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Antibacterial activity of subterranean termites used in South Indian folk medicine

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The investigation reports antimicrobial activity of the termite species most commonly used by the South Indian tribes for treating diseases likely to be associated with microorganisms. The antibacterial activities of 90% alcohol extracts of three species of subterranean termites, viz: *Microtermes obes* Holmgren, *Macrotermes estherae* (Desneux*), and Odontotermes formosanus* Shiraki, their mounds and nearby soil extracts collected from three different sites of South India were assayed against various bacterial strains. The antibacterial activity was most apparent in *Odontotermes formosanus* Shiraki, and its mound extracts, which inhibited all the bacterial strains studied. The highest antibacterial activity was encountered in *Odontotermes formosanus* (12.6 ±0.5 mm) and its mound extracts (14.3 ± 1.1 mm) against *E. coli* BL21. The MIC values also varied with the extracts and test organisms used and ranged from 10 µg/disc to 50 µg/disc. No activity was observed in the negative control as well as soil extracts collected from near by the termite mounds. Since, the traditional knowledge of indigenous people throughout the world has played an important role in identifying natural resources worthy of commercial exploitation and the search for new pharmaceuticals from naturally occurring biological material has been guided by ethnobiological data, the results are of significance to modern medicine.

Keywords: Antibacterial activity, Asthma, Ethnozoology, Ethnopharmacology, Termites, *Paniyan, Palliyan, Sholaga, Irular Kota* tribes

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Although the phenomenon of zootherapy is wide spread, it has only recently aroused interest of researchers. Some are focusing on its cultural aspects while others are studying the pharmacological effects of the substances involved. Of the 252 essential chemicals, which have been selected by the World Health Organization, 11.1% come from plants and 8.7% from animals[1]. There are about 16 times as many insect species as there are plant species. Yet very few researchers have concentrated on the medically useful properties of insects. Most research with insects revolves around getting rid of them or fighting the disease they spread. Ethnozoological studies conducted among South Indian tribes revealed that most of the tribes used the termite *Odontotermes formosanus* Shiraki to treat asthma, a disease likely to be associated with the microbes *Streptococcus pneumoniae, Haemophius influenza* and other viruses[2]. Apart from this, bacterial-antigen may produce an allergic response[3]. They used to roast the winged stage of termites in an earthen pot and consume as such in the evenings for 3 days to treat asthma. Termites are also reported to have antibacterial properties. Two novel antimicrobial peptides namely termicin and spinigerin, have been isolated from the fungus-growing termite *Pseudacanthotermes spiniger*[4,5]. Termites have not been studied in a large way mainly because of the difficulty in harvesting large numbers and extracting them from soil debris. Therefore, an attempt has been made to study the antibacterial potentialities of common subterranean termites, viz. *Microtermes obesi*, *Macrotermes estherae, and Odontotermes formosanus,* and their respective mounds.

Methodology

Three subterranean species of termites, viz. *Microtermes obesi*, *Macrotermes estherae, and Odontotermes formosanus* (Insecta: Isoptera) were collected from Kadayanallur, Tirunelveli district, Tamil Nadu, by digging the soil and was identified at Agricultural College, Mandya, Karnataka. Voucher specimens were deposited in the Environmental Biotechnology Division of Rajiv Gandhi Centre for

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Biotechnology, Kerala. The collected organisms were maintained with their mounds and were used for different studies. The soil from the mounds and nearby the mounds was also collected for the extraction of antimicrobial compounds. One gram each of different species of termites, their mounds and the soil near the mounds from three different sites were collected and suspended in 10 ml 90% alcohol separately and kept in a shaker at air tight container for a week. The samples were kept stagnant for 24 hrs to settle the debris completely. The supernatant was transferred to fresh pre weighed container and vacuum dried. The vacuum dried extract was weighed and re-suspended in absolute alcohol to a final concentration of 10 mg/ml and stored in refrigerator and used for antimicrobial assay. *Escherichia coli* O157 [American Type Culture Collection (ATTC); enterotoxigenic], *E. coli* JM 109 (Stratagene; constructed strain), *E. coli* mos blue (Amersham; constructed strain), E. *coli* BL21 (Invitrogen; constructed strain), *Pseudomonas putida* (ATTC; efficient in degrading many pesticides), *Klebsiella* sp [National Culture of Type Cultures (NCTC); environmental), *Strataphoromoans bhaumini* (NCTC; environmental), *Vibrio eltar* (NCTC; environmental) and *Vibrio classical* (NCTC; environmental) were used for the test. Pure cultures were maintained on the surface of agar slopes in screw capped test tubes in a refrigerator. They were sub-cultured at intervals of 1-2 months.

Bacterial cultures were revived in nutrient broth. For this, single colony from different bacterial strains was aseptically inoculated in 3 ml of nutrient medium and grown overnight in a shaker at 37ºC and 180 rpm. Hundred mL of overnight cultures was inoculated in 3 ml of fresh nutrient medium and grown for 4 hrs (0.6 OD A_{600}) and 10 mL was used for plating. Agar plates were prepared by pouring 20 ml of sterilized molten nutrient agar, not hotter than 45ºC, to petri plates of 88 mm diameter under asceptic conditions. The petri plates were left undisturbed for 15 min, to allow the medium to set. Ten mL of the broth culture was aseptically transferred to agar plates. Care was taken to uniformly spread the culture with the help of a sterilized spreader made up of glass rod. Antibacterial activity of the termite and its mound extracts was assessed following disc assay method[6]. A pilot screening of the extract was carried out by impregnating a 6 mm sterile Whatman number 1 filter paper disc with required mL of the extract dissolved in absolute alcohol so as to give a final disc load of 100µg/disc. These discs were allowed to dry fully. Once the entire alcohol was evaporated, they were placed on agar plates previously seeded with the respective bacterial strains. Three replicates were maintained for each treatment. Negative control discs were kept without any extracts but soaked in respective mL of absolute alcohol and dried. Chloramphenicol 30 mcg/disc supplied by HIMEDIA was used as positive control. The plates were then kept at 37ºC in an incubator for 24 hrs. The width of the inhibition zone was measured to the nearest millimeter, at 24 hrs by using HiAntibiotic Zone Scale supplied by HiMedia, and averaged. The extracts, which showed activity, were subjected to further assay with a series of extract concentrations (5, 10, 25, 50 µg/disc). The incubations and observations were carried out as above. The assay was repeated with different species of termites, respective termite mounds and the soil extracts of different places. Results were expressed as mean ± SE and subjected to the student's t-test. $P < 0.05$ was considered significant.

Results and discussion

Results on the antibacterial activity of various termite species and their respective mounds collected from different sites are presented (Table 1). All species of the subterranean termites and their mound extracts showed appreciable antibacterial activity against most of the bacterial strains. No activity was observed from the soil extracts collected from near the termite mounds. The antibacterial activity was most apparent in *Odontotermes formosanus* and its mound extracts, which inhibited all the bacterial strains studied compared to that *Microtermes obesi* and *Macrotermes estherae*. The activity score was highest in *Odontotermes formosanus* (12.6 ±0.5 mm) and its mound extracts (14.3 ± 1.1 mm) against *E. coli* BL21. The activity scores increased with increasing concentration of extracts and remained constant beyond the Minimum Inhibitory Concentration (MIC). The MIC values varied with the extracts and test organisms used and ranged from 10 µg/disc to 100 µg/disc. However, concentration of 25µg/disc and 50µg/disc displayed maximum inhibition zone among most of the treatments. Considerable inter-generic variation existed in the antibacterial activity of various termites and its mound extracts. This may be due to the individual variation or differences in the metabolic production. Interestingly, *Odontotermes*

Table 1—Inhibition zone width (mm ± SE) and MIC values* of various termite and termite mound extracts

Test organisms	Extract/organisms used				Positive control			
	Microtermes obesi		Macrotermes estherae		*Odontoterms formosanus*		Chloramphenicol (30 mcg/disc)	
	Termite	Termite mound	Termite	Termite mound	Termite	Termite mound	Termite	Termite mound
Escherichia coli O157	4 ±1 (10)	3.3 ± 0.5 (25)	3.6 ± 0.57 (10)	3.3 ± 0 (10)	11± 1.7 (25)	11.6 ± 0.5 (25)	10 .3 ± 1.5	11± 1.7
E. coli JM 109	3.6 ± 0.5 (25)	3.6 ± 0.5 (10)	3.6 ± 0.5 (25)	4.6 ± 0.5 (25)	8.3± 0.5 (25)	8.6 ± 0.5 (25)	8 ± 1	8 ± 1
E. coli mos blue	4.3 ± 0.5 (50)	3 ± 0 (25)	3.3 ± 0.5 (10)	-	10.3 ± 1.5 (50)	10.6 ± 2 (25)	9.2 ± 1.4	11 ± 1.7
E. coli BL21	3.3 ± 0.5 (50)	3.6 ± 0.5 (10)	3.3 ± 0.5 (20)	3.3 ± 0.5 (25)	12.6 ± 0.5 (25)	14.3 ± 1.1 (25)	11 ± 1.7	15 ± 6
Pseudomonas putida	4.3 ± 1.5 (25)	3.6 ± 0.5 (50)	3.3 ± 0.5 (50)	4.3 ± 0.5 (10)	8 ± 1 (10)	6.6 ± 0.5 (50)	8.3 ± 0.5	6 ± 1
Klebsiella sp	-	-	3.6 ± .5 (25)	4.3 ± 0.5 (25)	7 ± 1 (25)	6 ± 1 (10)	7 ± 1	5.6 ± 0.5
Strataphoromoans bhaumini	3.6 ± 0.5 (50)	3.3±0.5 (100)	4 ± 0 (50)	3.6 ± 0.5 (10)	3 ± 1 (25)	3.3 ± 0.5 (10)	3.6 ± 0.5	3.3 ± 0.5
Vibrio eltar	3 ± 0 (10)	3.3 ± 0.5 (25)	-	-	15 ± 6 (25)	18.3 ± 1.1 (25)	14.3 ± 1.1	18 ± 1
Vibrio classical	3 ± 0 (25)	3.3 ± 0.5 (25)	4.3 ± 0.5 (10)	5.6 ± 0.5 (25)	10.6 ± 1.1 (50)	13.3 ± 1.5 (25)	10.3 ± 1.5	14.3 ± 1.1

***Figures in parenthesis show MIC values (μg/disc)

formosanus was used by the *Kannikaran, Paniyan, Palliyan, Sholaga, Irular* and *Kota* tribes of South India for the treatment of Asthma, a disease associated with microbes[2]. Among the bacterial strains *Klebsiella* sp and *Vibrio eltar* were found to be less sensitive compared to other strains. No significant difference was observed between the MIC values of the termite and termite mound extracts for the bacterial strains studied.

Two novel antimicrobial peptides, termicin and spinigerin have been isolated from the fungus-growing termite *Pseudacanthotermes spiniger* (Insecta: Isoptera)[4]. Similar studies conducted on the termite *Pseudacanthotermes spiniger*, revealed that termicin, a cysteine-rich antifungal peptide has antibacterial properties also[5]. Antibacterial peptides are also reported from the ant *Myrmecia gulosa* and *Pachycondyla goeldii*[7,8]. Arthropods that live in close proximity to each other such as wasps, bees, mole crickets, scarab larvae, cicada nymphs and centipedes are subject to microbial attacks and epidemic diseases. To limit disease activity they incorporate antimicrobial compounds into their nests[9]. Soil dwelling ants also have been shown to use chemical defense against fungi and bacteria in their underground nests[10]. Termites, which live in mounds, are also subject to microbial attacks. To defend themselves against such microbial attacks they may be using chemical defence. The classification of toxic compounds by their role was difficult since the role was often combined[11]. Since, the tribes used the termites for the treatment of asthma and associated with viral infections, the compounds found within these organisms may have other pharmacological properties like antiviral activity or antifungal activity.

Conclusion

The study provides preliminary information on antimicrobial properties of subterranean termites used in South Indian folk medicine. Since, the traditional knowledge of indigenous people throughout the world has played an important role in identifying natural resources worthy of commercial exploitation and the search for new pharmaceuticals from naturally occurring biological material has been guided by ethnobiological data, the results carry much significance and provide insight into the potentialities of these arthropods in modern medicine.

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