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Research Article

ANTIBACTERIAL AND PHYTOCHEMICAL STUDIES OF VARIOUS EXTRACTS OF ROOTS OF DECALEPIS HAMILTONII WIGHT AND ARN

M.DEVI*[a] AND P.LATHA[b]

[a] Department of Biochemistry, Muthayammal College of arts and science, Rasipuram - 637408, Namakkal, Tamil Nadu, [b]Department of Biochemistry and Molecular Biology, Pondicherry University, Puducherry - 605014, India. Email: kmdevi6@gmail.com

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ABSTRACT

In the present study, the various root extracts of *Decalepis hamiltonii* were screened phytochemically for the presence of secondary metabolites and for in vitro antibacterial activity respectively. The in vitro antibacterial activity of the various extracts of *Decalepis hamiltonii* was studied against *Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Proteus mirabilis, Vibrio cholera, Shigella sonnie, Serritias sp, Staphylococcus aureus* and *Bacillus subtilis* by disc diffusion method. Streptomycin and Gentamycin were used as standard reference drugs while DMSO was included as a solubilizing agent as well as a negative control in this study. All the extracts were found to posses different degrees of antibacterial activity except aqueous extract.

Keywords: *Decalepis hamiltonii,* Phytochemical analysis, Antibacterial activity, Disc diffusion method.

INTRODUCTION

Decalepis hamiltonii Wight and Arn (swallow root) is a monogeneric climbing shrub endemic to the Deccan peninsula. *Decalepis hamiltonii* Wight and Arn commonly called as maredu kommulu or barre sugandhi or maradu gaddalu or makali beru belonging to the family Asclepediaceae. Its roots have been used in Ayurveda, the ancient Indian traditional systems of medicine to stimulate appetite, relieve flatulence and as a general tonic[1]. It is also useful as a blood purifier, preservative and as a source of bioinsecticide for stored food grains[2,3]. Its tubers are consumed as pickles and as a juice for its alleged health promoting properties. Earlier studies have shown that roots contains aldehyde, inositols, saponins, amyrins and lupeols[4,5,6] as well as volatile compounds such as such as 2-hydroxy-4-methoxybenzaldehyde, vanillin, 2-phenyl ethyl alcohol, benzaldehyde and others[7]. The roots have also been used as a substitute for Hemidesmus indicus in ayurvedic preparations of ancient Indian medicine[1]. It possess potent antioxidant properties[8], antiulcer[9], anti – inflammatory and antipyretic[10], gastroprotective[11] activities. In addition 4-hydroxyisopthalic acid, 14-aminotetradecanoicacid, 4-(1-hydroxy-1-methylethyl)-1-methyl-1,2- cyclohexane diol, 2-(hydroxymethyl)-3-ethoxybenzaldehyde, 2,4,8-trihydroxybicycle (3.2.1) octan-3-one, bis-2,3,4,6-galloyl-α/β-D-gluco pyranoside, bornerol and ellagic acid have been identified in swallow root[12,13]. The present study was to analyze the presence of photochemical and to evaluate the antibacterial activity of various extracts of *Decalepis hamiltonii* against several Gram positive and Gram negative bacterial strains in vitro.

MATERIALS AND METHODS

Plant material

The roots were collected from herbal suppliers in Chennai, India. The root (plant material) was identified and authenticated at Plant Anatomy and Research Center, Chennai, Tamil Nadu, India.

Preparation of plant extract

The roots were air dried under shade and powdered to 40 meshes coarse powder and stored in airtight bottles. 100g of *Decalepis hamiltonii* root powder was subjected to successive extraction with different solvents in increasing polarity viz. petroleum ether, benzene, chloroform, ethyl acetate, acetone, methanol, ethanol and distilled water by using soxhlet apparatus. The solvents were evaporated under reduced pressure and stored in desiccators at 4ºC.

Microorganisms used

The microbial strains were obtained from National Chemical Laboratory (NCL), Pune, India. The organisms were maintained on nutrient agar (Hi Media, India) slope at 4ºC and subculture before use. Among 9 microorganisms used, 7 Gram positive bacteria were *Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Proteus mirabilis, Vibrio cholera, Shigella sonnie, Serritia sp* and 2 Gram negative bacteria were *Staphylococcus aureus* and *Bacillus subtilis.*

Preliminary phytochemical analysis

The qualitative chemical analysis of various extracts were carried out for the presence of alkaloids, flavanoids, saponins, steroids, glycosides, phenols, thiols and resins using the method adopted in similar surveys[14].

Antibacterial activity

The antibacterial activity screening was performed by disc diffusion method[15] for various extracts. The Mueller Hinton Agar (Hi Media) was used as bacteriological medium. Mueller Hinton Agar plates were prepared by pouring 15ml of molten media into the sterile petriplates. The plates were allowed to solidify for 15 minutes and 0.1% inoculums suspension was swabbed uniformly and inoculums was allowed to dry for 5 minutes. Under aseptic conditions, 6mm diameter (whatman no 1) filter paper disc were impregnated with 10µl (contains 5mg/ disc) of various extracts of *Decalepis hamiltonii* dissolved in DMSO. The discs were overlaid on MHA plates and incubated at 37ºC for 24 hours. The diameter of zone of inhibition produced by the extracts was compared with standard drugs (10µg/ disc Gentamycin and 10µg/ disc Streptomycin). For each bacterial strain controls were maintained, where DMSO is used instead of extracts. The experiment was performed thrice to minimize the error and the mean values are presented and reported.

RESULTS AND DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents, the first step towards this goal is *in vitro* antibacterial activity. The extracts of higher plant can be very good sourcing of antibiotics against various bacterial pathogens[16]. The results of the photochemical screening of the roots of *Decalepis hamiltonii* are presented in Table 1. The phytochemical analysis revealed the presence of flavanoids, saponins, tannins, steroids, cardiac glycosides. The antibacterial activity of extracts against 9 bacterial strains was presented in Table 2. All the extracts were found to posses different degrees of antibacterial activities except aqueous extract. Petroleum ether extract showed a broad spectrum antibacterial activities may be due to the presence of steroids and glycosides than other extracts. Methanolic extract showed antibacterial activity to all microorganisms except *Staphylococcus aureus*. Acetone extract showed antibacterial activity

except to *Shigella sonnie*. Some of the extracts were ineffective in this study do not posses antibiotic properties or the plant extracts may have antibacterial constituents just not in sufficient concentration so as to be effective.

Various workers have already shown that Gram positive bacteria are more susceptible towards plant extracts as compared to Gram negative bacteria[17, 18]. These differences may be attributed to fact that the cell wall in Gram positive bacteria is of a single layer where as the Gram negative cell wall is multilayered structure[19]. Alternatively, the passage of the active compound through the Gram negative cell wall may be inhibited. Plant based antimicrobials have enormous therapeutic potential as they serve because of lesser side effects. Tannins are well known to possess general antimicrobial properties[20]. Tannins are quite resistant to microbial attack and are known to inhibit the growth of some microorganisms. It is this antimicrobial effect of tannins that slow down the rate of biodegradation of soil organic matter. Antimicrobial agents can damage pathogens in several ways. The major mode of actions is interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, and inhibition of a metabolic pathway[21].

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant - based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials[22]. However, the present study of in vitro antimicrobial evaluation of *Decalepis hamiltonii* forms a primary platform for further photochemical and pharmacological studies. The result of present study supports the traditional usage of the studied *Decalepis hamiltonii* and suggests that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious disease caused by pathogens.

Table 1: Photochemical screening for various extracts of root of *Decalepis hamiltonii*

Secondary metabolites	PE	B	C	E	A	M	Et	Aq
Alkaloids	_	_	_	_	_	_	_	_
Flavanoids	_	_	_	+	++	+++	++	_
Glycosides	+	_	_	+	+	+	+	_
Steroids	+	+	+	+	+	++	+	_
Saponins	+	_	_	_	+	+	_	_
Tannins	+	_	_	+	+++	+++	++	_
Phenols	+	_	_	+	+	+	_	_
Resins	_	_	_	+	+	+	_	_
Thiols	_	_	_	+	_	+	_	_
Carbohydrates	++	_	+	+	+	++	++	_

PE – Petroleum ether B – Benzene C – Chloroform E – Ethyl acetate; A – Acetone M – Methanol E – Ethanol Aq – Aqueous

Table 2: Antibacterial activity of various extracts of root of *Decalepis hamiltonii* against various bacterial strains by disc diffusion method

Microorganisms	PE	B	C	EA	A	M	Et	Aq	S	G	DMSO
Staphylococcus aureus	25	12	9	10	12	20	15	_	21	20	_
Bacillus subtilis	20	11	13	8	12	15	12	_	16	16	_
Escherichia coli	21	8	8	8	14	15	14	_	11	11	_
Salmonella typhi	20	10	9	8	12	16	10	_	15	10	_
Klebsiella pneumonia	18	_	10	10	12	11	_	_	14	13	_
Proteus mirabilis	23	8	8	_	18	14	12	_	16	15	_
Vibrio cholera	22	8	9	8	14	12	11	_	11	12	_
Shigella sonnei	17	8	9	9	12	14	12	_	_	_	_
Serritia spp	17	_	_	_	_	_	_	_	20	17	_

Values include cup border diameter (6mm)

Values are mean of three replicates

PE – Petroleum ether B – Benzene C – Chloroform E – Ethyl acetate; A – Acetone M – Methanol E – Ethanol Aq – Aqueous; S – Streptomycin G - Gentamycin

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