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Evaluation of Antimicrobial Activity of Earthworm *Lampito mauritii* Paste against Bacteria and Fungi

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Abstract

Earthworms lives in the soil along with other microorganisms, some of which can cause diseases to main and other organisms. This has made these worms to develop defence mechanisms against these organisms. It has been reported that earthworms can cure a number of diseases. This has prompted studies on using various species of earthworms to test their antimicrobial activities. Hence, the present study was attempted to study the antimicrobial activity of *Lampitomauritii* against bacterial and fungal organisms which was collected from Musiri area. Results indicate that among bacteria the highest inhibition was shown by *Escherichia coli* and the least by *Vibrio cholerae* and among fungi, the highest inhibition was shown by *Penicillium citrinum* and least by *Candida albicans*.

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Keywords

Antibacterial activity, Earthworm, *L. mauritii*, Bacteria, Fungi.

Introduction

Earthworms which play a major role in the proper functioning of the ecosystem live in an environment containing a large number of microorganisms like bacteria and fungi which includes some microbes which can cause diseases to other organisms including man. In addition, as substrate feeders, they also have a high intake of these organisms. Hence, they have developed humoral and cellular defence mechanisms (Bilej*et al*., 2001; Field *et al*., 2004).

From time immemorial, earthworms have been used as a therapeutic agent. It has been reported that earthworms can be used to treat a number of diseases like chronic bronchitis, peptic ulcer, herpes zoster, burn, scald, bladder calculi *etc.* (Liu, 1983; Liang, 1984; Mu, 1988; Kurek *et al*., 2007; Paul, 2014). Several authors have also reported earthworms to have antimicrobial properties (Hua *et al*., 2011; Hossam *et al*., 2012; Mathur *et al*., 2010; Chauhan *et al*., 2014; Esavani*et al*., 2017). This is an important property as today development of microbial resistance to various existing antimicrobial drugs has become a serious public health concern (Esavani*et al*., 2017). Hence, the present study was attempted to study the effect of earthworm paste of *Lampitomauritii*on various bacterial and fungal pathogens.

Materials and Methods

Collection of earthworms

Matured earthworms, *Lampito mauritii* were collected from the stock culture, Agricultural Earthworm Farm, Kulithalai, Tamil Nadu, India. They were soaked in N-

saline and solution was exchanged after every time so that the gut of earthworm gets thoroughly cleaned.

Preparation of earthworm extracts

About 20 to 30 g of the earthworms were homogenized separately in different solvents used according to decreasing polarity such as that in phosphate buffer (0.2 M, pH 7.0), 95% ethanol and petroleum ether. The homogenized mixtures prepared separately in different solvents were filtered and the filtrates obtained were condensed in water-bath –35°C. The crude extracts obtained were diluted in 10% DMSO for evaluation of antimicrobial activity (Mathur *et al*., 2010).

Culture media

The media for bacterial culture was Muller Hintan Agar (MHA) while Sabourand's Dextrose Agar (SDA) was used for fungal cultures.

Inoculum

The bacterial cultures inoculated in Muller Hinton Agar broth were incubated at 37°C for 18 hr. The suspension was checked to provide approximately 10^5cfs. Fungal cultures were inoculated in Sabouraud's Dextrose against/both were incubated at 37°C for 48 hr.

Microorganism used

Pure culture of five bacterial species (*Vibrio cholerae*, *Salmonella typi*, *Escherichia coli*, *Aeromonas hydrophila* and *Klebsiella pneumoniae*) and fungal culturing species (*Aspergillus niger*, *Pencillium citrinum*, *Candida albicans*, *Aspergillus nidulans* and *Cladosperium herbarium*) were obtained from Tiruchirappalli Biotech Centre, Thillai Nagar, Tiruchirappalli, Tamil Nadu, India.

Preparation of dried filter paper discs

Whatman filter paper No.1 was used to prepare discs approximately 6 mm in diameter, the disc were placed in a Petridish and were sterilized in a hot air oven. The discs were then loaded with 25 µl, 50 µl, 100 µl, 150 µl and 200 µl of each warm extracts concentration per disc.

Antibiotic discs

Two commercial antibiotic discs namely Amoxicillin and Fluconazole were purchased from H^+ media, Mumbai and were used as standard against bacterial and fungal pathogens selected for the experiment.

Antimicrobial activity determination

Determination of antibacterial activity

The disc diffusion method was used. Nutrient agar medium/Muller Hintan Agar (MHA) used for the bacterial cultures. The total of 6 mm diameter were punched the Whatman No.1 filter papers and immersed into the earthworm extracts and the disc was placed on the agar medium. Amaxylin (20 mg/ml) was used as the positive control. The plates were incubated at 37°C for 18 hr. The antibacterial activity was determined by measuring the diameter of zone of inhibition.

Determination of antifungal activity

Sabouraud's dextrose/agar (SDA) was used for the growth of fungal culture. The same procedure as that for assaying the antibacterial activity was adopted and fungal cultures were kept for 48 hr to determine the diameter of zone of inhibition. Flucanazole (20 µg/ml) was used as standard positive control.

Results and Discussions

The antimicrobial activity of *L. marutii*paste on the various bacterial organisms is presented in Table 1. In general, with increase an increase in the zone of inhibition for all the five bacteria that was tested. The highest inhibition was accorded at 200 µl concentration. Among the five organisms that were tested, the maximum zone of inhibition was shown towards the growth of *Escherichia coli* (24.6 ± 0.78 mm) which was closely followed by *A. hydrophila* (24 ± 0.72 mm), *S. typhi* (18.6 ± 0.54 mm), *K. pneumoniae* (15.6 ± 0.58 mm) and *V. cholerae* (14.7 ± 0.68 mm).

The antimicrobial activity with the antibiotic Amaxyline (40 µl) reveals that the maximum antimicrobial activity was with *A. hydrophila* (34.6 ± 0.26 mm) followed by *E. coli* (32.4 ± 0.64 mm), *K. pneumoniae* (30.4 ± 0.68 mm), *S.typhii* (24.4 ± 0.78 mm) and *V. cholerae* (21.2 ± 0.48 mm).

A comparison of the antimicrobial activity between the earthworm paste and Amoxyline reveals that Amoxyline had a higher inhibitory effect when compared with the earthworm paste for all the five bacteria tested. However,

their effect on microbes was different, while amoxyline had the highest inhibitory effect on *A. hydrophila*, earthworm paste had the highest inhibitory effect on *E. coli*.

The antimicrobial activity of *L. marutii* paste on various fungi is presented in Table 2. Here also as with bacteria, with increase in earthworm paste concentration the zone of inhibition also increased. However, the highest inhibition was shown by *P. citrinum* (14.8 ± 0.84 mm) followed by *A. nidulans* (13.8 ± 0.68 mm), *C. herbarum* (13.4 ±0.72 mm), *A. niger* (11.6 ± 0.92 mm) and *C. albicans* (8 ± 0.72 mm). With regard to fucanazole, also the maximum zone of inhibition was with *P. citrinum* (16.4 ± 0.49 mm) followed by *A. nidulans* (14.6 ± 0.29 mm), *C. herbarum* (13.8 ± 0.20 mm), *C. albicans* (13.6 ± 0.52 mm). A comparison of antimicrobial activity between the earthworm paste and Fucanazole indicates that Fucanzole recorded higher inhibition zone. However, the inhibition zones were comparable for *C. herbarum*.

Table.1 Antibacterial activity of earthworm paste of *Lampito mauritii*

S. No.	Organisms	Zone of inhibition in concentration (mm)					
		25 µl	50 µl	100 µl	150 µl	200 µl	Amaxyline (40 µl)
1.	*Vibrio cholerae*	6.5 ± 0.42	8.3±0.86	9.4±0.72	12.4±0.42	14.7±0.68	21.2±0.48
2.	*Salmonella typi*	5.6±0.68	8.6±0.72	12.8±0.42	15.2±0.62	18.6±0.54	24.4±0.76
3.	*Escherichia coli*	9.2±0.64	14.2±0.72	18.0±0.42	22.0±	24.6±0.78	32.4±0.64
4.	*Aeromonas hydrophila*	8.2±0.72	10.2±0.58	16.7±0.74	19.4±0.68	24.0±0.72	34.6±0.26
5.	*Klebsiella pneumoniae*	7.4±0.86	8.4±0.48	10.2±0.92	14.2±0.42	15.6±0.58	30.4±0.68

Table.2 Antifungal activity using earthworm paste *Lampito mauritii*

S. No.	Organisms	Zone of inhibition in concentration (mm)						Fucanazole 20 µl
		Control	25 µl	50 µl	100 µl	150 µl	200 µl	
1.	*Aspergillus niger*		1.2 ± 0.22	4.6 ± 0.72	8.6 ± 0.64	12.8 ± 0.64	11.6 ± 0.92	12.4 ± 0.52
2.	*Pencillium citrinum*		1.4 ± 0.40	4.8 ± 0.60	9.8 ± 0.72	12.2 ± 0.78	14.8 ± 0.92	16.4 ± 0.49
3.	*Candida albicans*		0.6 ± 0.72	2.9 ± 0.64	4.9 ± 0.94	6.2 ± 0.98	8.0 ± 0.92	13.6 ± 0.38
4.	*Aspergillus nidulans*		2.2 ± 0.34	5.8 ± 0.94	9.6 ± 0.98	13.0 ± 0.72	13.8 ± 0.92	14.6 ± 0.29
5.	*Cladosperium herbarium*		2.4 ± 0.36	5.8 ± 0.82	9.8 ± 0.72	12.0 ± 0.64	13.4 ± 0.92	13.8 ± 0.20

A perusal of literature reveals that Ramasamy *et al.* (2008) reported the coelomic fluid of *E. eugenia* showed maximum antibacterial activity against *Staphylococcus aureus* while Kathireswari *et al.*, (2014) while studying the antimicrobial activity with the coelomic fluid of *L. marutii* and *M. konkanensis* suggested inhibition zones varying from 7-17 mm and 9-15 mm for *A. hydrophila, B. subtilis* and *V. parahaemolyticus*. They also reported that the antimicrobial activity with the coelomic fluid of *D. impertusa* and *D. lennora* showed only moderate inhibition zones ranging from 3-6 and 3-8 mm for *A. hydrophila, B. subtilis* and *V. parahaemolyticus*. Comparing these levels with the present study shows higher inhibition rates against *A. hydrophila, E. coli* and *S. typhi*. Chauhan *et al.* (2014) while studying the effect of antimicrobial activity based on the earthworm *E. eugeniae* tissue extract reported inhibition zone of 29 ± 1 mm for *P. aeruginosa* and 24 ± 1 mm for *P. vulgaris* and *S. epidermis*. These levels are elevated when compared with the present study.

While Mathur *et al.* (2011) reported that petroleum ether extract of earthworm powder was found to possess maximum antifungal activity against *A. niger* when compared to *C. albicans.* With regard to the antifungal activity, Vasanthi *et al.*, (2013) reported that *E. eugeniae* paste showed antifungal activity against *C. albicans, A. niger, A. flavus, P. notatum* and *T. rubrum.* However, Chauhan *et al.*, (2014) reported that the tissue extract of *E. eugeniae* did not show any activity against *C. albicans* and *A. niger*. However, the present study clearly shows the antifungal activity against all the five fungi that were tested. Thus, the above results clearly indicate that earthworms can be used against microbes.

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