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Antibacterial Potential of *Decalepis hamiltonii* Wight & Arn. Callus Extract

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Abstract

Antibacterial potential of crude petroleum ether extract obtained from leaf callus tissue of *Decalepis hamiltonii* was evaluated against five bacterial pathogens using agar well diffusion method. Among the tested bacterial strains, inhibitory activity of the callus extract was minimum against *Klebsiella pneumonia* (5 mm) and it was followed by *Proteus vulgaris* (6mm). Maximum inhibitory activity was observed against *Salmonella typhi* (11mm). From this observation it is evident that phytochemical principles which are responsible for the curative (antibacterial) activity of *Decalepis hamiltonii* could have a constant expression pattern in a specialized set of cells even after their rapid division. *In vitro* micropropagated clones from the parental plant will have an efficient transmission of the antibacterial active constituents. Moreover, this observation may be a baseline for the large scale extraction of antibacterial principles and other curative chemicals through callus culture.

Key Words: *Decalepis hamiltinii*, Callus culture, antibacterial principles, herbal medicine

Introduction

Plants have been the traditional source for raw materials and finished medicines since the dawn of civilization. A rich heritage of knowledge on preventive and curative measures was even available in ancient scholastic work included in the Atharvana Veda, Charaka and Sushrutra. It has been estimated that about 20,000 plant species are known to have worldwide use as drugs. Phytochemical tests have been performed in about 5000 and nearly 1110 species are extensively utilized in Ayurveda (80%) and Allopathic medicine (33%) (Singh,1998; WHO,2006). Historically, therapeutic results have been mixed; quite often cures or symptom relief resulted. Poisonings occurred at a high rate, also. Currently, of the one-quarter to one-half of all pharmaceuticals dispensed in the United States having higher plant origins, very few are intended for use as antimicrobials, since we have relied on bacterial and fungal sources for these activities. Since the advent of antibiotics in the 1950s, the use of plant derivatives as antimicrobials has been visually nonexistent (Lemos *et al.*, 1990).

In India, herbal medicines have been the basis of treatment and cure for various diseases, physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Indian folk medicine comprises numerous herbal prescriptions for therapeutic purposes which may be as varied as healing wounds, treating inflammation due to infection, skin lesions, leprosy, diarrhoea, scabies, venereal diseases, snake bite and ulcers etc. The realization that many infectious pathogenic microorganisms are fast developing resistance against prevailing drugs has necessitated a search for new sources of anti-microbial compounds. In the course of their life cycle, plants encounter infection by a variety of viruses, bacteria, fungi, parasites specific to them. They are expected to synthesize a variety of secondary metabolites capable of providing them protection against the infectious agents (Kambu, 1990; Bahadur *et al*., 2007).

Antimicrobial evaluation, as can be seen from comprehensive range of organisms been tested. The Antimicrobial properties of plant volatile oils and their constituents from a wide variety of plants have been assessed (Benoit-Vical *et al*.,2001). These have included food spoiling organisms and food poisoning organisms spoilage and mycotoxigenic filamentous fungi and plant viruses. Although reports of antibacterial activity of indigenous plants have been known from many regions (Carson and Riely, 1995) they have not been systematically and adequately evaluated and validated, except in a few cases, thereby leading to confusion in drawing a meaningful

conclusions. Hence, in every developing country it is necessary that the documentation of medicinal plants be treated as a matter of extreme urgency (WHO, 2001).

Decalepis hamiltonii is a glabrous extensively climbing shrub growing in moist deciduous forests, scrub jungles of Deccan peninsula and the Western Ghats of India. This is an endemic and endangered medicinal plant of southern peninsula. This plant prefers to grow along rocky slopes, big rock boulders and rocky crevices and small mounds at an altitude from 300 - 1200 meters. The stem and branches are articulated, angled with swollen nodes and reaches a girth of 5 cm. The stem and tubers are aromatic and highly valued for medicinal properties. The tubers are fleshy, strongly fragrant, and pale brown in colour and grow up to 160 cm in length and 2.5 cm width. Dried samples are available in the country drug stores and local tribal markets. During summer the plant appears deciduous, vegetative structures become dry whereas the fruit follicles and tubers persist. Family - *Periplocaceae (*Kostel.) Schltr (Earleir under Asclepiadaceae).

Decalepis hamiltonii is Endemic to central peninsular India. Fairly common along the dry hill tracts of Eastern of Andhra Pradesh, and Western Ghats of Karanataka, and Tamil Nadu. Herb. Madras; Wall. Cat. 8247. Deccan peninsula: Balaghaut (Pulgart) mountains, Near Madras; Annamallay Hills, Wight (Hooker, 1885). Deccan. Horislegkonda at 4,500 ft (Gamble, 1957), Madanapalle in Chittoor at 3,000 ft. hills of Nellore (Ramaswami), Kanbakam hills of Chingleput, W. Ghats, Anamalayas (Wight, Barber) (Gamble, 1957). In Kerala, few plants have been noticed (Sasidharan and Sivarajan, 1996) in the deciduous belt of Marauyar, Chinnar, Iduki district. In Tamil Nadu several other new locations have been found with sparse distribution of *Decalepis hamiltonii:* Small Hillocks of Vedanthangal, Javvadhu hills, Sirumalai hills of Dindigul (K. Thangavel, Field No. 144, SPKCES Herbarium).

Recently, antidiabetic, hepatoprotective and antiatherosclerotic properties of root extract of Decalepis has been evaluated in rats and reported that the tuber extract could able to protect the rats from oxidative stress and also inhibit the activity of antioxidant enzymes causing liver damage (Naveen and Khanum, 2010). The tuber extracts and the purified compounds of *Decalepis* have found usage in controlling insect pests, Ayurvedic medicine and as a potent antioxidant (Nayar *et al*.,1978; Srivastava *et al*., 2007). An earlier study has shown that aromatic roots of this plant as possible source of insecticides have led to determine the pesticide properties and can be stored for long periods without being affected by microbes. This promoted many researchers to investigate its roots for insecticidal activity against three coleopteran stored product pests, viz; rice weevil, the lesser grain borer, and the rust-red flour beetle (George *et al*., *1998*, 1999). Further, the residual deposit of 2-hydroxy 4-methoxy benzaldehyde (HMB) has been assayed for contact toxicity on rice weevil (*Sitophilus oryzae), Rhyzopartha dominica* and *Tribolium castaneum*. The effective deposits (ED) for 50% and 95% mortalities were derived from a profit analysis of the data (George *et al*., (1999). Thus the aromatic chemicals of *Decalepis hamiltonii* have been established with insecticidal and pesticidal properties.

Thangadurai *et al* (2002) have evaluated and documented the antibacterial potential of the rhizome extract against bacterial strains (especially food pathogens) like *Bacillus subtilis, Candida albicuans, and Staphylococcus aureus.* In this present study, preliminary attempt was made to evaluate the active phytochemical constituents present in the callus tissues obtained from the leaf disc of *Decalepis hamiltonii.* Actively proliferating callus tissue was obtained from leaf disc explant of *Decalepis hamiltonii* by culturing the young leaf discs in sterile MS medium supplemented with 2µM Kinetin and 6 micromolar 2,4-D. Callus tissue was established in the same media combination. Crude petroleum ether extract was obtained from that callus and its antibacterial potential *in vitro* was evaluated against five selected bacterial pathogens using agar well diffusion method.

Materials and Methods

8mm fresh leaf discs (obtained from tender leaves) were inoculated after surface sterilization in sterile Murashige and Skoog (1962) medium supplemented with different concentrations of Kinetin and 2,4-D in combination. The best media combination was identified based on the callus formation efficiency and further cultures was done with such media combination alone for establishing the callus for further extraction. Established callus tissue (both friable and non friable) was used for the crude extract preparation. 1 gram of callus tissue was ground finely with 20ml of petroleum ether as solvent.Then the extract was filtered

aseptically using several layers of sterile cheese cloth. 50 and 100 microlitre of this filtrate (separately) was loaded in 6mm agar wells created in sterile nutrient agar Petri dishes spread with overnight grown pathogenic bacterial cultures separately. In each set triplicate Petri dishes were maintained. For negative control one well was loaded with 100 microlitre of petroleum ether and for positive control, 100 microlitre of gentamycin antibiotic solution (50mg/ml) was also loaded. Inoculated Petri dishes were incubated at 37ºC for 20- 24 hours. After the incubation, zone of inhibition around the wells were observed and recorded, average values were calculated and tabulated (Table-1).

Results

1g/20ml concentration was effective against all the bacterial strains used in this present study. In case of the extracts used in different volumes, 100µl was yielded with notable and interesting results i.e. a clear zone of inhibition. It could be the minimal volume for the exhibition of the antibacterial potential of callus extract. Based on this present observation, the minimal inhibitory concentration of the callus crude extract could be 5mg/ml. It reveals that the existing presence of the antibacterial principles needs to be more than the mother plant tissues comparatively.

Table-1: Inhibitory activity of *Decalepis hamiltonii* leaf callus extract (Test), Petroleum ether (- control) and Gentamycin antibiotic (+ control) against the selected bacterial strains (Zone of inhibition in mm - diameter of agar well ± SD. All values are average of triplicates)

S.No.	*Salmonella typhi*	*Proteus vulgaris*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Escherichia coli*
Test *(Decalepis hamiltonii)* callus extract)	11 ± 0.202	6 ± 0.143	5 ± 0.135	9 ± 0.164	10 ± 0.198
Negative control	3 ± 0.197	2 ± 0.204	3 ± 0.208	3 ± 0.186	3 ± 0.208
Positive control	8 ± 0.214	7 ± 0.210	7 ± 0.209	8 ± 0.182	7 ± 0.191

Discussion

Among the listed medicinal and aromatic plants in medicinal flora, *Decalepis hamiltonii* and its medicinal properties were reported by many authors and it will give a special attention to this species. Aromatic roots of this plant is useful as an appetizer, blood purifier, preservative and as a source of bio-insecticide for stored food grains (Ravishankar *et al.,* 2001). Tuberous roots are used to cure indigestion, deficient digestive power, dysentery, cough, bronchitis, leucorrhoea, uterine hemorrhage, skin diseases, fever, thirst, and vomiting, poisoning, chronic rheumatism, anemia, debility, dysuria and blood diseases. Tuber extract of *Decalepis hamiltonii* proved to be appetizer and better medicine for ulcer and diabetes. Callus extract exhibited more inhibitory effects against *S. typhi* and *E. coli*. These findings are supporting the previous reports by Rastogi (1993) and Majumdar (2002). In case of *K. peumoniae* and *P.vulgaris*, such a high efficiency of inhibition was not observed. This may be due to the development of multiple drug resistance by the respective bacterial strains during the course of their differential exposure. This modified pattern of inhibition reveals that this plant based medicine could be fully screened and the possible number of bacterial infections could be controlled by the application of this tuber or its derivatives. So that the broad spectrum as well as the blunt usage of herbal medicines for a non prescribed ailments will get regulated. Moreover, the possible side effects due to such blunt usage of herbal medicines may be gradually reduced.

Even in this less concentration i.e., 5mg/ml of MIC this callus extract was able to exhibit such a promising inhibition against almost all the tested pathogenic bacteria. This reveals that the specialized group of this plant cells are capable of synthesizing and storing such antibacterial active constituents in higher quantity. So, further studies regarding the specific tissues or cells involved in this synthesis/secretory phenomenon are being necessiated. If it so happens, the selection of the suitable explants for the *in vitro* production of antibacterials could become an easy and more convenient ones. From this present study it is evident that the antibacterial active constituent of this plant is having a

constant expression pattern and it is being detained into the derived callus. So, no doubt the similar medicinal properties of this parental plant will be transmitted fully to clonal plantlets could be produced through the *in vitro* micropropagation protocol. So, *in vitro* cultivation and extraction of active chemical constituents may be a promising one without the loss of their natural populations. In order to evaluate the constant transmission of antibacterial principles to the next progenies, further studies are underway to testify the antibacterial potential of the plantlets which are being produced through *in vitro* regeneration (Parr, 1989; Tripathi and Tripathi, 2003; Dass *et al*., 2008).

Further studies need to be undertaken to analyze the correlation between the chemical composition of the leaf based antibacterial substances and their specific activity against the particular group of bacterial pathogens as reported by Cimanga *et al.*, (2002).

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