsis hamiltonii Wight & Arn.* is a monogeneric climbing scrub native of the Deccan peninsula and forest areas of Western Ghats of India. It is used in treatment of loss of appetite,fever,skin disease,diarrhea and nutrition disorders(wealth of India,1990). In this present study the bioactive compounds from *Decalepsis hamiltonii* was extracted using different solvents – ethanol,methanol,chloroform and petroleum ether. Preliminary screening for the phytochemical compounds in the extracts were performed and the extracts were then examined for antibacterial and antifungal activity by disc diffusion method. Abundant flavanoids are detected in methanol and trace amount in other extracts. Moderate amount of tannin is indicated in all four extracts. The extract of roots with all four solvents showed considerable antibacterial activity. The broad spectrum antibacterial activity of *Decalepsis hamiltonii* may be due to the active compounds like flavanoids, tannins, etc.

**Keywords:** *Decalepsis hamiltonii,* Antimicrobial activity and Phytochemical analysis.

## Introduction

Medicinal plants are the most important source of life saving drugs for the majority of world's population. Over centuries cultures around the world have learned how to use plants to fight illness and maintain health.In India, around 20,000 medicinal plants have been recorded however traditional communities are using only 7,000 -7,500 plants for curing different diseases. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body.

Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional remedies such as herbs for their medicine.It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients. The most popular analgesic,aspirin,was originally derived from species of Salix and Spiraea and some of the most valuable anti-cancer agents such as paclitaxel and vinblastine are derived solely from plant sources.

*Decalepsis hamiltonii* is a monogeneric climbing scrub native of the Deccan peninsula and forest areas of Western Ghats of India.This is an endemic and endangered medicinal plant and grow largely in moist as well as dry deciduous forest,scrub jungles of southern parts of Deccan peninsula (Gamble and Fischer,1957).Pharmacognostical study of roots of *D. hamiltonii* was investigated by Nayar *et al*.(1978) during the drug preparation.Shefali et al (2009) reviewed the pharmacognosy,phytochemistry and

pharmacology of *D. hamiltonii*.The aromatic root of *D.hamiltonii* has potent bioinsecticidal,antimicrobial and anti-ulcer properties.The roots are being used in Ayurveda,the ancient Indian system,to stimulate appetite,relieve flatulence and as a general tonic(Vedavathy,2004).It is used in treatment of loss of appetite,fever,skin disease,diarrhea and nutrition disorders(wealth of India,1990). Recently antidiabetic,hepatoprotective and antiatherosclerotic properties of root extract of *D. hamiltonii* have been evalvated in rats and reported that the tuber extract could be able to protect the rats from oxidative stress and also inhibit the activity of antioxidant enzymes causing liver damage(Naveen and Khanum,2010)
In this present study the bioactive compounds from Drynaria quercifolia was extracted using different solvents and preliminary screening for the phytochemical compounds in the extracts were performed. The extracts were then examined for antibacterial and antifungal activity.

## Materials and Methods

### Description of the Plant

***Decalepsis hamiltonii Wight & Arn.***

This plant is an Angiosperm and belongs to family Asclepiadaceae. It is a mono generic climbing scrub native of Indian Peninsula. The roots are long, thick and aromatic. The liane stems with broad obavate leaves climb to canopy and produce white villous umbrella shaped flowers.

### Plant Collection and Processing:

The roots of *Decalepsis hamiltonii* was collected from Kollimalai, Namakkal District, Tamilnadu. The plant is identified as *Decalepsis hamiltonii Wight & Arn*. using the Herbarium Specimen at Ranipat Herbarium (RPT), St.Joseph College, Trichirapalli. The roots were washed with sterile distilled water. It was cut into small pieces and air-dried in shade. The dried material was powdered using grinder and stored in air tight containers.

### Plant Extract Preparation:

25 g of powdered root sample of *Decalepsis hamiltonii* was weighed and macerated with respective solvents. It was kept at room temperature for 72 hrs with stirring for every 24 hours. The suspension was filtered through Whatman filter paper # 1. The extracts were dried by evaporating in room temperature. The dried residue was stored in refrigerator and used for further studies. Finally four extracts were obtained with solvents methanol, ethanol, chloroform, petroleum ether respectively.

### Microbial Culture

Bacteria:

Organism used were *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhi, Vibrio cholera.*

Fungi:

*Candida albicans, Cryptococcus neoformans*
Media Used:

Nutrient agar, Mueller – Hinton agar, SDA medium (Hi media).

### Preparation of Antibiotic Disc:

20 mg of crude extract was dissolved in 1 ml of 20% DMSO. From this stock solution 10-20 µl of respective solvent extract is added to the disc separately in aseptic condition. Each disc now contains 0.2 mg of the extract. The disc were dried at room temperature and used for study of antibacterial activity.

### Phytochemical Screening:-

Chemical tests were carried out on ethanol, methanol, methanol-chloroform, chloroform extracts of *Decalepsis hamiltonii*, using standard procedures to identify the constituents as described by Sofowara (1993) Trease and Evans (1989), Harborne (1973) and E1.Tawil, (1983).

### 1.Alkaloids:

About 0.2 g of the extracts was warned with 2% $H_2SO_4$ for two minutes. It was filtered and few drops of Dragencloffs reagent were added. Orange red precipitate indicates the presence of alkaloids.

**2.Glycosides:**

The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

**3.Saponins:**

About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

**4.Flavonoids:**

Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless indicates the presence of flavonoids.

**5.Steroids:**

2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of $H_2SO_4$. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

**6.Coumarins:**

In a test tube 1 gm of plant sample was placed and covered with filter paper moistened with dil.NaOH, then heated on water bath for few minutes. The filter paper was examined under UV light, yellow fluorescence is indicative for the presence of coumarins.

**7.Tannins:**

Small quantity of extract was mixed with water and heated on a water bath. The mixture filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

**8.Terpenoids ( Salkowski test):**

0.2 g of the extract of the whole plant sample was mixed with 2 ml of chloroform **(**$CHCl_3$**)** and concentrated $H_2SO_4$. (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

**Antibacterial Susceptibility test:**

**Disc diffusion method:**

Kirby-Bauer method was followed for disc diffusion assay. In vitro microbial activity was screened using Mueller – Hinton agar (MHA) .The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 mins and 0.1 ml of the inoculum suspension was swabbed uniformly and the inoculums was allowed to dry for 5 mins. The discs loaded with the plant extract were placed on the surface of the medium and the compound was allowed to diffuse for 5 mins. The plates were then incubated at 37° C for 24 hours. Negative control was prepared using respective solvents. Gentamycin ( 10 µg/disc) was used as positive control. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in duplicate.

**Antifungal susceptibility test:-**

The fungistatic activity the extract was also evaluated by disk diffusion method. 100 µl of fungal suspension. ($10^{5}$ CUF ml-1) was pipette onto petri dishes containing SDA and spread uniformly. Discs impregnated with 20 µl of extracts were placed on the surface of solid agar petri dishes. The plates were incubated for 48-72 hrs and the diameter of zone inhibition around each disc was measured in millimeters.

## Results and Discussion

**Preparation of plant extract:**

*Decalepsis hamiltonii* roots were collected from Kollimalai,Nammakal.The air dried sample was powdered and extracted with four different solvents - ethanol,methanol,chloroform and petroleum ether.

Phytochemical screening of *Decalepsis hamiltonii:*

The results of phytochemical screening tests for 9 compounds are given in Table-2. Alkaloids were not present in any of the four extracts. Similarly saponins, coumarins and terpenoids were not detected in extracts with all the four solvents. Trace amount of glycosides is detected from ethanol and methanol extract but absent in chloroform and petroleum ether. Abundant

flavanoids are detected in methanol and trace amount in other extracts. Steroids are strongly detected in all four extracts. Moderate amount of tannin is indicated in all four extracts. Collectively more number of compounds are present in the methanol extract of roots of *Decalepsis hamiltonii* . This extract may be used for the detailed study of the medicinally active compounds in *Decalepsis hamiltonii*.

**Antibacterial activity of *Decalepsis hamiltonii:***

The results for test for antibacterial activity of rhizome extract of *Decalepsis hamiltonii* with different solvents by disc diffusion method is listed in Table-3. From the results it is shown that petroleum ether extract of the rhizome has greater inhibitory effect against both gram positive and gram negative bacteria. Petroleum ether extract is followed by extract with methanol in its antibacterial activity. Chloroform extract showed least effect. Among the bacteria used *Staphylococcus aureus* and *Bacillus cereus* showed high susceptibility to all the different extracts. The extracts were more active against Gram positive bacteria when compared to Gram negative.

The major antimicrobial component of essential oil responsible for the inhibition of microbial growth was identified as HMB(Phadke et al.,1994).The antimicrobial activity of the essential oil was tested against food borne pathogens.It exhibited strong antimicrobial activity against Bacillus cereus,Bacillus megaterium,Candida albicans, Escherichia coli,Micrococcus luteus(Thangadurai et al.,2002).Elizabeth et al(2005) also demonstrated that the D.hamiltonii possesses strong antimicrobial property.Mohana et al(2008) investigated the antimicrobial potential of D.hamiltonii with some other medicinal plants against human pathogenic bacteria. This table reveals that the extract of roots with all four solvents showed considerable antibacterial activity. The broad spectrum antibacterial activity of *Decalepsis hamiltonii* may be due to the active compounds like flavanoids, tannins, etc.

**Antifungal activity of *Decalepsis hamiltonii:***

The results for antifungal activity of root extracts of *Decalepsis hamiltonii* is given in Table -6.It is similar to that of its antibacterial activity. Petroleum ether extract is highly effective of all the four extracts. From the above results it can be confirmed that *Decalepsis hamiltonii* possess strong medicinal properties.Further studies are being carried out on the identification and isolation of phytochemical compounds from ethanol and methanol extract.

Table- 1: Phytochemical analysis of extract from *Decalepsis hamiltonii*

| S.No | Phytochemical | Solvent used | | | |
|---|---|---|---|---|---|
| | | Ethanol | Methanol | Chloroform | Petroleum ether |
| 1. | Alkaloids | - | - | - | - |
| 2. | Glycosides | + | + | - | - |
| 3. | Saponins | - | - | - | + |
| 4. | Flavanoids | + | +++ | - | ++ |
| 5. | Steroids | ++ | + | + | + |
| 6. | Coumarin | - | - | - | - |
| 7. | Tannins | ++ | ++ | - | ++ |
| 8. | Terpenoids | - | - | - | - |
| 9. | Phenolics | - | + | - | ++ |

**Table -2:** Antibacterial activity of rhizome extracts of *Decalepsis hamiltonii* in different solvents

| ORGANISM | ZONE OF INHIBITION(MM) | | | | |
|---|---|---|---|---|---|
| | ETHANOL | METHANOL | CHLOROFORM | PETROLEUM ETHER | CONTROL |
| *S. aureus* | 10 | 11 | 9 | 21 | 14(C) |
| *Bacillus cereus* | 12 | 15 | - | 15 | 7 (Ap) |
| *E.coli* | 14 | 15 | 8 | 14 | 11(G) |
| *K.pneumoniae* | 8 | 11 | 10 | 10 | 13 (C) |
| *P.aeruginosa* | 8 | 12 | - | 11 | 6 (Ap) |
| *Salmonella typhi* | 10 | 16 | - | 12 | 10(G) |
| *Vibrio cholera* | 11 | 12 | 9 | 12 | 12(T) |
| *Proteus mirabilis* | 12 | 12 | 8 | 13 | 15(Cp) |

Control: G –Gentamycin, Ap- Ampicillin,T – Tetracycline,C- Chloramphenicol,Cp-Ciprofloxacin

**Table -3:** Antifungal(yeast) activity of rhizome extracts of *Decalepsis hamiltonii* in different solvents

| Organism | Zone of inhibition(mm) | | | |
|---|---|---|---|---|
| | Ethanol | methanol | Chloroform | Petroleum ether |
| *Candida albicans* | 15 | 19 | 9 | 16 |
| *Cryptococcus neoformans* | 14 | 16 | 10 | 15 |

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