

Antimicrobial Activity of Methanolic Root Extract of *Decalepis hamiltonii* Wight & Arn

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ABSTRACT: The present study was quantitative analysis and antimicrobial activity of petroleum ether, chloroform, ethyl acetate, methanol root extracts. The gram positive and gram negative bacterial and fungal species by the disc diffusion method at various concentration (250µl-1000µl). The results was present in the phenolic and flavonoid content compared to extracts. All the root extract against to *Bacillus subtilis* and *Streptococcus pyogenes* and fungal species *Candida albicans* at highest inhibition at concentration of methanolic root extract of zone formation. The preliminary studies on *Decalepis hamiltonii* root extract exhibited their antimicrobial potential which could be exploited further and future pharmaceutical, therapeutic efficiency and food preservation and applications

1. INTRODUCTION

The world Health Organization (WHO) estimates that about 80% of the population living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. In almost all the traditional medicine, the medicinal plants play a major role and constitute the backbone of the traditional medicine. Indian material medical includes about 2000 drugs of natural origin almost all of which are derived from different traditional system and folklore practices [1]. The medicinal value of plants lies in some chemical substances or group of compounds that produce a definite physiological action in the human body. These chemical substances are called secondary metabolites. The most important of these bioactive groups of plants are alkaloids, terpenoids, steroids, flavonoids, tannins and phenolic compounds [2]. Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance. Among the infectious diseases, fungal infections account for a larger proportion of health problems in human beings, particularly among women and children [3]. The substances that can inhibit pathogens and have little toxicity to host cells could be considered candidates for developing new antimicrobial drugs [4]. These compounds are found in various medicinal plant parts such as stems, roots, leaves, barks, flowers, fruits and seeds [5]. *Decalepis hamiltonii* is endemic endangered medicinal plant species. It prefers to grow along rocky slopes, big rock boulders and rocky crevices and small mounds where there is thick vegetation at an altitude from 300 to 1200m. It has good medicinal importance and used in wide drug preparations. Pharmacognostical study of roots of *D. hamiltonii* was investigated for proper identification during drug preparation. The pharmacognosy, phytochemistry and pharmacology of *D. hamiltonii* were reviewed [6]. The aim of the present study is to study the antimicrobial activity of *D. hamiltonii* medicinal plant

2.0 MATERIAL METHODS

2.1 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

2.2 Microorganisms

Pure culture of *Escherichia coli* (ESBL-3984), *Staphylococcus epidermidis* (MTCC 96), *Streptococcus pyogenes* MTCC 102), *Bacillus subtilis* (MTCC 441), *Clostridium bifermentans* MTCC 165), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella pneumoniae* (ESBL-3971) of bacteria and *Candida albicans* (MTCC 227), *Aspergillus flavus* (MTCC 230), *Aspergillus niger* (MTCC 232), *Aspergillus fumigatus* (MTCC 241) and *Pseudomycelium* (MTCC 248) of fungi were used in this study.

2.3 Antibacterial and Antifungal assays

Disc diffusion method

The agar diffusion method [7] was employed for the initial assessment of antibacterial potential of the extracts. Petri plates were prepared by pouring 20 ml of MHA and SDA allowed solidifying for the use in susceptibility test against bacteria and fungi. Plates were dried and uniformly spread. The excess inoculums were drained and the plates were allowed to dry for 5 min. After drying, the disc with extracts were placed on the surface of the plate with sterile forceps and gently pressed to ensure the contact with the incubated agar surface. Ciprofloxacin (5µg/disc) for bacteria and Ketoconazole (10µg/disc) for fungi was used as positive control. 5 per cent DMSO was used as blind control in these assays. Finally, the inoculated plates were incubated at 37 °C for 24 h bacteria and 28 °C for 72 h fungi. The zone of inhibition was observed and measured in millimeters. Each assay in this experiment was repeated three times.

2.4 Total Phenols Content

Total phenolic content was carried out following the Folin-Ciocalteu method by [8]. One ml of crude extracts solution containing (1mg /ml) was added volumetric flask. 1 ml of Folin-Ciocalteu reagent and allowed to stand at 22 °C for 5 min; 7.5% of 0.75 ml of sodium bicarbonate solution was added and mixed thoroughly. The samples were measured spectrophotometrically (Hitachi U-20) at 765 nm using spectrometer after 90 min at 22 °C. The amount of total phenolic was determined as Gallic acid and equivalent and expressed as mg GAE/g dry weight.

2.6 Total Flavonoids Content

The flavonoids content was determined by aluminum trichloride method using catechin as a reference compound [9]. This method based on the formation of a complex flavonoid-aluminum having the absorptive spectrophotometrically (Hitachi U-20) maximum at 415 nm, after remained react at room temperature for 30 min. Briefly, 0.5 mL of each extracts (1:10 g/mL) in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The amount of total flavonoids was determined as mg CE/g dry weight

3. RESULTS

The present root extract of methanol extract of *Decalepis hamiltonii*. The total phenol content in methanol extract of all the solvent extract compare to methanol extract. The phenolic content was found in 13.05mg/ml methanol extract. There these extract were used to for further antioxidant and antimicrobial studies.

Table.1 Total phenol and flavonoid content of root extracts of *Decalepis hamiltonii*

S.No	Solvents	Total phenol	Total flavonoid
1	Methanol	13.05±1.01	6.4±0.70

-Mean of the three assays ± - standard deviation ** significant at $p < 0.05$

The total phenol content of different extracts are expressed in GAE, varied between 3.0 to 13.05 mg/ml. The highest activity was found in the methanol root extract of *Decalepis hamiltonii*. The level of flavonoid content expressed in Quercetin, varied from 1.6 to 6.4 mg/ml. The highest amount was found in the methanol root extract of *Decalepis hamiltonii*

Table. 2 Antibacterial activity methanolic root extract of *Decalepis hamiltonii*

S.No.	Bacteria	250 µl	500 µl	1000 µl	Antibiotic 5µg/disc
1	<i>Escherichia coli</i>	8.8±0.40	10.4±0.55	14.6±0.61	18±0.55
2	<i>Klebsiella pneumoniae</i>	7.4±0.45	10.2±0.97	13.5±0.50	16.6±0.77
3.	<i>Clostridium bifermentans</i>	11.5±0.35	12.4±0.40	15.2±0.50	17.4±0.35
4	<i>Pseudomonas aeruginosa</i>	11.4±0.51	14.3±0.55	16.5±0.91	22.9±0.35
5	<i>Staphylococcus epidermidis</i>	15.0±0.90	17.6±0.60	21.0±0.77	30.7±0.60
6	<i>Streptococcus pyogenes</i>	14.0±0.90	16.4±0.40	20.4±0.95	27.7±0.30
7	<i>Bacillus subtilis</i>	13.1±0.85	16.1±0.80	19.6±0.70	22.6±0.55

Values were expressed as Mean ± SD.

The antibacterial activity of *Decalepis hamiltonii* plant extracts leaves, stem and root were further investigated by time growth assays of both the gram positive and gram negative bacteria in the presence of plant extract the concentration of plant extract in this assays was 250,500,1000ug/ml concentration. The results were observed on the effect of methanolic root extract of *Decalepis hamiltonii* *Staphylococcus epidermidis* (21.0±0.95), *Streptococcus pyogenes* (20.4±0.95) and), *Bacillus subtilis* 19.6±0.70 were indicate high inhibition zone and minimum activity observed in *Escherichia coli* (14.6±0.61), *Klebsiella pneumoniae* 13.5±0.50 and *Clostridium bifermentans* 16.5±0.91

Table.3 Antifungal activity methanolic root of extract of *Decalepis hamiltonii*

S.No.	Fungi	250 µl	500 µl	1000 µl	Antibiotic 5µg/disc
1	<i>Aspergillus niger</i>	12.9±0.45	14.8±0.65	17.9±0.35	22.0±0.56
2	<i>Aspergillus flavus</i>	11±0.30	13±0.40	16±0.25	20.0±0.45
3	<i>Aspergillus fumigatus</i>	12±0.55	15±0.30	19±0.45	22.2±0.40
4	<i>Candida albicans</i>	16±0.30	18±0.30	22±0.36	25.0±0.65
5	<i>Pseudo mycelium</i>	15.2±0.36	16.8±0.61	20.2±0.50	24.4±0.30

Values were expressed as Mean ± SD.

The methanolic root extract produced high degree of inhibition followed by methanolic extract while antifungal activity observed in methanol and measurement mean zone of experiment compared to antibiotics. the zone inhibition were significant on the effect of methanolic root extract of *Decalepis hamiltonii* *Candida albicans* (22.0±0.50), *Aspergillus flavus* (16±0.25) and *Pseudo mycelium* 20.2±0.50 were indicate high inhibition zone and minimum activity observed in *Aspergillus niger* (17.9±0.61), *Aspergillus fumigatus* 19.0±0.45. Among all extracts have the root methanol extract exhibited good antifungal activity.

4. DISCUSSION

Many naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against pathogens [10]. Found that the antifungal active principal is a phenolic compound. Methanol extract of *A. procera* showed antifungal activity against seven fungal strains and ethyl acetate extracts showed activity against any five fungi [11] In addition these results confirmed the evidence in previous studies reported that the methanol is a better solvent for more constituent extraction of antimicrobial substance from medicinal plants compared to other solvents such as water, ethanol and hexane [12, 13-14). The antimicrobial activity of *D. hamiltonii* against human pathogenic bacteria and food borne fungi has been demonstrated by [15]

5. CONCLUSIONS

The results of preliminary phytochemical screening suggest that root extracts of *D.hamiltonii* are good sources of beneficial phytochemicals. The antimicrobial activities of the prepared extracts indicated that root extracts are more potent antimicrobial agent as compared to the root extracts. Among the various prepared extracts of root, methanol extracts were most effective against tested bacterial and fungal species. The statistical analyses performed with regard to antimicrobial activities of different extracts at various concentrations were corroborated with the present findings. Thus, further research is warranted to determine the efficacy of these extracts against various other pathogenic bacterial and fungal species. Moreover *D.hamiltonii* obtained from various geographical locations could also be explored; to evaluate better potential of the plant as a source of antimicrobials. Also, there is call for isolation and identification of active principles of the plant extracts responsible for antimicrobial activity in order to develop future pharmaceuticals.

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