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**PJBS**

ISSN 1028-8880

# **Pakistan Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Studies on Antimicrobial Activity of *Perionyx excavatus*

<sup>1</sup>S. Ahmed John and <sup>2</sup>N. Packialakshmi

<sup>1</sup>Molecular Genetics Research Laboratory, Post Graduate and Research Department of Botany,  
Jamal Mohamed College, Trichirappali-620020, India

<sup>2</sup>Department of Microbiology, Sri Sarada Niketan College, Karur-639005, India

**Abstract:** The antimicrobial activity of vermiextract was assessed against four human pathogenic microorganisms. Especially strains of *Proteus* species are a prominent cause of urinary tract infection in children. The present investigation deals with particular urinary tract pathogenic organisms like *E. coli*, *Proteus*, *Providencia*, *Morganella* species. Screening was performed by disc diffusion method. The zone of inhibition range from 17 to 19 mm against  $1.0 \times 10^8$  cells of each culture at 1 mg concentration of vermiextract. Vermextract was most effective against *Proteus* species. The MIC (Minimum Inhibitory Concentration) was estimated by microdilution method.

**Key words:** Antimicrobial activity, vermiextract, pathogenic microorganisms

### INTRODUCTION

Vermiculture is a new development in biotechnology based product. In olden days Earthworms mainly used in the agricultural field. But now a days it will be very useful in the medical field because Earthworms contains amazing antimicrobial activity, *Perionyx excavatus* is an earthworm belongs to the phylum Annelida and class Oligochaeta. This species contains high protein, nitrogen and fat content (Senapathi, 1993). The present day situation great caution is warranted due to existing pollution, pathogens and accumulation of pesticides. The peoples are affected some infections, mainly the Females are affected by the Urinary tract infections. This affected patients take the antibiotic continuously, they will be facing other problems like rheumatism, chest pain an ulcer complaint. The present study has been suggested that earthworms might contain antimicrobial substance effective in curing rheumatism (Weisbach, 1962). But so far there were no reports on the antimicrobial activity of *Perionyx excavatus*. Therefore the investigation deals with antimicrobial activity of *Perionyx excavatus*, against human pathogenic microorganisms.

### MATERIALS AND METHODS

*Perionyx excavatus* were collected from Tamil Nadu Agricultural University, Coimbatore. The species were cleaned with distilled water, crushed and the juice was collected. The milky white fluid was found to have corpuscles, which in turn gives the antimicrobial activity. The stock solution was prepared by the level of

2 mg mL<sup>-1</sup> and it was stored in a refrigerator. The stock was diluted to a desired concentration and then it was used.

**Sample collections:** Samples such as urine and pus were collected sterile bottle from the individuals suspected for various infections in the month of April to June 2005 at Government Hospital, Tiruchirapalli. The collected samples were preserved at 4°C until it could be cultured (Mou and Feldman, 1961). The samples were then subjected to various microbiological and biochemical tests for the detection of pathogens reference with the Bergey's Manual of Determinative Bacteriology (Breed *et al.*, 1965). Microbial strains were obtained from MTCC, Chandigarh, India. The study started with the Molecular Genetics Research Laboratory, Jamal Mohamed College, Trichy.

**Preparation of McFarland ephelometer standard:** A chemically induced precipitation reaction were used. The turbidity of a bacterial suspension was made approximately 1% chemically pure sulfuric acid and 1.175% aqueous solution of barium chloride was prepared. (Bailey *et al.*, 1982). McFarland standard tubes were store in dark at room temperature. The stability will be maintained for 6 months (Table 1).

**Disc diffusion method:** Muller Hinton Agar plates were prepared. Sterile filter paper discs were placed aseptically and various concentration of vermiextracts were placed on the filter paper discs (Bailey *et al.*, 1982). A negative control was maintained, the plates were incubated and the zone of inhibition were measured.

Table 1: McFarland standard preparation

Sample	Numbers					
Chemical	0.50	1.0	2.00	3.0	4.0	5.0
Barium chloride (mL)	0.05	0.1	0.20	0.3	0.4	0.5
Sulphuric acid (mL)	9.95	9.9	9.98	9.7	9.6	9.5
Approximate cell density ( $10^8 \text{ mL}^{-1}$ )	1.50	3.0	6.00	9.0	12.0	15.0

**Classical microbroth dilution method:** Different concentration of antimicrobial ranging from  $8 \mu\text{g mL}^{-1}$  to  $1024 \mu\text{g mL}^{-1}$  were prepared in the medium containing tryptone soya broth Bailey *et al.*, 1982).

**Dispensing antimicrobial dilution into the microtrays:** In the present work, 50  $\mu\text{L}$  of different vermiextract concentration from 64 to  $1024 \mu\text{g mL}^{-1}$  were distributed with calibrate dropping pipette or multichannel pipette. Tryptone soya broth without the vermiextract and with bacterial suspension was added to the 12th well, it act as growth control and 100  $\mu\text{L}$  of tryptone soya broth added to the 11th well of the first row, which in turn act as a medium control.

**Inoculation of the trays:** Before inoculation of the cultures they were adjusted to the 0.5 McFarland Standard, was further diluted in 1:50 dilution (0.1 mL of suspension in 4.9 mL of TSB). From this, 50  $\mu\text{L}$  of bacterial cultures were added to different concentration of diluted vermiextract (Bailey *et al.*, 1982).

**Incubation of the trays:** After inoculation, the trays were stacked one above the other to minimize evaporation. These stacks were then placed in aluminium box with tightly sealed tops. The base of each bin was covered with wet cotton, which gives the humidity in the box and those boxes were placed in an incubator at  $35^\circ\text{C}$  for 5 to 7 h (Bailey *et al.*, 1982).

**Chi-Square test ( $\chi^2$ ):** In this study Chi-Square test ( $\chi^2$ ) was applied (Snedecor and William, 1994). The purpose of Chi-Square test ( $\chi^2$ ) was to decide whether the set of observed data (antibiogram of microorganism) agrees with the standard antimicrobial disc susceptibility test (NCCLS, 2002).

## RESULTS AND DISCUSSION

The previous study reported that the earthworm contains up to 72% of protein, 8-17% carbohydrate, 7-9% fat, 4-8% ash, 2-4% minerals, with a good range of essential vitamins such as niacin which is a valuable component of animal feed (Alawdden and Ismail, 1986).

Table 2: Zone of inhibition (mm) in vermiextract against human pathogenic microorganisms

Microorganisms	Diameter of zone of Inhibition in mm		$\chi^2 = \sum (\text{O} - \text{E})^2/\text{E}$
	Standard (E)	Observed (O)	
<i>Proteus</i> sp.	21	17	0.19047
<i>Providencia</i> sp.	20	19	0.05
<i>Morganella</i> sp.	20	18	0.1 $\chi^2 = 0.34047$

Table Value,  $\chi^2_{(0.05)} = 5.991$

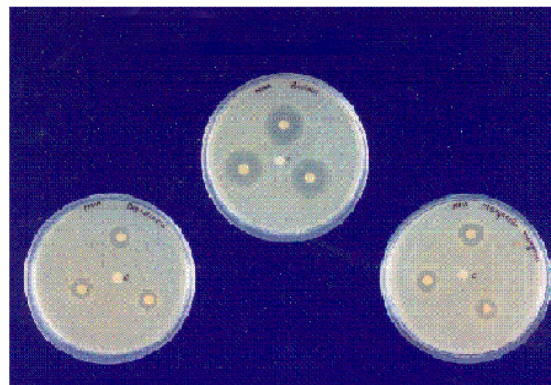


Fig. 1: Disc diffusion method

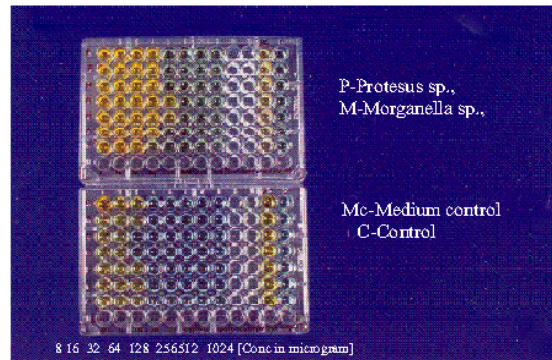


Fig. 2: Minimum Inhibitory concentration assay

The present study, the vermiextract was effective against *Proteus*, *Providencia* and *Morganella* species. The zone of inhibition of *Proteus* species ranged from 17 mm, while the *Providencia* and *Morganella* species. ranged from 19 and 18 mm, respectively (Table 2).

Minimum inhibitory concentration is defined as the lowest concentration of the antimicrobial agent that inhibits the growth of the microorganism. A variety of laboratory methods can be used to measure the *in vitro* susceptibility of bacteria to antimicrobial agents. Commonly used *in vitro* methods are such as micro dilution method, Agar dilution method (Ronald and Jones, 1983). This study deals with a modified microdilution

method with incorporation of indicators to determine the MIC's of vermiextract against pathogenic organisms (Fig. 1 and 2) showed that the minimum inhibitory concentration of *Proteus*, *Providencia* and *Morganella* species was less than 64 µg. In the previous study gentamicin, streptomycin and newer cefotazime gave 100% sensitivity (David *et al.*, 1992) From this study vermiextract also gives the sensitivity to the particular pathogen. The Chi-square value obtained has 0.34047 which was less than the calculated table value,  $\chi^2$  (0.05) = 5.991 at 5% level of significance. The above results lead to the conclusion that the data is consistent with the hypothesis and diameter of zone of inhibition obtained from observed data showed similarities with experimental data (Snedecor and William, 1994). National Institute of Industrial Research reported that the milky white fluid of coelom contains corpuscles of four different kinds, they were phagocytes, chloragogen, circular nucleated cells and mucocytes, they were also found to have antimicrobial activity. These patients can also be suggested to take the regular intake of vermijuice or backed earthworm with bread in the diet, will give remedy to the patients.

These findings concluded that earthworms were good meal for human consumption, continuous taking for the earthworm in the diet after one week the people recovered from the Wound and Urinary Tract Infection.

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