

**Phytochemical screening and Antibacterial activity of root extracts of *Decalepis hamiltonii* Wight & Arn.****Prakash P., G. Thiyagarajan, \*Rengarajan Manivasagaperumal**

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**ABSTRACT**

In the present investigation, preliminary phytochemical and antibacterial activity of petroleum ether, chloroform, ethyl acetate and methanol root extracts of *Decalepis hamiltonii* were examined. The phytochemical analysis revealed the presence of active ingredients such as glycosides, steroids, flavonoids, phenols, terpenoids, saponins and tannins in the root extracts of *Decalepis hamiltonii*. The antibacterial activity was performed by disc diffusion method using different concentrations of solvent extracts. Seven bacterial pathogen such as gram positive - *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus* and gram negative-*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* were used as test organisms. Among the four solvents used methanol extract was found to be more active against most of the tested pathogenic bacteria at higher concentrations, as they showed potential phytochemical constituents. Among all the tested organisms *Klebsiella pneumoniae* was found to be more resistant by all the extracts, where as *Escherichia coli*, *Pseudomonas aeruginosa*, and *salmonella typhi* showed moderate resistant and *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis* showed least resistant against all the extracts. The results of present study supports traditional usage *Decalepis hamiltonii* and also suggest that methanol root extract possess compounds with antimicrobial property that can be used as antimicrobial agents in new drug for the therapy of infectious diseases caused by pathogens.

**Keywords:** Antibacterial activity, disc diffusion method, *Decalepis hamiltonii*, successive extraction

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**INTRODUCTION**

Medicinal plants play a significant role in the life of people and are present in innumerable forms. In Indian traditions, all plants in this earth are considered as medicinal. However, the simplest definition of the medicinal plants would be "Medicinal plants are plants that provide people with medicines to prevent diseases, maintain health or cure ailments." In one form or another they benefit virtually everyone on the earth. In India, different parts of several medicinal plants or their extracts are used for the treatment of various diseases. Several antibiotics are used for the treatment of human infections, which have limited antimicrobial spectrum. They could develop drug resistance in pathogens and lead to serious ill effects. Hence plant derived antimicrobial properties have received considerable attention in years.

Several plants have been indicated in folk and other traditional system of medicine as specific agents. More than hundred species of therapeutically important higher plants are listed and described in ancient literature to have the antimicrobial activity. Efforts are thus directed to identify the plant products which have broad spectrum of antimicrobial property with no ill effects [1]. However, a majority of traditionally used Indian medicinal plants have not been systematically screened against various microbial pathogens. Considering all these in mind, the present study is concentrated on the medicinal plant *Decalepis hamiltonii* Wright & Arn an endemic, endangered, climbing shrub and native of southern peninsula. This plant has been used in Ayurveda, the ancient Indian traditional system of medicine to stimulate appetite,

relieve flatulence and as a general tonic [2]. It is also useful as a blood purifier, preservative and as a source of bioinsecticide for stored food grains [3- 4]. Earlier studies have shown that roots contain aldehyde, inositol, saponins, amyriinsandlupeols [5-6] as well as volatile compounds such as 2-hydroxy-4-methoxybenzaldehyde, vanillin, 2-phenyl ethyl alcohol, benzaldehyde and others [7]. These chemical preservatives act as antimicrobial compounds, which inhibit the growth of undesirable microorganisms. Hence the present study was to analyze the presence of phytochemical and to evaluate the antibacterial activity of different solvent extracts of root of *Decalepis hamiltonii* against some gram positive and gram negative bacterial strains.

## MATERIALS AND METHODS

### Preparation of plant extracts

Fresh and healthy *Decalepis hamiltonii* roots were collected from Kolli hills of Namakkal district, Tamilnadu. The plant was taxonomically identified by using flora of Madras presidency. In the laboratory, the roots were washed 2-3 times with running fresh water and then air dried under shade. After complete shade drying the plant material was grinded with mechanical grinder, the powder was kept in small labelled plastic bags. 100g of roots of *Decalepis hamiltonii* were subjected to successive extraction with different solvents in increasing polarity viz. Petroleum ether, chloroform, Ethyl acetate and Methanol using soxhlet apparatus. The solvents were evaporated under reduced pressure and stored in desiccators at 4°C.



### Morphological features of root of *Decalepis hamiltonii* wright & Arn Qualitative phytochemical analysis

The qualitative chemical analysis of various extracts were carried out for the presence

of alkaloids, glycosides, flavonoids, phenols, gums, terpenoids and tannins by using the following standard methods [8-11].

#### 1. Test for carbohydrate:

##### Barfoeds test:

To 1ml of filtrate, 1ml of Barfoed's reagent is added and heated on a boiling water bath for 2min. Red precipitate indicates presence of sugar

##### Barfoed reagent

Copper acetate, 30.5 g is dissolved in 1.8 ml of glacial acetic acid

#### 2. Tests for Saponins:

To 0.5g of plant extracts, distilled water was added and heated for few minutes. Foam formation indicates the presence of saponins.

#### 3. Tests for Tannins:

To 0.5g of plant extracts, 10ml of distilled water was added and filtered; to the filtrate 0.1% of ferric chloride solution was added. Formation of brownish green indicates the presence of tannins.

#### 4. Tests for Steroids:

To 0.5g of plant extracts, 2ml of acetic anhydride was added and 2ml of sulphuric acid was added. Formation of violet-blue colour indicates the presence of steroids.

#### 5. Tests for Flavonoids:

To 0.5g of plant extracts, acetone was added and heated in a water bath until the acetone gets evaporated, then filtered. The filtrate is cooled and 5ml sodium hydroxide was added. Presence of yellow colour indicates the presence of flavonoids.

#### 6. Tests for Alkaloids:

To 0.5g of plant extracts, 3ml of methanol was added with 10% acetic acid and ammonium hydroxide was added. Formation of precipitate indicates the presence of alkaloids.

#### 7. Tests for phenols:

To 0.5g of plant extracts, distilled water was added and heated, to that 2ml of ferric chloride was added. Blue/green colour formation indicates the presence of phenols.

#### 8. Tests for Glycosides:

To 0.5 g of plant extracts, 1ml of glacial acetic acid was added, then ferric chloride was added and 1ml of sulphuric acid was added. Reddish brown colour appears at the junction of two layers and the upper layer

turned bluish green indicates the presence of glycosides.

#### 9. Test for Gum and Mucilage [12]

The extract (100mg) is dissolved in 10ml of distilled water and to this; 25ml of absolute alcohol is added with constant stirring. White or cloudy precipitate indicates the presence of gums and mucilage.

#### 10. Test for Terpenoids

5 ml of each extracts was mixed in 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive result for the presence of terpenoids.

#### Antimicrobial Screening

##### Microorganisms used

Seven bacterial strains were used in the present study. The clinical isolates were obtained from the Department of Clinical Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai nagar.

The seven bacterial species used in the study were, the gram negative strains- *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *salmonella typhi* and gram positive bacteria - *Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*. They are identified according to standard phenotype test.

##### Antibacterial assay

##### Disc diffusion method:

The agar diffusion method [13] was followed for antibacterial susceptibility test. Petri plates were prepared by pouring 20ml of Muller Hinton Agar allowed solidifying for the use in susceptibility test against bacteria. Plates were dried and uniformly spread. The excess inoculum was drained and the plates were allowed to dry for 5min. After drying the discs with extract were placed on the surface of the plates with sterile forceps and gently pressed to ensure the contact with the incubated agar surface. Ciprofloxacin (10µg/disc) was used as positive control. 5 percent DMSO was used as blind control in these assays. The incubated plates were incubated at 37°C for 24hrs. The zone of inhibition was observed and measured in millimeters. Each assay in this experiment was repeated for three times.

## RESULTS

### Phytochemical screening

In the present study, the phytochemical screening was studied with petroleum ether, chloroform, ethyl acetate and methanol extract of the roots of *Decalepis hamiltonii*. The results revealed, that methanolic root extracts of *Decalepis hamiltonii* were rich in saponins, steroids flavonoids, phenols, terpenoids, and tannins followed by other extracts (Table 1).

### Antibacterial screening:

The antibacterial activity of different concentration of Petroleum ether, Chloroform, Ethyl acetate and Methanol extracts of the roots of *Decalepis hamiltonii* against bacterial pathogens are presented in Table 1. The mean zone of inhibition for Petroleum ether extract ranging from 6.4 to 13.0 mm against bacteria such as 13.0 mm against *S. aureus*, 12.4 mm against *B. cereus*, 12.1mm against *B. subtilis*, mild inhibitory effect on 11.2 mm against *P. aeruginosa*, 11.2 mm against *S. typhi*, 10.5 mm against *K. pneumoniae* and 9.8mm against *E. coli* at 1000µg concentration.

The mean zone of inhibition for Chloroform extract ranging from 6.2 to 12.8 mm against bacteria such as 12.8 mm against *S. aureus*, 11.9 mm against *B. cereus*, 10.3mm against *E coli*, 8.3 mm against *S. typhi*, 9.6 mm against *P. aeruginosa*, mild inhibitory effect on 9.3 mm against *K. pneumoniae* and 8.3 mm against *B. subtilis* at 1000µg concentration.

The mean zone of inhibition for Ethyl acetate extract ranging from 6.9 to 14.3 mm against bacteria such as 14.3 mm against *S. aureus*, 13.9 mm against *B. cereus*, 13.3 mm against *B. subtilis*, 12.8 mm against *S. typhi*, 12.2 mm against *E. coli*, mild inhibitory effect on 11.1 mm against *K. pneumoniae* and 10.1 mm against *P. aeruginosa* at 1000µg concentration.

The mean zone of inhibition for methanol extract ranged from 7.4 to 21.0mm against bacteria such as 21.0 mm against *S. aureus*, 20.4. mm against *B. cereus*, 19.6 mm against *B. subtilis*, 16.5 mm against *S. typhi*, 15.2 mm against *P. aeruginosa*, mild inhibitory effect on 14.6 mm against *E. coli* and 13.5 mm against *K. pneumonia* at 1000µg concentration.

## DISCUSSION

### Photochemical screening

Phytochemical constituents such as tannins, flavonoids and several other aromatic compounds or secondary metabolites of plants serve as defense mechanism against predation by many microorganisms. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins and steroids [14]. The presence of saponins, flavonoids, phenols and terpenoids in the root extract are very important and are used in analgesic, anti-Plasmodia and bactericidal activities [15]. Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development

### Antibacterial screening

The evaluation of antimicrobial potential by disc diffusion method indicated that all the bacteria tested organisms showed growth inhibition towards the root extract, with differing sensitivity. Among the bacterial pathogens, *S. aureus* is more sensitive when compared to other bacteria. Gram-positive bacteria were exhibited more sensitiveness to root extracts when compared to Gram-negative bacteria [16]. In addition, these results confirmed the evidences in previous studies reported that methanol and petroleum ether extracts were found to be most active against tested bacterial strains. The different solvent extracts of *A. serpyllifolia* against the tested strains are showed that the methanol, petroleum ether, and benzene extracts of both plants showed

a broad spectrum of activity, being active on Gram +ve and Gram -ve organisms [17].

The different solvent viz, petroleum ether, chloroform, acetone and methanol of stem extracts were tested for antibacterial activity. Among the four solvent tested all the test pathogens were highly sensitive to methanol extract followed by acetone, chloroform and petroleum ether extracts [18]. The different organic extract of *A. Aspera* exhibit significant antimicrobial activity against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *B. subtilis*, *M. luteus* and *S. aureus*. Methanol extracts from leaf part of *A. aspera* produced consistent level of inhibition of bacterial growth and followed by aqueous, chloroform and ethyl acetate. *In vitro* antibacterial studies of the four different leaf extracts of *Aristolochia bracteata* revealed that the methanol extract had significant activity against most of the organism and followed by aqueous extract, while the ethyl acetate extract possessed moderate activity [19]. In addition, these results confirmed the evidences in previous studies reported that methanol as a better solvent for more consistent extraction of antimicrobial substances from medicinal plant compared to other solvents, such as water, Ethanol and hexane [20-22].

### CONCLUSION

As the search for new antimicrobial agents is in demand, plant extracts may provide attractive alternate sources of antimicrobial drug against various microbial diseases. The present study provides the evidence that methanol root extract of *Decalepis hamiltonii* has antimicrobial property against bacteria.

**Table 1: Preliminary Qualitative Phytochemical analysis of root extracts of *Decalepis hamiltonii* Wight & Arn**

No.	Name of the Test	Root			
		Petroleum ether	Chloroform	Ethyl acetate	Methanol
1.	Carbohydrate	+	-	-	-
2.	Saponins	+	-	-	++
3.	Tannins	+	-	-	++
4.	Steroids	+	+	+	++
5.	Flavoids	+	-	+	++
6.	Alkaloids	-	-	-	-
7.	Phenol	+	+	+	++
8.	Glycosides	+	-	+	+
9.	Gums	+	-	+	+
10.	Terpenoids	+	+	+	++

++= Strong positive +=Positive -=Negative

**Table 2: Antibacterial activity of different solvent extracts of root of *Decalepis hamiltonii* (250, 500, 1000 µg / disc)**

S. No	Micro organisms	Mean Zone of inhibition (mm) <sup>b</sup>												Antibiotic 10µg/disc
		Petroleum ether			Chloroform			Ethyl acetate			Methanol			
		250µg	500 µg	1000 µg	250 µg	500µg	1000 µg	250 µg	500 µg	1000 µg	250 µg	500 µg	1000 µg	
1	<i>E.coli</i>	6.4±0.36	8.5±0.21	9.8±0.61	6.8±0.35	7.5±0.51	10.3±0.81	7.2±0.30	8.8±0.40	12.2±0.75	8.8±0.40	10.4±0.55	14.6±0.61	18±0.55
2	<i>K. pneumoniae</i>	6.8±0.50	8.0±0.35	10.5±0.61	6.2±0.25	7.1±0.36	9.3±0.76	6.9±0.50	8.8±0.40	11.1±0.55	7.4±0.45	10.2±0.97	13.5±0.50	16.6±0.77
3	<i>P.aeruginosa</i>	6.5±0.55	9.3±0.55	11.2±0.40	7.0±0.60	8.3±0.20	9.6±0.45	7.2±0.45	9.3±0.72	10.1±0.65	11.5±0.35	12.4±0.40	15.2±0.50	17.4±0.35
4	<i>S. typhi</i>	6.9±0.45	8.5±0.65	11.2±0.69	6.7±0.50	7.1±0.36	10.2±0.47	9.5±0.88	11.3±0.51	12.8±0.60	11.4±0.51	14.3±0.55	16.5±0.91	22.9±0.35
5	<i>S.aureus</i>	8.7±0.50	10.4±0.40	13±0.50	7.8±0.30	9.5±0.60	12.8±0.35	11.8±0.25	12.6±0.50	14.3±0.55	15.0±0.90	17.6±0.60	21.0±0.77	30.7±0.60
6	<i>B.cereus</i>	7.4±0.45	9.2±0.66	12.4±0.79	7.7±0.50	8.3±0.20	11.9±0.43	10.9±0.25	12.3±0.51	13.9±0.55	14.0±0.90	16.4±0.40	20.4±0.95	27.7±0.30
7	<i>B.subtilis</i>	6.5±0.55	8.7±0.56	12.1±0.65	6.6±0.45	7.4±0.20	8.3±0.45	7.4±0.81	9.3±0.47	13.3±0.41	13.1±0.85	16.1±0.80	19.6±0.70	22.6±0.55

a – diameter of Zone of inhibition (mm) including disc diameter of 6mm, b – mean of three assays; ± standard deviation; Ciprofloxacin (10 µg/disc) ± SEM (standard Error Mean).

*E. coli* - *Escherichia coli*, *K.p* - *Klebsiella pneumoniae*, *P.a* - *Pseudomonas aeruginosa*, *S.t* - *Salmonella typhi* (**Gram negative bacteria**)  
*S.a* - *Staphylococcus aureus*, *B.c* - *Bacillus cereus*, *B.s* - *Bacillus subtilis* (**Gram positive bacteria**)

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