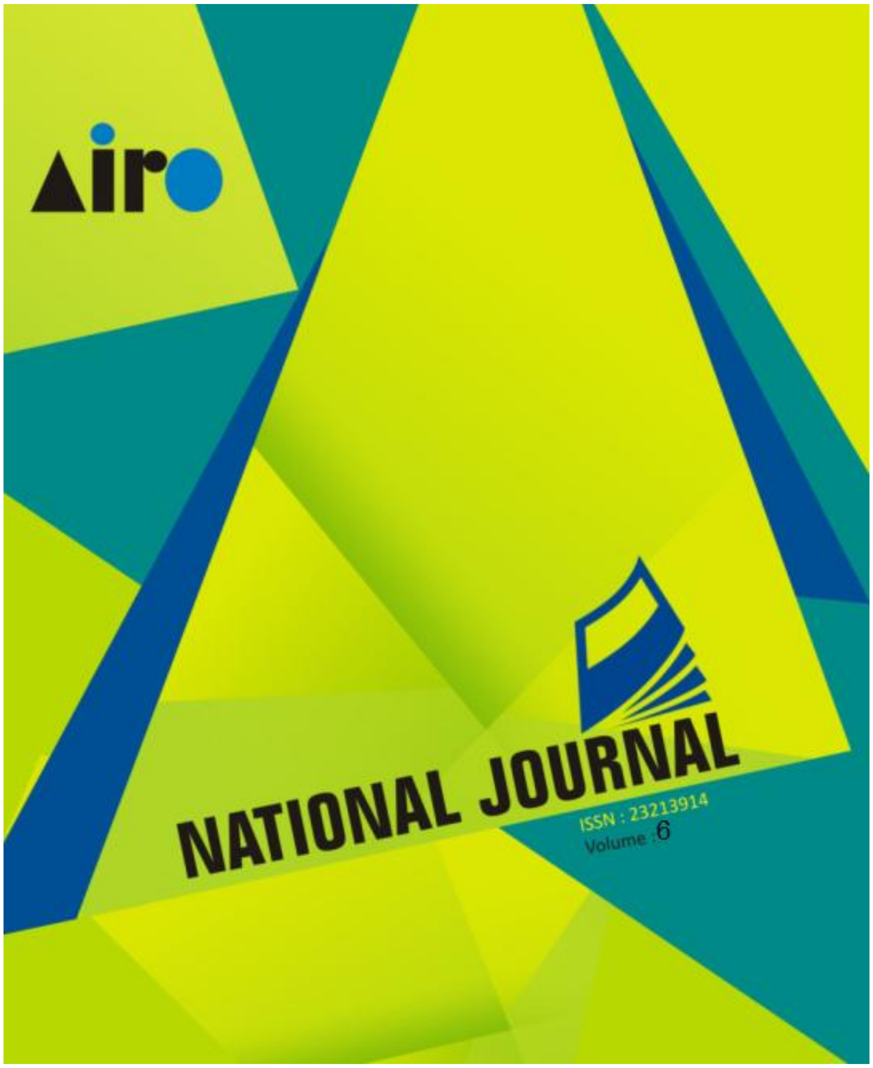
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ROLE OF PIPER BETLE LEAF IN ANTIFUNGAL ACTIVITIES

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ABSTRACT

Mycosis constitutes a common health problem, especially in tropical and subtropical developing countries; dermatophytes, Malassezia species and Candida species being the most frequent pathogens in humans and animals. In recent years, there has been an increasing search for new antifungal agents. However, since many of the available antifungal drugs have undesirable side effects or are very toxic (amphotericin B), produce recurrence, show drug-drug interactions (azoles) or lead to the development of resistance (fluconazole, 5- flucytosine), some shows ineffectiveness and have become therefore less successful in therapeutic strategies.

Therefore it is necessary to search for more effective and less toxic novel antifungal agents that would overcome these disadvantages. Interestingly, plants are widely employed in folk medicine, mainly in communities with inadequate conditions of public health and sanitation.

KEYWORDS:

Plants, Leaves, Piper Betle

INTRODUCTION

Several medicinal plants have been extensively studied in order to find more effective and less toxic compounds. Piper betle L., (Piperaceae) has been extensively used in traditional herbal remedies in India, China, Taiwan, Thailand and many other countries. It is reported for various pharmacological activities such as antimicrobial, antioxidant, antimutagenic, anticarcinogenic, antiinflammatory etc. It also acts as a stimulant, a breath freshener, a carminative, a sialagogue, a cardiac tonic, a pain killer in joint pain, an aphrodisiac, an astringent, an antiseptic, a digestive and pancreatic lipase stimulant, wound healing.

Hydroxychavicol is the major phenolic component, isolated from the aqueous extract of P. betle L., leaf has been reported to possess antinitrosation, antimutagenic, anticarcinogenic activities. It also has a tendency to act as an antioxidant, and a chemopreventive agent. Other useful properties include antiinflammatory, antiplatelet and antithrombotic without impairing haemostatic functions. There have been reports on the antibacterial activities of hydroxychavicol, but so far the report on its antifungal activity is lacking.

There have been reports on the antifungal activities of P. betle. Pongpech and Prasertsilpe found that P. betle gel inhibited growth of dermatophytes that cause ringworm and growth of Candida species more effectively than tolnaftate and with a similar inhibitory effect to that of clotrimazole. Recently, Trakranrungsie et al also reported the antidermatophytic activity of P. betle extract against M. canis, M. gypseum and T. mentagrophyte by broth dilution method and showed that P. betle exhibited more effective antifungal properties with average IC50 and IC90 values ranging from 110.44 to 119.00 µg/ml and 230.40 to 492.30 µg/ml "respectively".

Hydroxychavicol is one of the major constituents of P. betle. It has been extensively reported for its antibacterial activity. However its antifungal activity has not been reported so far. Here in this study we have for the first time reported the antifungal potential of hydroxychavicol.

Methods

Hydroxychavicol was isolated in the pure form from the chloroform extraction of the aqueous leaf extract of P. betle L., (Piperaceae) as described previously. Amphotericin B was purchased from Sigma Chemical Co. (St. Louis, MO), and terbinafine was obtained as kind gift from Lupin Laboratories, Pune, India.

Propidium iodide (Sigma), a small cationic, nucleic acidbinding fluorochrome largely excluded by intact cell membranes was used to stain the yeast cells. Sodium deoxycholate (Sigma), an anionic detergent, was used to facilitate diffusion of propidium iodide into the yeast cell membranes which were damaged by the antifungal agent.

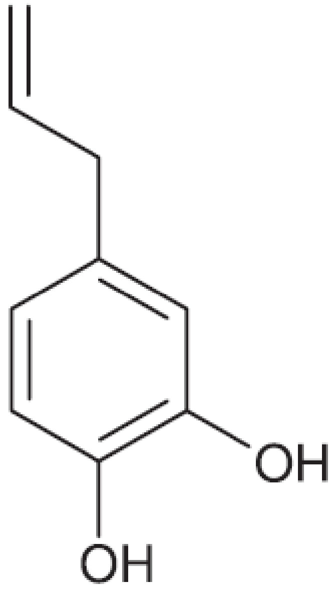


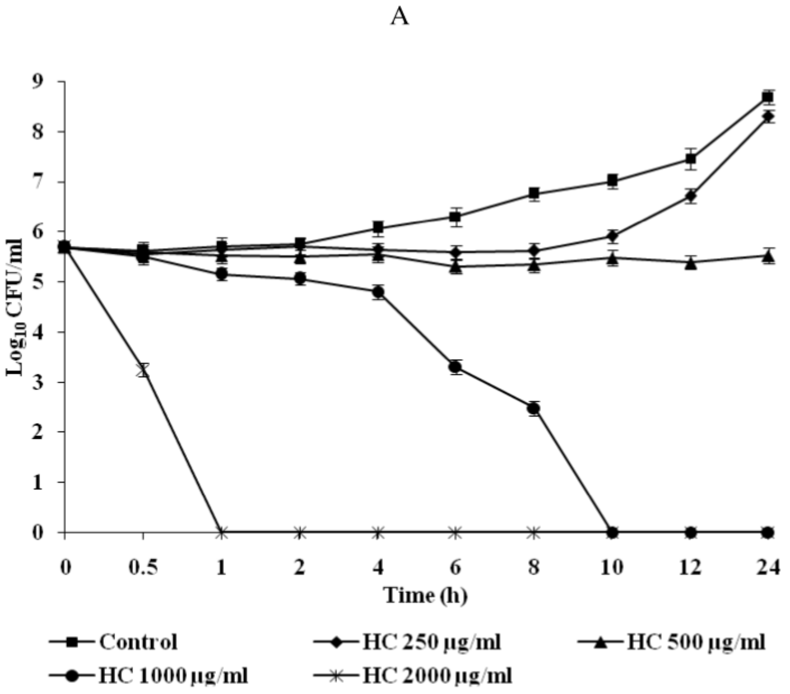
Figure 1: Structure of hydroxychavicol

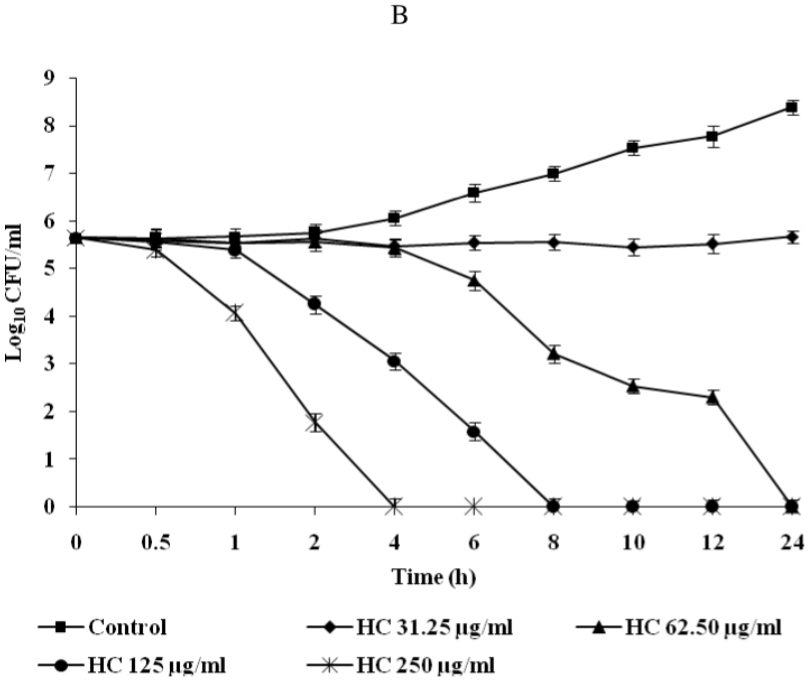
A total of 124 fungal strains were tested for their susceptibility to hydroxychavicol. These strains comprised of Candida albicans (ATCC 90028, ATCC 10231 and 23 clinical isolates), Candida glabrata (ATCC 90030 and 7 clinical isolates), Candida krusei (ATCC 6258 and 3 clinical isolates), Candida parapsilosis (ATCC 22019 and 5 clinical isolates), Candida tropicalis (ATCC 750 and 11 clinical isolates), Cryptococcus neoformans (ATCC 204092 and 2 clinical isolates), Aspergillus flavus (MTCC 1973, MTCC 2799 and 10 clinical isolates), Aspergillus fumigatus (MTCC 1811 and 17 clinical isolates), Aspergillus niger (ATCC 16404 and 6 clinical isolates), Aspergillus parasiticus (MTCC 2796), Epidermophyton floccosum (MTCC 613 and 1 clinical isolate), Microsporum canis (MTCC 2820 and 3 clinical isolates), Microsporum gypsium (MTCC 2819 and 2 clinical isolates), Trichophyton mentagrophytes (ATCC 9533 and 7 clinical isolates), and Trichophyton rubrum (MTCC 296 and 9 clinical isolates). Reference strains were procured from the American Type Culture Collection (ATCC, Manassas, VA, USA), and Microbial Type Culture Collection (MTCC, Chandigarh, India). The clinical isolates were obtained from the Department of Microbiology, Acharya Shri Chander College of Medical Sciences, Sidhra, Jammu, India.

Suspensions of the yeasts and Aspergillus species were prepared in sterile normal saline (0.85%) containing 0.05% polysorbate 20 (NST) from 24 h (48 h for C. neoformans) and 7-day-old cultures “respectively” grown on potato dextrose agar (Difco Laboratories, Detroit, Mich) at 35°C [16,17]. A stock inoculums suspension of each dermatophytes was prepared from fresh, mature (7-dayold) cultures grown on potato dextrose agar with 2% inhouse rice flour slants at 28°C. The densities of these suspensions were adjusted with a spectrophotometer (Multiskan spectrum, Thermo electron, Vantaa, Finland) at a wavelength of 530 nm to a transmittance of 65 to 70% to yield an initial inoculum of 1 × 106 to 5 × 106 cfu/ml. All adjusted suspensions were quantified by plating on Sabouraud dextrose agar (SDA; Difco Laboratories) plates.

MIC and MFC determination of hydroxychavicol

The MIC was performed by broth microdilution methods as per the guidelines of Clinical and Laboratory Standard Institute (formerly, the National Committee for Clinical Laboratory Standards) [16,17], with RPMI 1640 medium containing L-glutamine, without sodium bicarbonate and buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (RPMI) (both from Sigma). Stock solution of hydroxychavicol was prepared in 100% dimethyl sulfoxide (DMSO; Sigma) and twofold serial dilutions were prepared in media in amounts of 100 μl per well in 96-well U-bottom microtiter plates (Tarson, Mumbai, India). The above-mentioned fungal suspensions were further diluted in media, and a 100 μl volume of this diluted inoculums was added to each well of the plate, resulting in a final inoculum of 0.5 × 104 to 2.5 × 104 cfu/ml [19] for yeasts and 0.4 × 104 to 5 × 104 cfu/ml for dermatophytes and Aspergillus species. The final concentration of hydroxychavicol ranged from 3.90 to 2000 μg/ml. The medium without the agents was used as a growth control and the blank control used contained only the medium. Amphotericin B and terbinafine served as the standard drug controls.





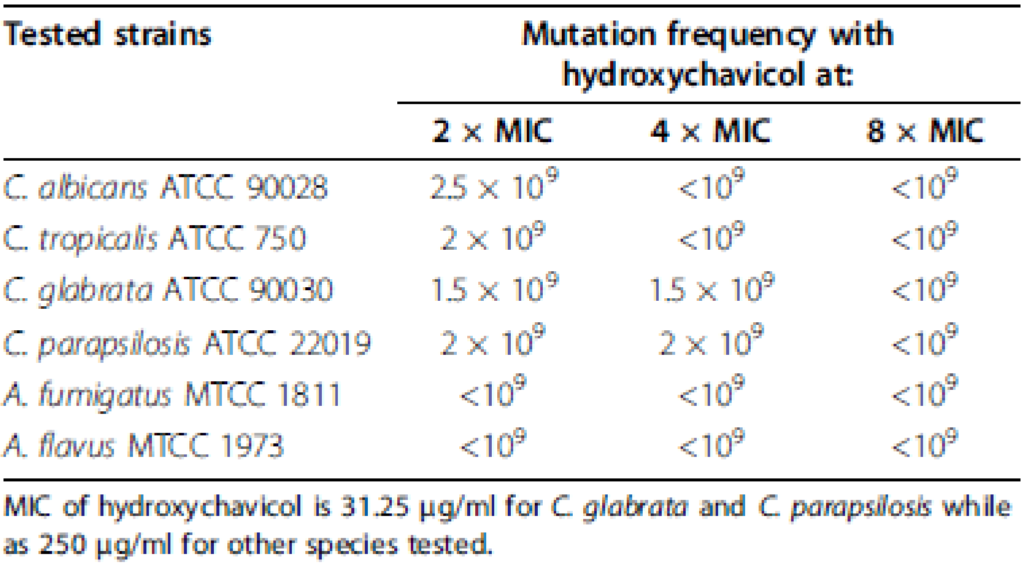
The microtiter plates were incubated at 28°C for 7 days for dermatophytes, and at 35°C for 48 h for Candida species (72 h for C. neoformans) and Aspergillus species. The plates were read visually, and the MIC was defined as the lowest concentration of the antifungal agents that prevented visible growth with respect to the growth control.

The MFC was determined by plating a 100 µl volume on SDA from the wells showing no visible growth. The plates were incubated as described above in MIC. The minimum concentration of hydroxychavicol that showed ≥ 99.9% reduction of the original inoculums was recorded as the MFC.

Results

The MICs and MFCs of hydroxychavicol were evaluated in vitroagainst 58 strains of yeasts, 39 strains of Aspergillus species and 27 strains of dermatophytes and all values are listed in Table 3.1. Hydroxychavicol exhibited the MICs range between 15.62 to 500 µg/ml for yeasts, 125 to 500 µg/ml for Aspergillus species and 7.81 to 62.5 µg/ml for dermatophytes, where as the MFCs were found to be similar or two fold greater than the MICs. Among all the fungal species tested, dermatophytes were found to be the most susceptible species to hydroxychavicol.

Table 1: Frequency of mutation with hydroxychavicol

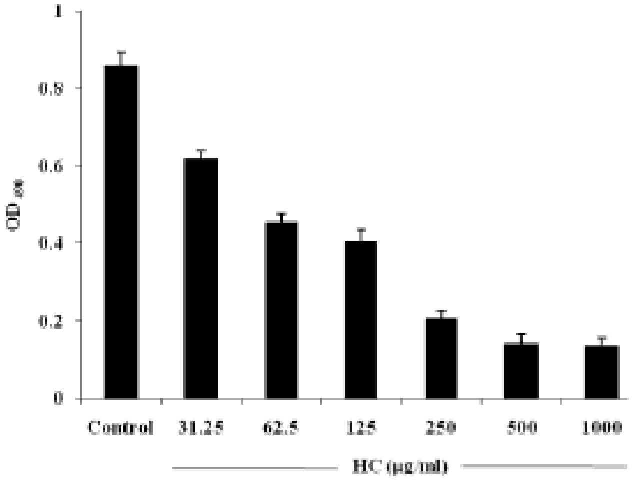


Tested strains	Mutation frequency with hydroxychavicol at:		
	2 × MIC	4 × MIC	8 × MIC
C. albicans ATCC 90028	2.5×10^9	$<10^9$	$<10^9$
C. tropicalis ATCC 750	2×10^9	$<10^9$	$<10^9$
C. glabrata ATCC 90030	1.5×10^9	1.5×10^9	$<10^9$
C. parapsilosis ATCC 22019	2×10^9	2×10^9	$<10^9$
A. fumigatus MTCC 1811	$<10^9$	$<10^9$	$<10^9$
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 exhibited fungicidal activity against both Candida species and the reduction in the number of cfu per milliliter was greater than 3 log units

(99.9%). The fungicidal endpoint for C. albicans was achieved after 10 and 1 h at 4 × MIC (4 × 250 µg/ml) and 8 × MIC (8 × 250 µg/ml) of hydroxychavicol (Fig. 3.2A). In C. glabrata, killing was observed at a lower concentration of hydroxychavicol due to its lower MIC. There was concentration dependent killing observed in case of C. glabrata, with two, four and eight times the MIC exhibited fungicidal activity in 10, 8 and 4 h “respectively ”.



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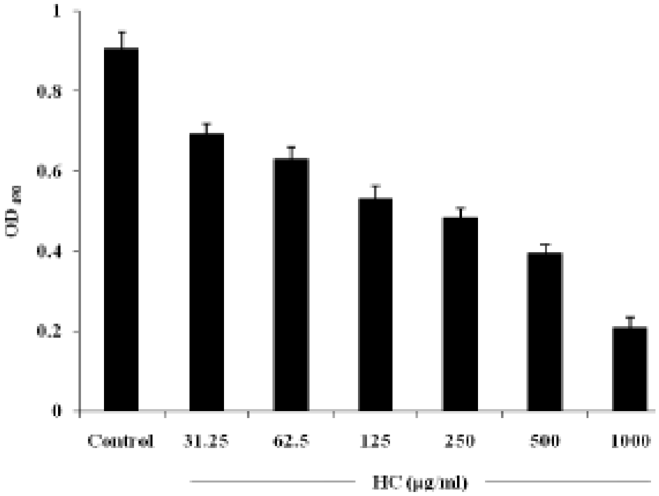

Figure 2: Inhibitory effect of hydroxychavicol (HC) on the biofilm formation (A) and reduction (preformed) (B) of C. albicans ATCC 90028 biofilms.

Discussion

In this study, we evaluated the antifungal activities of hydroxychavicol against various fungal species.

Hydroxychavicol demonstrated fungicidal effects against all the fungal species tested including Candida spp., Aspergillus spp. and dermatophytes. The fungicidal effect was most pronounced in dermatophytes including T. rubrum (MICs and MFCs were 15.62 - 62.5 µg/ml) which is the etiological agent of 80 to 93% of all clinical infections produced by dermatophytes. Hydroxychavicol also exhibited concentration dependent killing and extended PAFE of > 8 h. In the concentration range of 250-1000 µg/ml it completely suppressed the emergence of mutants of various Candida and Aspergillus species tested.

C. albicans is most commonly associated with biofilm formation, and the increase in Candida infections in the last decades has almost paralleled the increase and widespread use of a broad range of medical implant devices (such as stents, prostheses, implants, endotracheal tubes, pacemakers, and catheters), mainly in populations with impaired host defenses. Biofilm formation on medical devices can negatively impact the host by causing the failure of the device and by serving as a reservoir or source for future continuing infections. Hydroxychavicol was effective in inhibiting the C. albicans generated biofilm with 80% inhibition of biofilm was observed at the MIC concentration (250 µg/ml). However the reduction of the preformed biofilm was seen at four fold greater concentrations.

Conclusions

The results presented in this study are the first information of hydroxychavicol for antifungal activity. Hydroxychavicol exhibited a broad range antifungal activity against clinically significant human fungal species. Further studies are therefore warranted in order to explore of this natural compound for topical use in fungal infections particularly dermatomycoses.

REFERENCES

- Jacobson S (2004). The wrong solution. Emerg. Med., 36(8): 13.
- Prajapati DS, Purohit SS, Sharma AK, Kumar T (2001). In: Handbook of medicinal plants-Complete source book, Agrobios., p. 478.
- Prabhu MS, Patel K, Saraawathi G, Srinivasan K (1995). Effect of orally administered betel leaf (*Piper betle* leaf Linn.) on digestive enzymes of pancreas and intestinal mucosa and on bile production in rats. Indian J. Exp. Biol., 33: 752–756.
- Pongpech P, Prasertsilpe V (1993). The study of antimicrobial activity of *Piper betle* cream and gel against some fungi, yeast and bacteria. J. GPO, 19: 8–22.
- Razak FA, Othman RY, Haji ZHA (2006). The effect of *Piper betle* and *Psidium guajava* extracts on the cell-surface hydrophobicity of selected early settlers of dental plaque. J. Oral Sci., 48: 71–75.
- Rang HP, Dale MM, Ritter JM, Moore PK (2003). In: Pharmacology, 5th Edn., Churchill Livingstone, p. 229.
- Santhanam G, Nagarjan S (1990). Wound healing activity of *Curcuma aromatica* and *Piper betle*. Fitoterapia, 61: 458–459.
- Singh S, Govil JM, Singh VK (2003). In: Recent progress in medicinal plants. Phytochem. Pharmacol., Stadium Press LIC., 2: 239.