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Research Article

ANTI BACTERIAL ACTIVITY OF *DERRIS SCANDENS***B. Lakshmi Satya*¹ and T. Praveen Kumar²**

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Abstract:

The antimicrobial activity of ethanol, methanol extracts of Derris scandens whole plant which is used as an herb in Thai traditional medicine was evaluated against various gram positive and gram negative bacteria. The whole plant was extracted by using maceration method for both solvents. The antimicrobial activity and MIC of plant was determined by using agar diffusion method. The medium selected was nutrient agar medium. The organisms selected are Strains of Staphylococcus aureus(MTCC-96), Pseudomonas aeruginosa(MTCC-424), Escherichia coli (MTCC-443), and Bacillus subtilis (MTCC-736) which are purchased from IMTEC. Gentamycin (0.001%) is used as a standard drug. From the results it was obtained that the ethanolic and methanolic extract of whole plant of Derris scandens showed significant inhibition zones. Among all organisms more significant inhibition zone was observed for P.a, E.c, P.v for both extracts. After observing measurable inhibition zones the work was continued to determine MIC for both extracts of whole plant by taking concentrations of 5%, 10%, 15% and 20%. Both extracts of D.s had given an MIC at 15% for all organisms. In conclusion the methanol extract of whole plant of D.s had showed good inhibitory effect than ethanol extracts on growth of P.a, E.c, P.v. which are nosocomial infection bacteria.

Keywords: Anti-bacterial, Derris Scandens, Ethanol, Methanol, disc diffusion, MIC.

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INTRODUCTION:

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (1). Over 50% of all modern clinical drugs are of natural product origin (2) and natural products play an important role in drug development programs in the pharmaceutical industry (3). There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. This burgeoning worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (4-5). Much work has been done on ethno medicinal plants in India. Interest in a large number of traditional natural products has increased. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumor and antimicrobial agents.

The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products. In the present work a

few selected medicinal flora were screened for potential antibacterial activity.

MATERIALS AND METHODS:

Culture of Bacteria:

Strains of *Staphylococcus aureus* (MTCC-96), *Pseudomonas aeruginosa* (MTCC-424), *Escherichia coli* (MTCC-443), and *Bacillus subtilis* (MTCC-736) were obtained from IMTEC, Chandigarh, and were maintained on agar medium at 4 °C for further experiments.

Culture Media and chemicals:

The media used was Nutrient agar medium. For extraction of plant material ethanol and methanol were used. All of them were obtained from SD-fine chemicals.

As a standard drug Gentamycin was used. DMSO for dissolving the plant extracts.

Composition of Nutrient Agar:

Peptone: 0.3%
MeatExtract: 0.5%
SodiumChloride: 0.5%
Agar: 2%
Distilledwater: upto 100ml
Ph: 7.2(+/-) 0.2

Methodology:

Processing of plant material:

Plant parts were washed thoroughly with distilled water and dried under shade. The dried parts were finely grinded using electrical grinder and subjected for sieving. The sieved material was preserved in an air tight container for further use. The pulverized plant material (20g) was extracted in each 500ml of methanol and ethanol by maceration process. The extracts were kept for distillation and allowed to dry. The plant was authenticated by Madhavashetty, Botanist, S.V. University, Tirupathi.

Preparation of Extract:

Maceration method is based on the immersion of crude drug in bulk solvent. The rate of extraction depends upon

- The rate of transport of solvent into the mass to be leached.
- The rate of solubilization of soluble constituents into solvent.
- The rate of transport of solute out of the insoluble material.

In the process of maceration, about 20g of powdered solid material was placed in a closed vessel and 150ml of distilled water (menstrum) is added. It is allowed to stand for 7 days with occasional shaking. After 7 days, the liquid is strained off; the solid

residue (marc) is pressed to recover as much solvent as possible.

Preparation of Standard Drug:

Gentamycin 0.001%: It was prepared by dissolving 10mg of drug in 100ml in sterile distilled water which had given 0.01%. Then it was subjected for 1 in 10 dilutions.

Preparation of Samples: Plant extract was first dissolved in DMSO to prepare dilutions

20% drug extract: 2g of drug was dissolved in 10ml of sterile distilled water and used as a stock culture.

15% drug extract: 1.5 ml of stock culture was taken into a sterile test tube and diluted with 0.5 ml sterile water under aseptic conditions.

10% drug extract: 1 ml of stock culture was taken into a sterile test tube and diluted with 1 ml sterile water under aseptic conditions.

5% drug extract: 0.5 ml of stock culture was taken into a sterile test tube and diluted with 1.5 ml sterile water under aseptic conditions.

Determination of Antibacterial Activity:

Test Organisms:

The selected type bacterial strains were provided by laboratory. The selected micro-organism was listed below: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *proteus vulgaris*.

Preparation of Inoculum:

For determination of antibacterial activity, the inoculum was prepared by using 24hr cultures which were cultured on nutrient agar slants and the stock cultures were maintained at 4°C. Active cultures for experiments were prepared by transferring a loopfull of culture on nutrient agar slant and incubated at 37°C for 24 hours. After observing growth a suspension was prepared by using sterile water under aseptic conditions.

Agar Cup-Plate Method:

The sterile nutrient agar medium was prepared according to composition and sterilized by autoclaving at 121°C at 15 lb/inch² pressure for 15-20 min. the medium is then subjected to cooling. When the medium is at 40°-50°c seeded with a loopfull of 24hrs culture. The tube was mixed gently between the palms and immediately poured into the sterile Petri plates (6 Inch). The plates were then allowed to solidify by keeping on a flat surface. Once all the plates got solidified cavities were made with a depth of 3 to 4 mm by using a sterile cork borer. In each plate four cavities were made. Two cavities were filled with the drug extract and one cavity was

filled with a std drug Gentamycin and one cavity was filled with solvent.

After placing all the solutions the petri plates were allowed to diffuse for 1hr which minimizes the differences due to time variation in placing the solution. The plates were incubated at 37°C for 24hours and zone of inhibition was observed.

Estimation of MIC:

- The methanolic and ethanolic extracts of whole plant were taken and various concentrations like 5%, 10%, 15%, 20% were prepared by dissolving them in DMSO.
- MIC was determined by agar cup plate method where two cavities were filled with respective concentration, one is filled with gentamycin and one is filled with solvent.
- All plates were kept for incubation and observed for the inhibition zones.

RESULTS AND DISCUSSION:

Extraction of plant material: 25g of plant material when extracted with each 500ml of methanol and ethanol solvent had given a yield of 2% and 4% respectively.

Antibacterial Activity: After 24hrs of incubation the inhibition zone diameters were measured and tabulated.

it is observed that the methanolic extract of the plant *Derris scandens* had shown a good inhibitory activity on *Proteus vulgaris* and the ethanolic extract had shown on *Escharechia coli*. Both extracts had produced inhibition zones with sufficient diameter. Methanolic extract had produced very less activity on *Bacillus subtilis* and *Pseudomonas aeruginosa*. As they have shown antibacterial activity the work was further continued to determine the MIC of the plant extracts on various organisms as show in figure no: 1

Estimation of MIC:

The methanolic and ethanolic extracts of both the plants were estimated for their MIC where the concentrations of 5%, 10%, 15% and 20% were placed in the petriplates and incubated. After incubation the inhibition zone diameters were measured and tabulated.

The Methanolic extract of *Derris scandens* had given MIC at 15% con all organisms except *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Onboth of that organisms it had given MIC at 10% conc as shown in figure no: 2

The Ethanolic extract of *Derris scandens* had given MIC at 15% concon all organisms except *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Onboth of that organism it had given MIC at 10% conc as shown in figure no :3

Table 1: Derris Scandens

Name of the organism	Inhibition zone diameter(mm)		Gentamycin (100mg/lit)
	Methanol extract	Ethanol extract	
<i>Bacillus subtilis</i>	-	09	20
<i>Escherichia coli</i>	19	16	21
<i>Staphylococcus aureus</i>	11	08	24
<i>Proteus vulgaris</i>	18	16	20
<i>Pseudomonas aeruginosa</i>	15	13	20

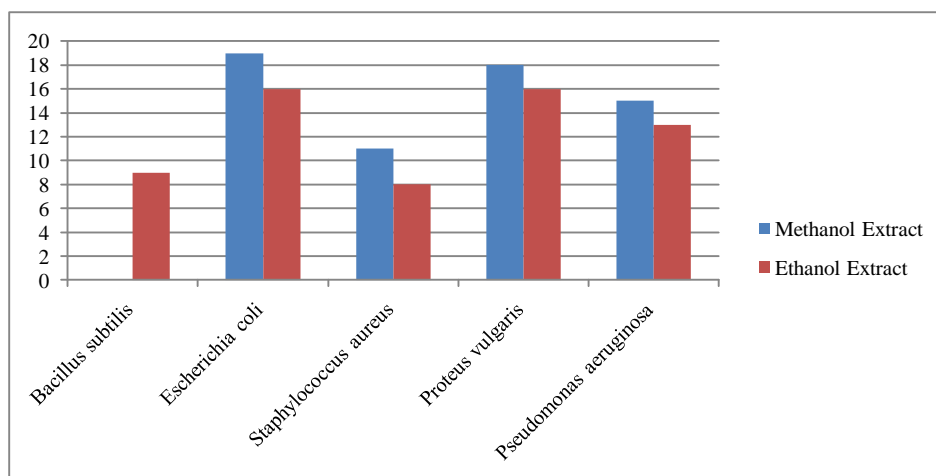


Fig. 1: Derris Scandens

Table 2: Methanolic Extract of *Derris Scandens*

Name of the organism	Methanolic extract of <i>Derris scandens</i>			
	Inhibition zone diameter in mm			
	5%	10%	15%	20%
<i>Bacillus subtilis</i>	-	-	9	10
<i>Escherichia coli</i>	-	-	11	13
<i>Staphylococcus aureus</i>	-	10	12	13
<i>Proteus vulgaris</i>	-	-	09	12
<i>Pseudomonas aeruginosa</i>	-	7	10	14

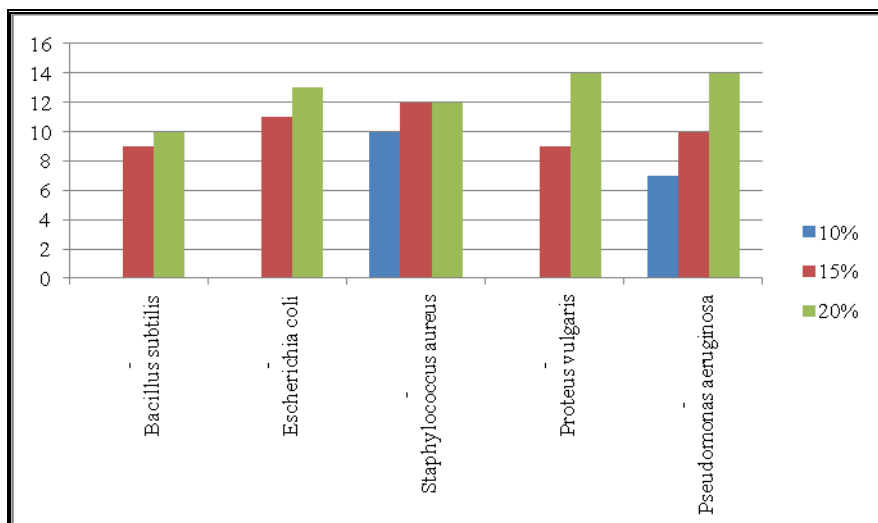


Fig. 2: Methanolic Extract of Derris Scandens

Table 3: Ethanolic Extract of Derris Scandens

Name of the organism	Ethanolic extract of <i>Derris scandens</i>			
	Inhibition zone diameter in mm			
	5%	10%	15%	20%
<i>Bacillus subtilis</i>	-	-	12	15
<i>Escherichia coli</i>	-	-	16	18
<i>Staphylococcus aureus</i>	-	10	15	18
<i>Proteus vulgaris</i>	-	-	13	16
<i>Pseudomonas aeruginosa</i>	-	7	10	17

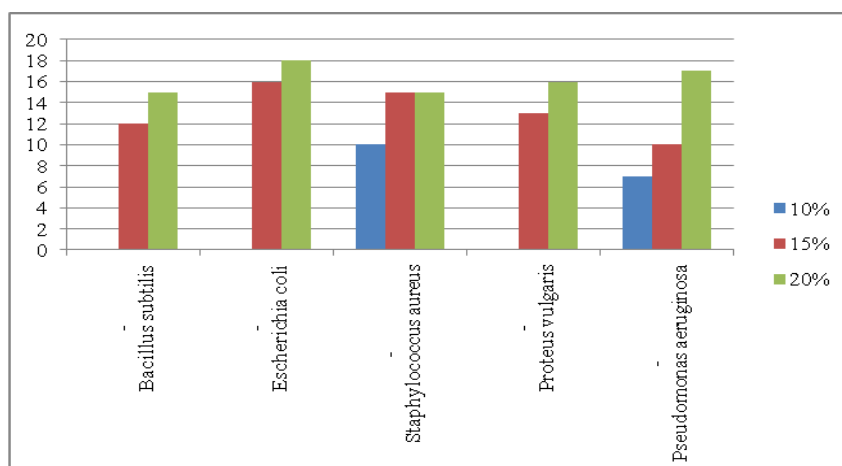


Fig. 3: Ethanolic Extract of Derris Scandens

CONCLUSION:

All results from the above studies show different anti-bacterial activity when whole plant were used against many groups of microorganisms. Difference in results depends on factors including extraction solvents, type of microorganism studied, plant parts, types of herbs used. This study focused on a systematic evaluation of the anti-bacterial activity of *Derris scandens* on *staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* using two extraction solvents (ethanol ,and methanol).

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