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Antifungal Activity of Piper Betle Leaf

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Abstract – Mycosis is a widespread health issue, especially in the tropical and subtropical developing countries; dermatophytes, species Malassezia and species Candida are the most common pathogens in humans and animals. There has been a growing search for new antifungal agents in recent years. However, as many of the current antifungal medications have adverse side effects or are extremely toxic (amphotericin B), induce recurrence, demonstrate drug-drug interactions (azoles) or contribute to resistance production (fluconazole, 5- flucytosine), others display ineffectiveness and have thus been less active in therapeutic strategies.

Therefore, more powerful and less toxic novel antifungal agents need to be looked to address these drawbacks. It is important to note that plants are commonly used in folk medicine, especially in communities with insufficient public health and sanitation conditions.

Key Words: Plants, Leaves, Piper Betle

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INTRODUCTION

Several medicinal plants have been thoroughly researched to identify more powerful and less poisonous compounds. In India, China, Taiwan, Thailand and many other countries, Piper betle L. (Piperaceae) has been commonly used in traditional herbal remedies. Different pharmacological activities such as antimicrobial, antioxidant, antimutagenic, anticarcinogenic, antiinflammatory etc are recorded. It also serves as a stimulant, a air freshener, a carminative, a sialagogue, a heart tonic, a joint pain reliever, an aphrodisiac, an astringent, an antiseptic, a stimulant of the digestive and pancreatic lipase, wound healing.

Hydroxychavicol is the main phenolic ingredient extracted from the aqueous extract of P. betle L., leaf antinitrosation, antimutagenic, anticarcinogenic activity has been reported. It also helps to act as an antioxidant, and as a chemical-preventive agent. Such beneficial effects include anti-inflammatory, antiplatelet and antithrombotic agents without haemostatic functions being compromised. Reports have been made on hydroxychavicol's antibacterial activities, but the research on its antifungal function is missing so far.

Reports have been made on P. betle's antifungal activities. Pongpech and Prasertsilpe find that P.betle gel inhibits dermatophyte development that allows ringworm and Candida species to develop more efficiently than tolnaftate and has a similar inhibitory effect to clotrimazole. Trakranrungsie et al recently confirmed P.betle extract's antidermatophytic activity against M, too. Channis, M. Gypseum, T. Mentagrophyte by broth dilution process and demonstrated that P.betle showed more efficient antifungal properties with average values of IC50 and IC90 ranging from 110.44 to 119.00 µg / ml and 230.40 to 492.30 µg / ml , respectively.

Hydroxychavicol is one of P. betle 's main constituents. The antibacterialactivity has been widely published. But its antifungal activity has not yet been published. Here we first reported the antifungal ability of hydroxychavicol in this study.

PRACTICES

The pure form of hydroxychavicol was obtained from the chloroform extraction of the aqueous leaf extract of P. betle L., (Piperaceae) as mentioned above. Amphotericin B was bought from Sigma Chemical Co. (St. Louis, MO), and terbinafine was purchased from the Lupin Laboratories, Pune, India as a kind gift.

To dye the yeast cells, propidium iodide (Sigma), a thin cationic, nucleic acid binding fluorochrome that was mainly omitted by intact cell membranes, was used. Sodium deoxycholate (Sigma), an anionic detergent, was used to promote the dissemination of propidium iodide through the membranes of yeast cells that were weakened by the antifungal agent.

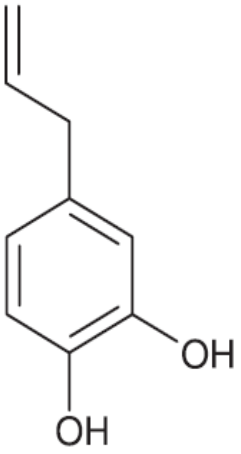


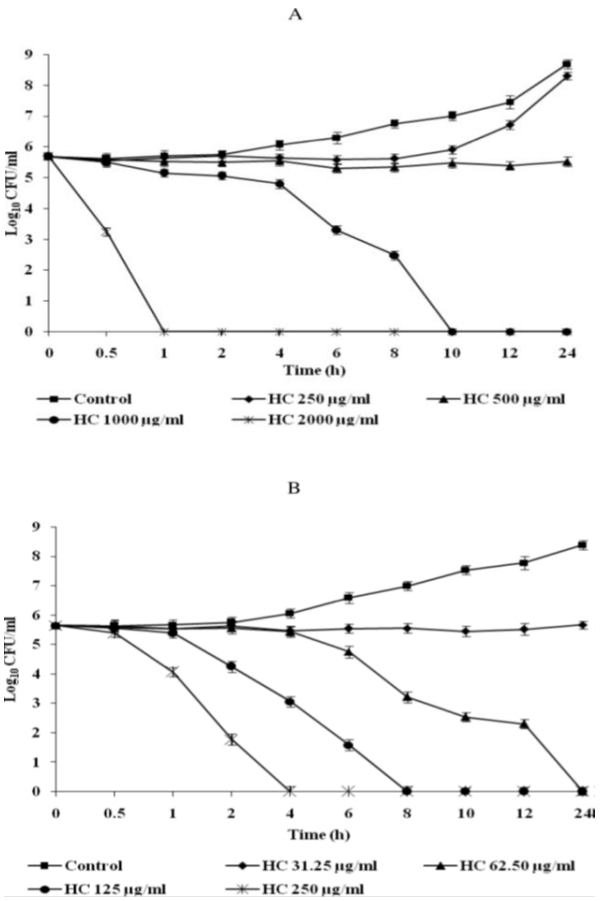
Figure 1:Structure of hydroxychavicol

Check for their resistance to hydroxychavicol, a total of 124 fungal strains. These strains consisting of Candida albicans (ATCC 90028, ATCC 10231 and23 scientific isolates), Candida glabrata (ATCC 90030and 7 scientific isolates), Candida krusei (ATCC 6258 and 3 clinical isolates), Candida parapsilosis (ATCC 22019and 5 clinical isolates), Candida tropicalis (ATCC 750and 11 clinical isolates), Cryptococcus neoformans(ATCC 204092 and 2 clinical isolates), Aspergillus flavus(MTCC 197) The American Type Culture Collection (ATCC, Manassas, VA, USA), and Microbial Type Culture Collection (MTCC, Chandigarh, India) produced reference strains. The clinical isolates were collected from Acharya Shri Chander College of Medical Sciences, Department of Microbiology, Sidhra, Jammu, India.

Suspensions of the yeasts and Aspergillus species were prepared in sterile standard saline (0.85 percent) containing 0.05 percent polysorbate 20 (NST) from 24 h (48 h for C. neoformans) and 7-day-old crops grown 'respectively' on potato dextrose agar (Difco Laboratories, Detroit, Mich) at 35 °C [16,17]. A stock inoculum suspension of each dermatophyte was prepared from young, mature (7-day-old) crops grown on potato dextrose agar with 2 per cent in-house slants of 28 ° C rice flour. The densities of these suspensions have been modified by means of a spectrophotometer (Multiskan continuum, Thermo electron, Vantaa, Finland) at a wavelength of 530 nm to a transmittance of 65 to 70 percent toyield with an initial inoculum of between 1 and 106 to 5 cfu / ml. Both modified suspensions were quantified by putting the dextrose agar on Sabouraud (SDA; Difco Laboratories) plates.

HYDROXYCHAVICOL CALCULATED BY MIC AND MFC

The MIC was carried out using broth microdilution techniques in compliance with the recommendations of the Clinical and Laboratory Quality Institution (formerly the National Committee for Clinical Laboratory Standards)[16,17], with an RPMI 1640 medium containing L-glutamine, without sodium bicarbonate and buffered to pH 7.0 with 0.165 M morpholine propanesulfonic acid (RPMI) (both from Sigma). Hydroxychavicol stock solution was prepared in 100% dimethyl sulfoxide (DMSO; Sigma), and in 96-well U-bottom microtiter plates (Tarson, Mumbai , India) two-fold serial dilutions were prepared in media in amounts of 100 µl per line. The above-mentioned fungal suspensions were further diluted in media and a volume of 100 µl of this diluted inoculum was applied to each platform well, resulting in a final inoculum of 0.5 µl 104 to 2.5 µl 104 cfu / ml[19] for yeasts and 0.4 µl 104 to 5 µl for dermatophytes and Aspergillus organisms. The final hydroxychavicol concentration varied between 3.90 and 2000 µg / ml. The medium was used as growth control without the officers, and the blank control was used Just provided content. The normal safety monitoring were for amphotericin B and terbinafine.



For dermatophytes, the microtiter plates were incubated at 28 ° C for 7 days, and for the genus Candida (72 h for C. neoformans) and Aspergillus at 35 ° C for 48 h. The plates were visually read, and the MIC was described as the lowest antifungal agent concentration that prevented significant growth as regards growth control.

The MFC was estimated by plating the wells with a volume of 100 µl on SDA that showed no noticeable rise. In MIC the plates have been incubated as mentioned above. The minimum hydroxychavicol concentration showing a reduction of 99.9 percent of the initial inoculums was reported as the MFC.

RESULTS

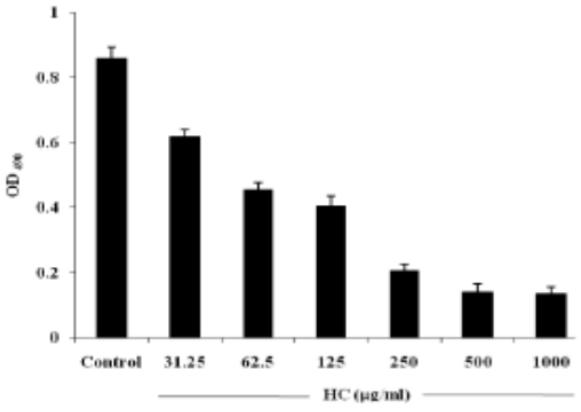
The hydroxychavicol MICs and MFCs were tested in vitro against 58 yeast strains, 39 Aspergillus strains and 27 dermatophyte strains and all values are described in Table 3.1. Hydroxychavicol displayed a spectrum of MICs between 15.62 and 500 µg / ml for yeasts, between 125 and 500 µg / ml for Aspergillus bacteria, and between 7.81 and 62.5 µg / ml for dermatophytes, where the MFCs were found to be equivalent or twofold greater than MICs. Dermatophytes were found of all the fungal species tested to be the most susceptible to hydroxychavicol.

Table 1: Frequency of mutation with hydroxychavicol

Tested strains	Mutation frequency with hydroxychavicol at:		
	2 × MIC	4 × MIC	8 × MIC
C. albicans ATCC 90028	2.5×10^{9}	$<10^{9}$	$<10^{9}$
C. tropicalis ATCC 750	2×10^{9}	$<10^{9}$	$<10^{9}$
C. glabrata ATCC 90030	1.5×10^{9}	1.5×10^{9}	$<10^{9}$
C. parapsilosis ATCC 22019	2×10^{9}	2×10^{9}	$<10^{9}$
A. fumigatus MTCC 1811	$<10^{9}$	$<10^{9}$	$<10^{9}$
A. flavus MTCC 1973	$<10^{9}$	$<10^{9}$	$<10^{9}$

MIC of hydroxychavicol is 31.25 µg/ml for *C. glabrata* and *C. parapsilosis* while as 250 µg/ml for other species tested.

Hydroxychavicol demonstrated fungicidal activity against all Candida species and more than 3 log units (99.9 percent) decreased the amount of cfu per millilitre. The target fungicidal to C. At 4 MIC (4 µg / ml) and 8 MIC (8 µg / ml) for hydroxychavicol (Fig. 3.2A), albicans were obtained after 10 and 1 h. To C. Glabrata, killing of hydroxychavicol was observed at a lower concentration owing to its lower MIC. In case of C, concentration dependent killing was observed. Glabrata demonstrated fungicidal activity in 10, 8 and 4 h 'respectively, two, four and eight times the MIC.



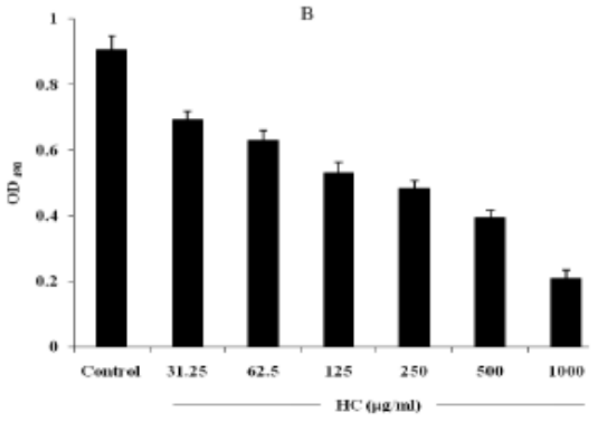


Figure 2: Inhibitory effect of hydroxychavicol (HC) on the biofilm formation (A) and reduction (preformed) (B) of C.albicans ATCC 90028 biofilms.

DISCUSSION

In this analysis we tested hydroxychavicol's antifungal activity against different fungal species.

Hydroxychavicol has shown fungicidal activity against all studied fungal species including Candida spp., Aspergillus spp. And dermatophysiologists. In dermatophytes like T the fungicidal effect was most pronounced. Rubrum (MICs and MFCs) was 15.62-62.5 µg / ml, an etiological factor in 80 to 93 percent of all dermatophyte-related clinical infections. Hydroxychavicol also demonstrated concentration-dependent killing and PAFE increased by > 8 h. It fully prevented the development of mutants of different Candida and Aspergillus species examined within the concentration range of 250-1000 µg / ml. C. Albicans are more frequently associated with the development of biofilms, and the rise of Candida infections of recent decades has also paralleled the growth and universal usage of a broad variety of surgical implant devices (such as stents, prostheses, valves, endotracheal tubing, pacemakers, and catheters), often in communities with compromised host defences. The accumulation of biofilms on medical devices will adversely impact the host by causing the system to malfunction and by acting as a repository or source for potential ongoing infections. Hydroxychavicol effectively inhibited C. Biofilm-generated albicans were observed at MIC concentration (250 µg / ml) with 80 percent biofilm inhibition. At four fold higher concentrations, however, the reduction of the preformed biofilm was seen.

CONCLUSIONS

The findings reported in this analysis are the first knowledge for antifungal activity about hydroxychavicol. Hydroxychavicol demonstrated wide spectrum of antifungal activity against clinically important species of human fungi. Therefore further

studies are needed to explore this natural compound for topical use in fungal infections , especially dermatomycosis.

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