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Antimicrobial peptides from the Coleoptera family Scarabaeidae against Candida and Cryptococcus pathogenic yeasts.

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Objectives: Host defense peptides (HDP) are produced by a diversity of beetles. The aims of this work were (1) to find new promising peptides from the Coleoptera family Scarabaeidae with potential biomedical applications, (2) to modify physicochemical and structural characteristics of one of the most promissory peptides in order to improve its antimicrobial properties, and (3) to evaluate the *in vitro* activity of the HDPs against reference strains of pathogenic *Candida* and *Cryptococcus* yeasts.

Materials and Methods: From the Scarabaeidae family transcriptome, 14 promising HDPs were identified. Subsequently, we designed 19 new sequences from Act8 peptide modifying the net charge, hydrophobic angle, and the general composition of amino acids, among other properties, in order to improve the HDPs antifungal activity. The *in vitro* antifungal susceptibility of the 33 HDPs against *C. albicans* SC5314, *C. krusei*, ATCC 6558, *C. parapsilosis* ATCC 22019, *C. glabrata* ATCC 2001, *C. tropicalis* ATCC 750, *C. neoformans* H99, and *C. gattii* HD0058-1-2029 isolates were evaluated by broth microdilution, with a concentration ranging from 0.19 to 50 µg/ml.

Results: All 14 peptides identified showed *in vitro* activity against *C. krusei*, *C. parapsilosis*, and *C. glabrata*. One peptide showed *in vitro* activity against *C. albicans*, 6 against *C. tropicalis*, 11 against *C. neoformans* and 13 against *C. gattii*. As well the 19 modified peptides showed *in vitro* activity against *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. neoformans*, and *C. gattii*. A total of 15 modified peptides showed *in vitro* activity against *C. albicans*, and 3 against *C. glabrata*. MIC ranges per species and per peptide are shown in Table 1.

Conclusions: The HDPs herein analyzed showed a significant *in vitro* antifungal activity against six *Candida* and two *Cryptococcus* pathogenic species. Our findings encourage further work with *in vivo* experimental models in order to better understand the action mechanisms of these antimicrobial peptides. HDPs from different species are becoming a promising therapeutic alternative in the control of fungal infections.

Peptide name	Minimum inhibitory concentration (MIC in µg/mL) against different fungal strains evaluated							
	Candida spp				Cryptococcus spp			
	C. krusei ATCC 6558	C. parapsilosis ATCC 22019	C. albicans SC5314	C. tropicalis ATCC 750	C. albicans ATCC 1453	C. glabrata ATCC 2001	C. neoformans H99	C. gattii H0058-I-2029
Act1	1.56	3.12	>50	50	>50	3.12	50	6.25
Act2	0.78	1.56	>50	50	>50	1.56	25	3.12
Act2.1	0.78	1.56	>50	>50	>50	1.56	>50	1.56
Act2.2	0.78	1.56	>50	12.5	>50	1.56	12.5	1.56
Act3	0.78	3.12	>50	>50	>50	1.56	50	3.12
Act4	0.78	1.56	>50	50	>50	3.12	50	1.56
Act5	1.56	3.12	25 - 50	>50	>50	3.12	25	1.56
Act6	0.78	1.56	>50	>50	>50	3.12	50	3.12
Act7	0.78	1.56	>50	>50	>50	3.12	50	3.12
Act8	0.78	1.56	>50	>50	>50	3.12	50	3.12
domcec	3.12	3.12	>50	>50	>50	6.25	>50	>50
ConCec	0.78	0.39	>50	25	>50	1.56	25	1.56
ox322	1.56	1.56	>50	>50	>50	3.12	50	3.12
sat122	1.56	0.39	>50	50	>50	3.12	50	3.12
8-1	12.5	25 - 50	>50	6 - 12	>50	>50	1.5	0.31 - 0.6
8-2	1.5	12 - 25	25	6 - 12	50	>50	1.5	0.31 - 0.6
8-10	12.5	6 - 12	50	1.5 - 3.0	50	>50	0.62	0.31
8-11	12.5	6 - 12	50	3 - 6	50	>50	0.62	0.31
812	12.5	6 - 12	12.5	1.5 - 3.0	50	>50	0.62	0.31
8-13	6	1.5 - 3.0	12.5	1.5 - 3.0	12.5	25	0.31	0.31
8-14	25	25	>50	25	>50	>50	3 - 6	0.6 - 1.5
8-15	6	25 - 50	50	12.5 - 25	>50	>50	0.62	0.31
8-16	0.62	12	25	6 - 12	50	>50	1.5	0.62
8-17	0.31	12	50	3 - 6	50	>50	0.62	0.31
8-18	0.31	6	25	6 - 12	50	>50	0.62	0.31
8-19	0.31	3	25	3 - 6	12 - 25	25	0.62	0.31
8-20	0.31	3	12.5	3 - 6	12 - 25	25	0.31	0.31
8-21	12.5 - 25	50	50	12 - 25	>50	>50	3.5	0.61 - 1.5
8-22	12.5 - 25	50	50	12 - 25	>50	>50	1.5	0.62
8-23	12.5 - 25	25	50	6 - 12	>50	>50	1.5	0.62
8-24	6 - 12.5	25	>50	12 - 25	>50	>50	12.5	3.5
8-25	6 - 12.5	12	50	6 - 12	>50	>50	3	1.5
8-26	6 - 12.5	12	>50	6 - 12	>50	>50	3	1.5

P071

Isolate profiling and antifungal susceptibility determination for terbinafine and itraconazole among dermatophytes in a tertiary care hospital of western rajasthan

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Objective: To determine the species distribution of causative agents causing dermatophytosis, and their antifungal susceptibility pattern for terbinafine and itraconazole in *trichophyton* spp among samples collected in patients with dermatophytosis clinical suspicion during the period of December 1, 2020 to January 31, 2022.

Materials and Methods: This is a prospective study conducted in the Department of Microbiology of a tertiary care super specialty and referral Centre of western Rajasthan from December 1, 2020 to January 31, 2022.

Skin scraping, nail clipping, and hair pluckings were collected in mycology lab from clinically suspected cases of dermatophytosis presenting to the department of dermatology for conventional identification, and antifungal susceptibility testing.

The specimens were subjected to direct KOH and calcofluor white microscopy and conventional fungal culture on SDA at 25°C and 37°C.

The cultures positive for dermatophytes were speciated by microslide culture lactophenol cotton blue mount, hair perforation test, and urease test.

The isolates identified as *Trichophyton* spp were taken up for antifungal susceptibility testing against terbinafine and itraconazole by microbroth dilution according to CLSI-M38 A2 guidelines. Further terbinafine resistance gene evaluation for detection of C1191A and T1189C single nucleotide polymorphism in Squalene epoxide by Amplified Refractory Mutation System-Polymerase chain Reaction (ARMS-PCR) is undergoing for *trichophyton* spp.

Results: Over the 14-month study period, the laboratory processed total of 174 specimens: 134 skin scraping, 36 nail clipping, and 4 hair pluckings. Of them, 106 (61.62%) specimens were microscopy positive and 111 (63.79%) were culture positive. Out of the 111 culture-positive agents isolated, 94 (84.68%) were found to be dermatophytes. On isolate profiling of 94 dermatophytes *T. mentagrophyte* was found to be most common 45 (48%) followed by *T. rubrum* 27 (29%), *T. tonsurans* 20 (21%), *T. verrucosum* 1 (1.1%), and *Microsporum* spp 1 (1.1%). Antifungal susceptibility of 93 *Trichophyton* spp against terbinafine showed resistance among 58.06% isolates with 83.33% isolates among terbinafine resistant cases showing ≥ 4 µg/ml minimum inhibitory concentration. There was no resistance detected for itraconazole with microbroth dilution.

Conclusion: A total of (54.02%) skin, hair, nail infections were found to be caused by dermatophytes.

On isolate profiling, *T. mentagrophyte*, *T. rubrum*, and *T. tonsurans* were found to be predominant species among our isolates showing altered trend of local isolates from *T. tonsurans* being second most common spp isolated in past.

On antifungal susceptibility >55% isolates showed resistance for Terbinafine with >80% having higher MIC of ≥ 4 µg/ml on the contrary there was no observed resistance for itraconazole.

There is a need for encouraging dermatologists for prescribing routine fungal microscopy, culture, and AFST for dermatophytes in Western Rajasthan, to reduce the indiscriminate use of antifungals.

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Initial results of an international effort in screening new agents against *Candida auris*

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Candida auris is an emergent fungal pathogen. A global concern regarding this yeast is its resistance to many currently available antifungal drugs, virulence factors, capacity to spread in hospital environments, and its misidentification, resulting in high rates of morbidity, and mortality.

Objective: In response to this challenge, new effective options of antifungals against *C. auris* are urgent. Therefore, our consortium evaluated the *in vitro* activity of two agents with novel mechanisms of action, and negligible toxicity in studies to date: diphenyl diselenide (PhSe)₂ and nikkomycin Z (NikZ), alone and in association with conventional antifungals (azoles, echinocandins, polyenes) against *C. auris*.

Methods: A total of 11 isolates of *C. auris* were included in this *in vitro* study, 10 from South Asian clade I and 1 from South Africa clade III. *In vitro* tests (dilution and interaction assays) were performed according to the CLSI M27-E4 protocol. Interactions between (PhSe)₂ or nikkomycin Z, and amphotericin B (AmB), fluconazole (FCZ), micafungin (MYC), or caspofungin (CSP) were evaluated by checkerboard assays, resulting in Fractional Inhibitory Concentration Indexes (FICI). Tests were read after incubation for 48 h at 35°C. The minimal inhibitory concentration (MIC) was defined as the lowest concentration