



Solvent Based Effectiveness of Antibacterial and Phytochemical Derivatized from the Seeds of *Harpullia arborea* (Blanco) Radlk. (Sapindaceae)

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ABSTRACT: Phytochemical investigation was carried out on the crude methanol and aqueous extracts of the seeds of *Harpullia arborea* (Blanco) Radlk. (Sapindaceae). The antimicrobial activity of the extract was tested against standard strains and clinical isolates of some bacteria using the disc diffusion method. Preliminary phytochemical studies revealed the presence of glycosides, steroids, saponins and resins as the chemical class present in the extracts. The extracts showed inhibitory activity against clinical isolates of *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris*. The results showed that the methanol extract was more potent than the aqueous extract @ JASEM

Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Infectious diseases are the leading cause for the loss of cattle and human beings worldwide. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. Natural products, either pure compounds or standardized plant extracts, provide unlimited opportunities for new drug leads. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures with antimicrobial traits with diverse chemistry and better modes of action for challenging infectious diseases (Parekh and Chanda, 2007). Hence increased attention focused towards folk medicine and their efficacies (Benkeblia, 2004). Failures of chemotherapies and antibiotic resistance exhibited by microbial agents have led to the screening of several medicinal plants for their potential antimicrobial efficacies (Colombo and Bosisio, 1996).

India exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition (Parekh and Chanda, 2007). It is an emporium of medicinal plants and is one of the richest countries in the world with regard to genetic resources of medicinal plants. Plant derived drugs have become a popular alternative medicine in developing countries. Synthetic antibacterial drugs widely used at present are sometimes causing toxicity and adverse drug reactions. Further more herbal medicines and supplementations are considered less toxic than the synthetic compounds. The barks, fruits and seeds of *Harpullia arborea* are traditionally used by the ethnic communities of Cannore (Kerala) as leech repellent, hair wash and excellent source of an antirheumatic activity (Bakshi, *et al.*, 2001). However, folk medicines administrations have mode of administrations not

been studied in the seeds of *Harpullia arborea*. Keeping this in view, the present study was undertaken in our laboratory to investigate the antibacterial activities of methanol and aqueous extracts of seeds of *Harpullia arborea* against various strains of bacteria.

MATERIALS AND METHODS

Plant Materials: The seeds of *Harpullia arborea* were collected from Coimbatore district and were shade dried, powdered and extracted in soxhlet apparatus successively with methanol 48hrs and aqueous 28hrs respectively due to their nature of polarity. After extraction, the methanol and aqueous extracts were filtered through Whatmann No.1 filter paper and stored for further use.

Microorganisms: The microorganisms *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris* were procured from the Department of Microbiology, Kovai Medical Centre and Hospital, Coimbatore and used for the study.

Antibacterial test

Preparation of culture medium and inoculation:

The petriplates and the nutrient agar medium as well as potato dextrose medium were sterilized for 20 minutes at 120 °C. The rest of the procedure was carried out in laminar air flow. Approximately 20 ml of the media was poured into the sterile petriplates and allowed to get solidified. After the media gets solidified the bacterial organisms were swabbed in the medium using cotton swabs.

Disc Diffusion Method: Antimicrobial activity of the plant extracts was tested using the disc diffusion method of Peach and Tracey, 1950. Sterile nutrient agar plates were prepared for bacterial strains and inoculated by a spread plate method under aseptic

conditions. The filter paper disc of 5 mm diameter (Whatmann No.1 filter paper) were prepared and sterilized. The seed extracts to be tested were prepared in various concentrations of 25%, 50%, 75%, and 100% and were added to each disc of holding capacity 10 microlitre. The sterile impregnated disc with seed extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Filter paper discs soaked in solvent were used for negative controls. All the plates were incubated at 37 °C for 24 hours. After incubation, the size (diameter) of the inhibition zones was measured.

RESULTS AND DISCUSSION

The preliminary phytochemical studies revealed the presence of glycosides, steroids, saponins and resins (Table 1). The antimicrobial activities of

these crude extracts were due to the presence secondary metabolites or compounds like Glycosides, saponins, tannins, flavonoids, terpenoids, alkaloids this has already been confirmed by many researchers (Okeke *et al.*, 2001).

Table 1. Preliminary phytochemical constituents of the *Harpullia arborea*

Constituents tested for	Observation •
Flavonoids	---
Alkaloids	---
Glycosides	++
Steroids	++
Phenols	----
Tannins	----
saponin	+++
Resins	+++

•+++appreciable amount, ++ moderate amount, + trace amount, ---completely absent.

Table 2. Antibacterial activity of *Harpullia arborea* seeds using disc diffusion method

EXTRACTS	STUDY OF BACTERIA	INDICATOR TEST	ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS (µl)*				
			Control	25	50	75	100
METHANOL EXTRACT	<i>Bacillus subtilis</i>		5	12	14	16	20
	<i>Salmonella typhi</i>		5	8	14	18	22
	<i>Pseudomonas aeruginosa</i>		6	10	14	16	19
	<i>Escherichia coli</i>		6	9	11	18	20
	<i>Proteus vulgaris</i>		5	7	12	14	22
AQUEOUS EXTRACT	<i>Bacillus subtilis</i>		5	7	9	9	14
	<i>Salmonella typhi</i>		5	6	7	8	11
	<i>Pseudomonas aeruginosa</i>		6	7	10	11	12
	<i>Escherichia coli</i>		6	8	11	11	12
	<i>Proteus vulgaris</i>		5	8	9	11	14

*Diameter in mm along with disc diameter (5mm).

The results of the methanol and aqueous extract of seeds of *Harpullia arborea* exhibited antibacterial activity against all the tested strain viz. *Bacillus subtilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris* as shown in Table 2. The zones of inhibitions were produced by both the aqueous and methanol extracts against all the test organisms. Methanol extracts were more active than the aqueous extract against all the microorganisms. The zones of inhibition were ranging from 6-22mm in diameter. The highest zone of inhibitions (22mm) noted in methanol extract against *Proteus vulgaris* and *Bacillus subtilis* in 100µl concentrates. The

extracts of higher plants can be very good source of antibiotics (Fridous *et al.*, 1990) against various bacterial pathogens. Plant having antimicrobial compounds have enormous therapeutic potential as they can act without any side effect as often found with synthetic antimicrobial products.

It is concluded that the seed extracts of *Harpullia arborea* have greater potential as antibacterial compounds against micro flora and that they can be used in the treatment of infectious diseases caused by resistant pathogenic microorganisms in cattle and human beings.

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