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Phytochemical studies and antibacterial activity of *Decalepis hamiltonii* Wight & Arn, an endangered medicinal plant

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Abstract
Decalepis hamiltonii Wight & Arn. belongs to family Asclepiadaceae and commonly called as Maredu kommulu, Maredu gaddalu and Barre sugandhi. It is an endemic endangered climbing shrub and mostly all parts of the plant (root, stem and leaves) are medicinally used. Its tuberous roots are generally used as health drink mostly in the southern part of India and are well known for its medicinal properties. In the present investigation the presence of phytochemical constituents and antibacterial activity of the root, leaf and stem extracts of the plant are studied. Methonolic extract of different plant parts were obtained and assessed for the presence of various phytochemicals and antibacterial activity. These activities were determined by using standard protocols with some modifications. The phytochemical evaluation revealed the presence of Alkaloids, Flavonoids, Phenols, Steroids, Tannins, Terpenoids, Saponins and Glycosides. The antibacterial activity was observed against test organisms like *Escherchia coli, Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* The study supports the traditional usage of *Decalepis hamiltonii* and suggests that some of the plant extracts possess compounds with antimicrobial properties that can be used to develop in new drugs for the therapy of infectious diseases caused by pathogens.

Keywords: *Decalepis hamiltonii*, Methanolic extract, phytochemical analysis, Antibacterial activity, Pathogens

Introduction
Indian traditional system of medicine is based on various systems of medicine such as Ayurveda, Siddha, Unani and Homoeopathy. During the last few years the graph of standardization of medicinal plants of potential therapeutic significance has been increased. The evaluation of all medicinal plants is based on phytochemical and pharmacological approaches leading to drug discovery and it is referred to as "natural product screening." [1] Secondary metabolites from the plants are responsible for their action or pharmacological activity [2, 3].

Decalepis hamiltonii Wight &Arn., is an endemic and endangered medicinal plant that grows largely in moist as well as dry deciduous forests, Scrub jungles, Southern part of Deccan peninsula and the Western Ghats of India. [4] It is locally called as Maredu kommulu or Barre sugandhi or Maredu gaddalu or Makaliberu belonging to the family Asclepediaceae. *D. hamiltonii* is utilized in tribal and traditional Indian and Chinese medicine for treatment of a wide range of ailments including digestive system, lungs and circulatory system [5, 6].

The parts of the plant used are the tuberous roots, which are fragrant and sweet, with vanilla like taste and odour. *D. hamiltonii*, the type sp, is the most widespread and utilized species of the genus [7]. The roots of *D. hamiltonii* are used in folk medicine and ayurvedic preparation [8] in treating indigestion, to stimulate appetite, to relieve flatulence and to act as a general tonic [9]. Roots are also used to cure dysentery, cough, bronchitis, leucorrhoea, uterine hemorrhage, skin disease, fever, indigestion, vomiting, chronic rheumatism, anemia, 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 effect [10].

Medicinal plants have been used as therapeutic agents in traditional system for treatment of human diseases for thousands of years [11]. Herbal medicines have become more popular in the treatment of many diseases due to lesser side effect. These medicinal plants constitute the main source of new health care products and pharmaceuticals [12]. Phytochemical screening of root,

stem and leaf extract of *D. hamiltonii* has revealed the presence of numerous chemicals including alkaloids, flavanoids, tannins, steroids, glycosides, and saponins.[13] These secondary metabolites serve as defense mechanism against many microorganisms and act as antimicrobial compounds. In the present study to explore the medicinal importance the root, stem and leaves of *D. hamiltonii* have been analyzed for the presence of phytochemicals and antibacterial activity against gram+*ve* and gram-*ve* bacterial strains.

Materials and methods

Collection of Plant Material: The plant material collected from Sheshachalam hills, Tirupathi, Chittor District, Andhra Pradesh. Plant was identified and authenticated at Plant Anatomy and Taxonomy Laboratory, Department of Botany, Sri Venkateshwara University, Tirupathi, Andhra Pradesh and conserved in Botanical garden Department of Botany, Osmania University, Telangana. The healthy root, stem, and leaves were brought to the lab, and maintained at Microbial Physiology lab, Department of Botany, University College of Science, Saifabad, Osmania University, Hyderabad, India (Fig 1).

Fig 1: *Decalepis hamiltonii* planted in Botanical garden, Department of Botany, University College of Science, Saifabad, Osmania University, Hyderabad.

Preparation of plant extract

The root, stem and leaf samples were washed thoroughly under running tap water to remove soil particles and finally washed with sterile distilled water. These samples were shade dried and ground in to fine powder (Fig-2). The powdered materials were stored in air tight polythene bags until use. 25 gm of powdered material was extracted by Soxhlet apparatus with 100 ml of methanol. The extract was then filtered through Whatman no 41 filter paper and the filtrate was concentrated through evaporation using rotary evaporator at 50^0C. One gram of the extract diluted with 100 ml of methanol and this solution was employed in phytochemical analysis and antibacterial activity.

Fig 2: A&B: Roots and Dried powder, C&D: Stem and Dried powder and E&F: Leaves and Dried powder.

Phytochemical Screening: The qualitative chemical analysis of methanolic extract was carried out for the presence of alkaloids, flavanoids, saponins, steroids, glycosides, phenols, tannins and terpenoids using standard procedures. [14]

Test for identification of Alkaloids: About 3 ml of methanolic extract was taken in a test tube and 5 ml of 1% dilute HCl was added, stirred and kept on a water bath for 20 minutes. The solution obtained was cooled and filtered. 2-3 drops of Mayer's reagent was added to the filtrate. A cream coloured precipitate indicated the presence of alkaloids.

Test for identification of Flavonoids: About 3 ml of methanolic extract was taken in a test tube, 1ml of 10% sodium hydroxide was added. A yellow colouration of the solution indicated the presence of flavonoids.

Test for identification of Phenols: 5 ml of methanolic extract was taken in a test tube and 2 drops of ferric chloride solution was added. Formation of green coloured precipitate indicated the presence of phenols.

Test for identification of Saponins: About 2 ml of methanolic extract was taken in a test tube was shaken 2 minutes. Fronthing which persisted was taken as evidence for the presence of saponins.

Test for identification of Steroids: About 1 ml of methanolic extract was taken in a test tube and 2 ml of concentrated sulphuric acid was added by the sides of the test tube and red colour at lower layer indicates the presence of steroids.

Test for identification of Tannins: About 1 ml of the methanolic extract was taken in to test tube and 1 ml of freshly prepared 10% ferric Chloride solution was added. Appearance of blue color indicates the presence of tannins.

Test for identification of Terpenoids: About 1 ml of the methanolic extract was mixed with 2 ml of chloroform and 2ml concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface showed the presence of Terpenoids.

Test for identification of Glycosides: 3 ml of the methanolic extract was taken in to the test tube and 2 ml of chloroform was added. Sulfuric acid was added careful to form a lower layer. Appearance of reddish brown colour indicates the presence of glycosides.

Antibacterial assay

Diffusion method

The antibacterial activity was performed by disc diffusion method for root, stem and leaf extracts. The Mueller Hinton Agar (Hi Media) was used as bacteriological medium. Mueller Hinton Agar plates were prepared by pouring 15 ml of molten media into the sterile petriplates. The plates were allowed to solidify for 15 minutes and 0.1% inoculum was swabbed uniformly and was allowed to dry for 5 minutes. Under aseptic conditions, 6 mm diameter (whatman no 1) filter paper disc were impregnated with 10 µl (contains 2 mg/disc) of root, stem and leaf extracts of *D. hamiltonii.* The discs were overlaid on MHA plates and incubated at 37 °C for 24 hours. The diameter of zone of inhibition produced by the extracts was compared with standard drugs (10 µg/disc Streptomycin). The experiment was performed thrice to minimize the error and the values are reported.

Results and Discussion

The present study contributes valuable information of phytochemical analysis and antibacterial activity in *D. hamiltonii.* The results revealed the presence of the phytochemicals like Alkaloids, Flavonoids, Phenols, Steroids, Tannins, Terpenoids, Saponins and Glycosides in the root extract, whereas Saponins, Glycosides and Tannins were absent in leaf and stem extracts of *D. hamiltonii* (Table-1). *D. hamiltonii* root, stem and leaf extracts were found to possess different degrees of antibacterial activities. The antibacterial activity of root, stem and leaf extracts against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* was quantitatively assessed by the presence or absence of inhibition zone (Table-2 & Fig-3). The Phytochemical constituents or secondary metabolites of plant serve as antimicrobial compounds. The biological function of alkaloids and their derivatives are important and are used as antibacterial agents. There are several reports on screening of phytochemicals in different plant extracts of Asclepiadaceae. [15, 16, 17] Hence the phytochemical studies are useful in the detection of bioactive compounds, drug discovery and development.

Table 1: Phytochemical analysis of methanolic extracts of root, stem and leaves from *D. hamiltonii.*

S.NO	Test for Phytochemicals	Test results		
		Root	Stem	Leaves
1	Alkaloids	+ve	+ve	+ve
2	Flavonoids	+ve	+ve	+ve
3	Phenols	+ve	+ve	+ve
4	Steroids	+ve	+ve	+ve
5	Tannins	+ve	-ve	-ve
6	Terpenoids	+ve	+ve	+ve
7	Saponins	+ve	-ve	-ve
8	Glycosides	+ve	-ve	-ve

+ *ve* Presence of the compound.
- *ve* Absence of the compound.

Table: 2 Zone of inhibition with methanolic Extract of *Decalepis hamiltonii*

Plant part used	Zone of inhibition in centimeters.			
	S. aureus	*P. aeruginosa*	*E. coli*	*K. pneumonia*
Control (Streptomycin)	1.2	1.3	1.6	1.1
Leaf	1.1	1.0	1.4	1.0
Stem	1.4	1.4	1.5	1.2
Root	1.4	1.5	1.6	1.2

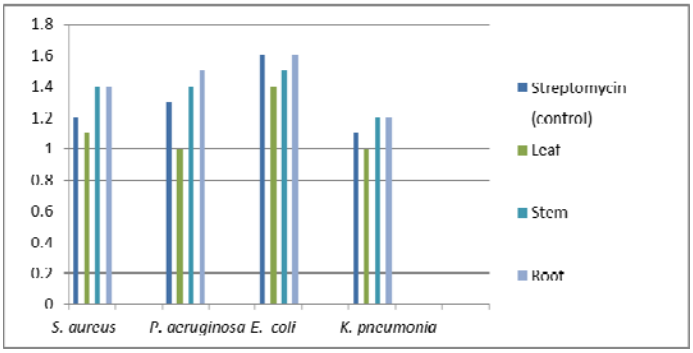

Fig 3: Zone of inhibition with Methanolic Extract of *Decalepis hamiltonii*

Conclusion

The present research has emphasized that medicinal plants are potential renewable natural resources with a beneficial role in human health care. The results revealed that the extracts of *Decalepis hamiltonii* root, stem and leaf showed good antibacterial activity against gram positive and gram negative pathogens. This property was attributed by the presence of bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids, gylcosides and phenolic compounds. The present study provides an insight that India is enriched with valuable assets of therapeutic plants which has to be worked out.
This information is valuable for the preparation of drugs in pharmaceutical industry and there is a need for more intensive research since play a great role in healthcare.

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