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INVESTIGATIONAL STUDY TO EVALUATE THE ANTIMICROBIAL ACTIVITY OF FRESH PASTE OF EARTHWORM *Octochaetona paliensis.*

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ABSTRACT Earthworms are annelids that are metamerically segmented oligochaetes, In the recent past, the coelomic fluid and paste of few species of these worms have been studied to exhibit antibacterial and antifungal activities. In this study we experiment to understand the antibacterial (bactericidal or bacteriostatic) and antifungal activity of a paste preparation from a species of earthworm named O.paliensis (Octochaetona paliensis) against standard ATCC (American Type Culture Collection) strains of microbes like S.aureus (Staphylococcus aureus), E.faecalis (Enterococcus faecalis, E.coli (Escherichia Coli), P.aeruginosa (Pseudomonas aeruginosa) and C.albicans (Candida albicans). Strain specific antibiotics were used as controls on a petriplate, that was incubated overnight at 37°C for bacteria and 25°C for fungi accordingly and results tabulated. It was concluded that the paste of O.paliensis has definite activity against the standard strains of microbes as compared to the strain specific antibiotics and significant, appreciable activity against the fungal lawn cultures of C. albicans as compared to ketoconazole. Hence the paste of O.paliensis needs to be further studied with different modifications and restructuring of processes and detailed molecular studies are mandated that will clearly explain us the structure of the compound that is exhibiting the antimicrobial activity. This will enable us to exploit such substances for novel discovery of antibacterial and antifungal agents helpful to curb the growing menace of antimicrobial resistance.

KEYWORDS : Earthworms; antimicrobial activity; earthworm paste; octochaetona paliensis; antimicrobial substances; antimicrobial resistance.

INTRODUCTION:

Earthworms are metamerically segmented tube shaped annelids. They commonly dwell in soil and feeds on dead and live organic matter[1].Earthworm Belongs to class olegochaeta and possess a coelomic cavity which is filled with coelomic fluid and proved to have excellent therapeutic properties since the ancient times [2].In Asia earthworms are used for nutritional purpose as well as they are used in traditional form of medicine[3].Various studies on earthworms have shown anti-inflammatory, antibacterial, antifungal, antihypertensive, antipyretic, anti-asthmatic, anti-allergic, and anti-oxidative properties [4]. Paste of some species of earthworms like lampito mautritii have shown to exhibit antiulcer properties besides being a good anti-oxidative substance [5].

In India there are many traditional medicinal practices such as sidda where earthworms are used for treating various ailments but however there is no scientific data or evidence to prove their claim. Earthworms have been extensively used in traditional Chinese medicine for many years till date. However with the advancement in biotechnology and allied technical advancements, the focus is shifted towards research on earthworms in the pharmaceutical industries [6]

Present study aims to evaluate the antimicrobial activity of earthworm paste prepared from the species octochaetona paliensis.it is a brown coloured worm with a pinkish hue, with its body length measuring 35-92mm in length and 2-3.5mm in thickness.

There are no evidence based researches that has been carried out in these earthworms that demonstrate its antimicrobial activities against standard ATCC strains of microbes. In the present study we aim to test the antimicrobial activity of O. paliensis paste against four standard ATCC strains of bacteria and one fungi that are clinical and therapeutic importance.

Fig.1.picture of octochetona paliensis

MATERIALS AND METHODS

Collection of Earthworms

These earthworms in the current study were collected using digging and hand sorting method [6]. These worms were kept in a separate collection and storage basket with their habitat soil, it was provided with a lid in which holes were made for proper aeration. The required temperature and moisture was maintained necessary for their viability. This method for collection of earthworms is widely used for sampling [7]. The earthworms for the present study is octochetona paliensis, these were collected from a cotton field located in Gulbarga district, Karnataka, India. These earthworms were identified with the help of experts and standard manuals.

Preparation of earthworm paste.

The worms for the present study were collected and kept in plastic culturing trays. For the purpose of preparation of paste, these worms were washed with running tap water, dried with blotting paper and kept in a plastic container containing tissue papers for feeding and enable their gut clearance for about 18 to 24 hours. Later the gut cleared mature clitellate worms were selected and washed with distilled water and were kept in a container containing NACL for one hour, after one hour the solution was changed and the process was continued for another hour, this procedure helps to clear the body surface and debris on the surface of the earthworms . These worms were then washed with distilled water, tap dried using blotting paper and minced. Later it was made into a fine paste which was ready for further investigation [8]

Collection of pathogenic bacteria for antimicrobial studies

ATCC strains of microbes maintained at Rajarajeswari Medical College and Hospital Bangalore were selected with their known pathogenesis and drug resistance profile. The pathogenic bacteria such as Staphylococcus aureus, Enterococcus Faecalis, Escherichia Coli, Pseudomonas aeuroginosa and pathogenic fungi Candida albicans are tested against the paste prepared from the earthworm O.paliensis.

Table: 1 Microbial strains tested against the paste of Octochaetona paliensis

SL. NO	LIST OF MICROBE TESTED (ATCC STRAINS)
1	Staphyloccoccus aureus (ATCC 25923)
2	Enterococcus Faecalis (ATCC 2912)
3	Escherichia Coli (ATCC 25922)
4	Pseudomonas aeuroginosa (ATCC 27853)
5	Candida Albicans (ATCC 1023)

Antibacterial assay

The invitro antimicrobial activity was screened against Staphylococcus aureus (ATCC 25923), Enterococcus Faecalis (ATCC 2912), Escherichia coli (ATCC 25922) and Pseudomonas aeuroginosa (ATCC 27853) by using Muller Hinton Agar (MHA). To prepare the MHA plates, sterile petri plates were taken and 15ml of molten media was poured into it, these plates were allowed a resting time of 5 minutes for solidification. Using a sterile swab, 0.1 % of the inoculum was swabbed uniformly over the petri plates and allowed to dry for five minutes The bacterial inoculum was matched with 0.5 McFarland 10^8 CFU/ml

Antifungal assay

The invitro antifungal activity was screened against Candida albicans (ATCC 10231), This microorganism was inoculated on Saboraud Dextrose broth during 24 hours at 25 degrees C. The inoculate absorbance was established between 1 McFarland 10^8 CFU/ml by adding Saboraud Dextrose broth before the fungal incorporation was carried out (λ=530 nm). Subsequently this fungal strain was seeded on the Sabouraud Dextrose agar by using 4% dextrose, later the 6mm sterile Whatman filter paper disc was seeded onto the agar plate.

Well diffusion technique

With the help of the cork borer one well in center of each solidified agar plate was prepared. Every petri plate contained 50 µg of paste prepared from O.paliensis and one standard ATCC strain of microorganism (Bacteria or fungi) as enlisted in the table.1 and discs of routinely used antibiotics specific to every microbe as mentioned in table 2 that were seeded on the plates and incubated for 24 hours at 37 C for bacteria and 25 C for fungi. The diameter of the zone of inhibition was measured with standard zone reader scale and recorded accordingly.

RESULTS

The paste obtained from O. paliensis was tested against four standard ATCC strains of bacteria and one strain of fungi. viz, S.aureus, E.faecalis, E.coli, P.aeruginosa and C.albicans respectively. The observed zone of inhibition exhibited by the paste of octochetona paliensis were compared with the standard antibiotics used for each strain and tabulated accordingly.

Table.2.showing zone of inhibition of standard antibiotics against ATCC strains of four bacteria and bacteriostatic zone of inhibition of earthworm O.paliensis paste.

STANDARD ATCC STRAINS OF BACTERIA	STANDARD ANTIBIOTICS	ZONE OF INHIBITION IN MILLIMETERS (mm)	BACTERIOSTATIC/BACTERICIDAL ZONE OF INHIBITION BY PASTE OF O. paliensis
S. aureus	Amp.-10mg.	30	8mm
	Gen-10	18	
	Cot-25mg.	20	
	Amc.-10	14	
E. faecalis	Amp.-10mg.	28	12mm
	CIP.-5mg	24	
	P.-10mg.	25	
	HLG.-120	32	
E. coli	Amp.-10mg.	34	10mm
	CIP.-5mg	20	
	Caz-30	29	
	Ak.-30	20	
P.aeruginosa	Tob-10	26	14mm
	Het.-30mg	20	
	Caz.-30mg.	30	
	Ak-30	25	

Table 3. showing zone of inhibition zone of standard antibiotics against ATCC strain of c. albicans and fungistatic zone of inhibition of earthworm O.paliensis paste

Standard ATCC Strains Of Fungi	Standard Antibiotics	Zone of Inhibition in Millimeters (mm)	Fungistatic/Fungicidal Zone of Inhibition By Paste of O. Paliensis
C.albicans	KT-10mg	14mm	36mm

After overnight incubation, the following results were obtained and tabulated as mentioned in table.2. For S.aureus, the paste of O.paliensis have exhibited 8mm bacteriostatic zone of inhibition against the standard antibiotics, where ampicillin 10 µg/disc has shown 30 mm zone of inhibition followed by gentamicin 10 µg/disc, cotrimoxazole 25 µg/disc and amikacin 10 µg/disc has shown 18mm, 20mm and 14mm zone of inhibition respectively. Similarly for E. faecalis, the paste of O.paliensis have shown 12mm bacteriostatic zone of inhibition against standard antibiotics, where High level gentamicin 120 µg/disc has shown 32mm zone of inhibition followed by Ciprofloxacin 5 µg/disc, Penicllin 10 µg/disc, and ampicillin 10 µg/disc has shown 24mm, 25mm and 28 mm zone of inhibition respectively. For E.coli, the paste of O.paliensis have shown 10mm bacteriostatic zone of inhibition against standard antibiotics, where Ampicillin 10 µg/disc has shown 34mm zone of inhibition followed by Ciprofloxacin 5 µg/disc, Ceftazidime 30 µg/disc, and Amikacin 30µg/disc has shown 20mm, 29mm and 20 mm zone of inhibition respectively. Similarly in P aeruginosa , the paste of O.paliensis have shown 14mm bacteriostatic zone of inhibition against standard antibiotics, where Ceftazidime 30 µg/disc has shown 30mm zone of inhibition followed by Tobramycin 5 µg/disc, Netilmicin 30 µg/disc, and Amikacin 30µg/disc has shown 26mm, 20mm and 25 mm zone of inhibition respectively.

In C.albicans the paste of O.paliensis have shown 10mm bacteriostatic zone of inhibition against standard antibiotics, where Ampicillin 10 µg/disc has shown 34mm zone of inhibition followed by Ciprofloxacin 5 µg/disc, Ceftazidime 30 µg/disc, and Amikacin 30µg/disc has shown 20mm, 29mm and 20 mm zone of inhibition respectively.

Fig. 2 Antibacterial activity of paste of O.paliensis against standard ATCC strains of P.aeruginosa with a four standard antibiotic as a postive control.

Fig. 3 Antibacterial activity of paste of O.paliensis against standard ATCC strains of E.coli with a four standard antibiotic as a postive control.

Fig. 4 Antibacterial activity of paste of O.paliensis against standard ATCC strains of S.aureus with a four standard antibiotic as a postive control.

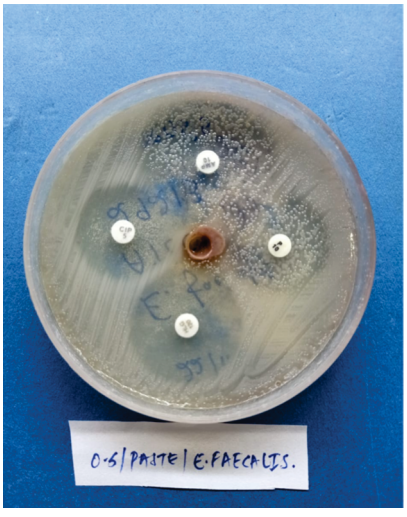

Fig. 5 Antibacterial activity of paste of O.paliensis against standard ATCC strains of E.faecalis with a four standard antibiotic as a postive control.

Fig. 6 Antifungal activity of paste of O.paliensis against standard ATCC strains of C.albicans with a four standard antibiotic as a postive control.

DISCUSSION

Numerous studies done in the recent past have demonstrated antimicrobial activity in the recent past. Although coelomic fluid of different species of earthworms has been studied, antimicrobial potency of freshly prepared earthworm past has been seldom known. Medzhitov R, in his article published by the Nature 1997, has explained in detail the molecular mechanisms that enable an earthworm to kill microorganisms [11], the specific substance that is responsible for antimicrobial activity in a paste needs to be studied further.

In this study we attempted to evaluate the antimicrobial activity of earthworm paste prepared from O.paliensis for its antimicrobial activity, the results obtained from the experiment shows that, when the paste of o.paliensis was observed against lawn culture of S.aureus with standard comparator antibiotics, it exhibited 8mm zone of discoloration, that is more of bacteriostatic zone than bactericidal, whereas ampicillin in the strength of 10 µg/disc has shown 30 mm zone of inhibition followed by gentamicin 10 µg/disc, cotrimoxazole 2µg/disc and amikacin 10 µg/disc has shown 18mm, 20mm and 14mm zone of inhibition respectively. So the bacteriostatic activity of paste of O.paliensis was near to amikacin activity on S.aureus that showed 14mm zone of bactericidal activity.

The petriplate containing lawn culture of E.faecalis inoculated with paste of O.paliensis as compared with standard antibiotics, High level Gentamicin exhibited maximum zone of inhibition (32mm) and Ciprofloxacin in the strength of 5 µg/disc exhibited 24mm zone of inhibition and the paste in question showed 12 mm zone of inhibition, hence its evident that although the paste of O.paliensis demonstrated some activity against E.faecalis, its potency is not much appreciable against other standard antibiotics in the petriplate, this could be further studied by technique modifications that may enhance the potency of the paste in further studies.

Against E.coli, the paste of O. paliensis has exhibited 10 mm zone of inhibition as compared to Amikacin and Ciprofloxacin that has shown 20 mm zone of inhibition, almost double the values exhibited by the paste and maximum zone was exhibited by Ampicillin as 34mm zone of bactericidal inhibition. This shows that the paste of O.paliensis has definite activity against E.coli, but its potency against other antibiotics used in the study is comparatively lesser.

The petriplate containing lawn culture of P.aeruginosa inoculated with paste of O.paliensis as compared with standard antibiotics shows maximum activity with Ceftazidime (30mm) and minimum by Netilmicin (20mm) which is close in comparison to activity exhibited by the paste of O.paliensis (14mm) zone of bacteriostatic inhibition. Other antibiotics, Tobramycin has shown (26mm) followed by Amikacin (25mm).

In petriplate containing lawn culture of C.albicans, the paste of O.paliensis was compared with a standard antibiotic Ketoconazole, wherein Ketoconazole exhibited 14mm zone of clear fungicidal activity, whereas it was observed that the paste has shown 36 mm zone of clear discoloration , that is presumably fungistatic. Hence it can be assumed that paste of O.paliensis has certainly shown good activity against the fungi C.albicans although the substance that is responsible for such activity needs further detailed studies to delineate its antifungal activity and subsequently its molecular mechanism that is responsible for such actions.

CONCLUSION

From this study, we conclude that the paste of O. paliensis at standard concentration of 50 µg/well has demonstrated visible activity against standard ATCC strains of clinically relevant bacteria like S.aureus, E.faecalis, E.coli, P.aeruginosa to variable extent and significant activity against fungi C,albicans when compared to standard strength of Ketoconazole 10 µg/disc. Hence the paste of O.paliensis needs to be further studied with different modifications and restructuring of processes. Detailed molecular studies needs to be carried out that will clearly explain us the structure of the component that is exhibiting such activity, and enable us to exploit such substances for novel discoveries of antibacterial and antifungal agents, to curb the growing menace of antimicrobial resistance and stagnant trend of discovery of antimicrobial substances.

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CONFLICT OF INTEREST

The authors doesn't declare and competing conflict of interest.

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