In vitro antifungal activity of hydroxychavicol isolated from *Piper betle* L

Intzar Ali1, Farrah G Khan3, Krishan A Suri2, Bishan D Gupta2, Naresh K Satti2, Prabhu Dutt2, Farhat Afrin4, Ghulam N Qazi4, Inshad A Khan1*

**Abstract**

**Background:** Hydroxychavicol, isolated from the chloroform extraction of the aqueous leaf extract of *Piper betle* L., (Piperaceae) was investigated for its antifungal activity against 124 strains of selected fungi. The leaves of this plant have been long in use tropical countries for the preparation of traditional herbal remedies.

**Methods:** The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of hydroxychavicol were determined by using broth microdilution method following CLSI guidelines. Time kill curve studies, post-antifungal effects and mutation prevention concentrations were determined against *Candida* species and *Aspergillus* species respectively. Hydroxychavicol was also tested for its potential to inhibit and reduce the formation of *Candida albicans* biofilms. The membrane permeability was measured by the uptake of propidium iodide.

**Results:** Hydroxychavicol exhibited inhibitory effect on fungal species of clinical significance, with the MICs ranging from 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. There was concentration-dependent killing of *Candida albicans* and *Candida glabrata* up to 8 × MIC. Hydroxychavicol also exhibited an extended post antifungal effect of 6.25 to 8.70 h at 4 × MIC for *Candida* species and suppressed the emergence of mutants of the fungal species tested at 2 × to 8 × MIC concentration. Furthermore, it also inhibited the growth of biofilm generated by *C. albicans* and reduced the preformed biofilms. There was increased uptake of propidium iodide by *C. albicans* cells when exposed to hydroxychavicol thus indicating that the membrane disruption could be the probable mode of action of hydroxychavicol.

**Conclusions:** The antifungal activity exhibited by this compound warrants its use as an antifungal agent particularly for treating topical infections, as well as gargle mouthwash against oral *Candida* infections.
antiinflammatory [5] etc. It also acts as a stimulant, a
breath freshener, a carminative, a salicyclogen, a cardiac
tonic, a pain killer in joint pain, an aphrodisiac, an
astringent, an antiseptic [5-7], a digestive and pancreatic
lipase stimulant [8], wound healing [9].

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 [10]. It also has a tendency to
act as an antioxidant, and a chemopreventive agent [10].
Other useful properties include antiinflammatory, anti-
platelet and antithrombotic without impairing haemo-
static functions [11]. There have been reports on the
antibacterial activities of hydroxychavicol [12,13], but so
far the report on its antifungal activity is lacking.

The present study was sought to investigate the effects
of hydroxychavicol on fungal pathogens. In addition its
effect on membrane permeability of C. albicans was also
examined.

Methods

Antifungal agents

Hydroxychavicol (Fig. 1) was isolated in the pure form
from the chloroform extraction of the aqueous leaf
extract of P. betle L., (Piperaceae) as described pre-
viously [12]. Amphotericin B was purchased from Sigma
Chemical Co. (St. Louis, MO), and terbinafine was
obtained as kind gift from Lupin Laboratories, Pune,
India.

Fluorochrome dye

Propidium iodide (Sigma), a small cationic, nucleic acid-
binding fluorochrome largely excluded by intact cell
membranes was used to stain the yeast cells [14].
Sodium deoxycholate (Sigma), an anionic detergent, was
used to facilitate diffusion of propidium iodide into the
yeast cell membranes which were damaged by the anti-
fungal agent [15].

Fungal strains and growth conditions

A total of 124 fungal strains were tested for their sus-
cceptibility 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 flaveus
(MTCC 1973, MTCC 2799 and 10 clinical isolates),
Aspergillus fumigatus (MTCC 1811 and 17 clinical iso-
lates), Aspergillus niger (ATCC 16404 and 6 clinical iso-
lates), Aspergillus parasiticus (MTCC 2796),
Epidermphyton floccosum (MTCC 613 and 1 clinical
isolate), Microsporum canis (MTCC 2820 and 3 clinical
isolates), Microsporum gypseum (MTCC 2819 and 2 clin-
ic 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 Collec-
tion (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.

“Inoculum” preparation

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. neo-
formans) and 7-day-old cultures “respectively” grown on
dextrose agar (Difco Laboratories, Detroit, Mich)
at 35°C [16,17]. A stock inoculums suspension of each
dermatophytes was prepared from fresh, mature (7-day-
old) cultures grown on potato dextrose agar with 2% in-
house rice flour slants at 28°C. The densities of these
susensions 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 [18]. All adjusted suspensions were quantified by
plating on Sabouraud dextrose agar (SDA; Difco Labora-
tories) 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
Figure 2 Time-kill curve plots for Candida species following exposure to hydroxychavicol (HC). C. albicans ATCC 90028 (A), C. glabrata ATCC 90030 (B). Each time point represents the mean log$_{10}$ ± standard deviations of two different experiments performed in duplicate. $P$ values < 0.001 (Student's t-test).
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 inoculum was added to each well of the plate, resulting in a final inoculum of 0.5 × 10^4 to 2.5 × 10^5 cfu/ml [19] for yeasts and 0.4 × 10^4 to 5 × 10^4 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 [18], and at 35°C for 48 h for Candida species (72 h for C. neoformans) and Aspergillus species [16,17]. 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 final concentration of hydroxychavicol that showed ≥ 99.9% reduction of the original inoculums was recorded as the MFC [19].

**Time kill curve studies**

Time-kill curve studies were performed as described by Ernst et al [20], using RPMI. C. albicans ATCC 90028 and C. glabrata ATCC 90030 were used as the test strains in this study. One milliliter of the adjusted inoculum suspension (= 5 × 10^6 cfu/ml) was added to nine ml of RPMI with or without hydroxychavicol, providing the starting inoculum of ≈ 5 × 10^5 cfu/ml. The range of hydroxychavicol concentrations tested was one to eight times the MICs for test strains i.e. 250 to 2000 μg/ml for C. albicans and 31.5 to 250 μg/ml for C. glabrata. The culture flask were incubated with agitation at 35°C. At predetermined time points (0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h following the addition of hydroxychavicol), a 100 μl aliquot was removed from each culture flask and serially diluted in sterile normal saline containing 0.1% polysorbate 80 (Sigma) for the inactivation of hydroxychavicol. A 20 μl aliquot was plated onto a Sabouraud dextrose agar with lecithin and polysorbate 80 (BBL, Becton Dickinson and Company, Cockeysville, MD) plate for colony count determination. When the colony counts were expected to be less than 1000 cfu/ml, samples of 20 μl or 100 μl were taken directly from the test solution and plated or subcultured without dilution. Plates were then incubated at 35°C for 24 to 48 h. The lower limit of accurate and reproducible quantification was 50 cfu/ml for each of the strains.

**Postantifungal effect (PAFE)**

The PAFE of hydroxychavicol was performed in RPMI by the method described by Craig and Gudmundsson [21]. C. albicans ATCC 90028, C. tropicalis ATCC 750, C. glabrata ATCC 90030 and C. parapsilosis ATCC 22019 were used as the test strains in this study. One milliliter of the adjusted inoculum suspension (= 5 × 10^7 cfu/ml) was added to nine ml of RPMI with or without hydroxychavicol, providing the starting inoculum of ≈ 5 × 10^6 cfu/ml. The hydroxychavicol concentrations ranged from one to four times the MIC. After exposures to the hydroxychavicol for 2 h, samples were diluted to 1:100 in prewarmed medium to effectively remove the hydroxychavicol. The diluted cultures were then incubated with agitation (200 rpm) at 35°C and sampling was done after 0, 2, 4, 6, 8, 10, 12, 16 and 24 h for colony counts. The colony counts were determined as described above in time-kill curve studies. The PAFE was calculated by the following equation: PAFE = T - C, where T represents the time required for the count in the test culture to increase 1 log_{10} cfu/ml above the count observed immediately after drug (hydroxychavicol) removal and C represents the time required for the count of the untreated control flask to increase by 1 log_{10} cfu/ml.

**Selection of resistant mutants in vitro**

The first step mutant frequency of reference strains of C. albicans ATCC 90028, C. tropicalis ATCC 750, C. glabrata ATCC 90030, C. parapsilosis ATCC 22019, A. flavus MTCC 2799 and A. fumigatus MTCC 1811 were selected, using previously described method [22]. A fungal suspension containing 10^9 cfu (100 μl) was plated on SDA containing hydroxychavicol at concentrations equal to two, four and eight times the MIC. Mutation frequency was calculated by counting the total number of colonies appearing after 48 h of incubation at 35°C on the hydroxychavicol containing plate and by dividing the number by the total number of cfu plated.

**Minimum biofilm inhibitory concentrations (MBICs)**

The effect of hydroxychavicol on C. albicans ATCC 90028 biofilm formation was examined by the microbroth dilution method, similar to MIC assays for planktonic cells [16] as described above. The fungal suspension was prepared from the overnight culture grown in yeast nitrogen base (Difco Laboratories) medium supplemented with 100 mM glucose [23], and the
cells were harvested in the late exponential growth phase, washed twice with sterile phosphate-buffered saline (PBS; pH 7.2; Ca\(^{2+}\) and Mg\(^{2+}\) free [Hi Media]) and the turbidity of the suspension was adjusted to 4 McFarland standard (≈ 5 × 10\(^5\) cfu/ml). The suspension was diluted in RPMI to obtain ≈ 5 × 10\(^6\) cfu/ml as the final inoculums. Twofold serial dilutions of hydroxychavicol were prepared in RPMI in the wells of a 96-well flat-bottom polystyrene microtiter plate (NUNC, Roskilde, Denmark) containing the same media in a volume of 100 μl per well. A 100 μl of above-mentioned suspension was added to each well; the final concentrations of hydroxychavicol ranged from 1.95 to 2000 μg/ml. Amphotericin B (at a final concentration range from 0.0156 to 16 μg/ml) was used as control drug. Following incubation at 35°C for 48 h, absorbance at 490 nm was recorded to assess culture growth. The culture supernatants from each well were then decanted, and planktonic cells were removed by washing the wells with sterile PBS. Biofilm formation was quantified by tetrazolium salt (XTT) reduction assay (see below).

### Minimum biofilm reduction concentrations (MBRCs)

The effect of hydroxychavicol was also examined on preformed *C. albicans* ATCC 90028 biofilm by the method as described previously [24]. Biofilms were prepared by inoculating the wells of a polystyrene microtiter plate in a manner similar to that described above. After incubation at 35°C for 48 h, the culture supernatant from each well was decanted, and the planktonic cells were removed by washing the wells with PBS. Two fold serial dilutions of hydroxychavicol were prepared in RPMI, and 200 μl of each dilution was added to the biofilm in the wells. The plate was further incubated at 35°C for 48 h. The final concentrations of hydroxychavicol ranged from 1.95 to 2000 μg/ml. Amphotericin B (at a final concentration range from 0.0156 to 16 μg/ml) was used as control drug. After the completion of incubation, the plates were decanted and washed three times with 200 μl of sterile PBS to remove loosely adherent cells. Biofilm reduction was quantified by XTT-reduction assay described below.

### XTT-reduction assay

XTT (tetrazolium salt 2, 3-bis (2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide) reduction assay was performed by the method as described by Jin et al., [23]. The XTT (Sigma) solution was prepared in PBS (1 mg/ml), filter-sterilized through a 0.22-μm-pore-size filter (Millipore, Bangalore, India) and stored at -80°C until required. Menadione (Sigma) solution (0.4 mM prepared in acetone) was filtered and mixed with XTT solution at a ratio of 1 to 5 by volume before the assay. 200 μl of PBS and 12 μl of the XTT-Menadione solution were added to each of the washed wells and the plate was incubated in the dark for 2 h at 35°C. Following incubation, 100 μl of the solution was transferred to a fresh microtiter plate and, the color change in the solution was measured spectrophotometrically using a microtitre plate reader (Multiskan spectrum, Thermo electron, Vantaa, Finland) at 490 nm.
Propidium iodide uptake assay
The disruptive effect of hydroxychavicol on *Candida albicans* ATCC 90028 cell membranes was assessed by using hydroxychavicol-mediated propidium iodide uptake. One-milliliter volumes of ∼5 × 10⁷ cfu/ml cell suspensions of *C. albicans* in sterile MilliQ water were incubated with two to eight times the MIC (500 to 2000 μg/ml) of hydroxychavicol at 35°C for 60 min under agitation in the dark chamber. Fifteen minutes prior to the completion of incubation, 10 μl each of propidium iodide and sodium deoxycholate solution were added at a final concentration of 25 μg/ml and 2.5 mg/ml "respectively" [14,15]. Amphotericin B at eight times the MIC (4.0 μg/ml) was used as the positive control and, the cells without hydroxychavicol served as the negative (growth) control, treated in similar fashion. After incubation, 50 μl aliquot was transferred into fluorescence-activated cell sorting (FACS) tube (Becton Dickinson Biosciences, CA) containing 950 μl of sterile MilliQ water. Each tube was analyzed using a FACScan flow cytometer (BD-LSR; Becton Dickinson) with Cell Quest Pro software for data acquisition and analysis.

Results
Antifungal susceptibility results
The MICs and MFCs of hydroxychavicol were evaluated in vitro against 58 strains of yeasts, 39 strains of *Aspergillus* species and 27 strains of dermatophytes and all values are listed in Table 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.

Time kill curve studies
The killing activities of hydroxychavicol for *C. albicans* ATCC 90028 and *C. glabrata* ATCC 90030 are shown

![Image](image.png)

Table 2: PAFE values of hydroxychavicol for *Candida* species after 2 h of exposure

| Species              | PAFEs (h) (mean ± SD) at the following multiple of the MIC: |
|----------------------|------------------------------------------------------------|
|                      | 1 × MIC | 2 × MIC | 4 × MIC |
| *C. albicans* ATCC 90028 | 5.53 ± 0.3 | 6.34 ± 0.2 | 8.64 ± 0.3 |
| *C. tropicalis* ATCC 750 | 4.4 ± 0.6 | 6.4 ± 0.4 | 8.70 ± 0.2 |
| *C. glabrata* ATCC 90030 | 3.08 ± 0.4 | 3.76 ± 0.6 | 8.04 ± 0.1 |
| *C. parapsilosis* ATCC 22019 | 2.0 ± 0.1 | 4.0 ± 0.2 | 6.25 ± 0.3 |

Table 3: Frequency of mutation with hydroxychavicol

| Tested strains          | Mutation frequency with hydroxychavicol at: |
|-------------------------|---------------------------------------------|
|                         | 2 × MIC | 4 × MIC | 8 × MIC |
| *C. albicans* ATCC 90028 | 2.5 × 10⁹ | <10⁹ | <10⁹ |
| *C. tropicalis* ATCC 750 | 2 × 10⁸ | <10⁹ | <10⁹ |
| *C. glabrata* ATCC 90030 | 1.5 × 10⁹ | 1.5 × 10⁹ | <10⁹ |
| *C. parapsilosis* ATCC 22019 | 2 × 10⁸ | 2 × 10⁹ | <10⁹ |
| *A. fumigatus* MTCC 1811 | <10⁹ | <10⁹ | <10⁹ |
| *A. flavus* MTCC 1973 | <10⁹ | <10⁹ | <10⁹ |

MIC of hydroxychavicol is 31.25 μg/ml for *C. glabrata* and *C. parapsilosis* while as 250 μg/ml for other species tested.
in Fig. 2. 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. 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”.

PAFE studies
Hydroxychavicol produced significant PAFE against all the Candida species tested (Table 2). Increase in the concentration of hydroxychavicol resulted in extended PAFE for all the Candida spp. tested. This increase in PAFE was more prominent for C. albicans and C. tropicalis, where a PAFE of >8 h was exhibited in these organisms at four times the MIC concentration of hydroxychavicol.

Frequency of emergence of hydroxychavicol resistant mutants
The frequencies of mutant selection of C. albicans, C. tropicalis, C. glabrata, C. parapsilosis, A. fumigatus, and A. flavus, are summarized in Table 3. Hydroxychavicol completely suppressed the emergence of mutants at two times its MIC for A. fumigatus and A. flavus, four times the MIC for C. albicans and C. tropicalis, and eight times the MIC for C. glabrata and C. parapsilosis “respectively”. This concentration of hydroxychavicol at which no mutant was selected can be defined as the mutation prevention concentration.

Biofilm susceptibility assay
Hydroxychavicol exhibited an inhibitory effect on the biofilm formation and reduction of preformed biofilm of C. albicans ATCC 90028. The 50% and 80% biofilm inhibition as well as biofilm reduction are represented in Fig. 3. The MBIC50 and MBIC80 values of hydroxychavicol were 125 μg/ml and 250 μg/ml, where as the MBRC50 and MBRC80 values were 500 μg/ml and 1000 μg/ml. Reductions of preformed biofilms values were four fold greater than the concentration required to inhibit biofilm formation.

Effect of hydroxychavicol on membrane permeability
Exposing the cell suspension of C. albicans ATCC 90028 to two to eight times (500 to 2000 μg/ml) the MIC of hydroxychavicol for 60 min increased the cell permeability to the fluorescent nucleic acid stain, propidium iodide due to the disruption of membrane integrity. This resulted in the increase in fluorescence in comparison to untreated control (Fig. 4). This increase in fluorescence was proportional to the increase in the hydroxychavicol concentrations.

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 fungidal 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 [3]. 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 [25]. 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.

There have been reports on the antifungal activities of P. betle. Pongpech and Prasertsilpe [26] 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 [27] 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”.

Hydroxycavicol is one of the major constituents of P. betle. It has been extensively reported for its antibacterial activity [12,13]. 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 hydroxycavicol.

Propidium iodide is a fluorescent nucleic acid stain that is unable to penetrate the cell membrane structures of healthy cells. However, cells with damaged or permeabilised cell membranes do not exclude propidium iodide. Therefore, propidium iodide staining of cells indicates cytoplasmic membrane (bacteria) and plasma membrane (yeast) damage [28]. Sodium deoxycholate was used in this study as it is reported to enhance the diffusion of propidium iodide across the cell wall to pass through the damaged yeast cell membranes [29,15]. Interestingly, the growth controls did not show dye uptake in the presence of deoxycholate as the deoxycholate is nontoxic to C. albicans [29]. The increased uptake of propidium iodide in the hydroxycavicol treated cells of C. albicans in our study, further confirmed the earlier findings that hydroxycavicol alters the cell membrane structure, resulting in the disruption of the permeability barrier of microbial membrane structures [30].

The clinical applications of hydroxycavicol were challenging to interpret in this study due to a lack of pharmacokinetic and safety studies. However its comparable cytotoxicity profile with that of thymol widely used natural phenolic as food preservative and oral care agent in the earlier study [12] is indicative of the safety of this compound.

Conclusions

The results presented in this study are the first information of hydroxycavicol for antifungal activity. Hydroxycavicol 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.

Abbreviations

MIC: minimum inhibitory concentration, MFC: minimum fungicidal concentration, ATCC: american type culture collection, MTCC: microbial type culture collection; CFU: colony forming unit; MOPS: morpholinopropansulfonic acid; DMSO: dimethyl sulfoxide; PAF: postantifungal effect; MBIC: minimum biofilm inhibitory concentration; XPBC: minimum biofilm reduction concentration; XTT: 2, 3-bis (2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide.

Acknowledgements

We are thankful to Lupin Pharmaceuticals (Pune, India) for providing terbinafine. Research fellowship was provided to Mr. Inzrar Ali by Council of Scientific and Industrial Research (CSIR), New Delhi, India (P-81101).

Author details

1Clinical Microbiology Division Indian Institute of Integrative Medicine, Canal Road, Jammu-180 001, India. 2Natural Product Chemistry Division, Indian Institute of Integrative Medicine, Canal Road, Jammu-180 001, India. 3Department of Microbiology, Acharya Shri Chander College of Medical Sciences, Sidhra, Jammu-180 017, India. 4Department of Biotechnology, Faculty of Science, Hamdard University, Hamdard Nagar, New Delhi-110 062, India.

Authors’ contributions

IA was carried out all experimental work, data acquisition and analysis, literature search and writing the manuscript. IAK and FA were responsible for study concept, designing and coordinating the research, supervising the work and revising the manuscript. FGK is the collaborator from the Medical College and provided the clinical inputs in the manuscript. KAS, BDG, NKS and PD involved in extraction and characterization of hydroxycavicol from Piper betle. GQN was involved in critical evaluation of the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 7 August 2009 Accepted: 3 February 2010 Published: 3 February 2010

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Cite this article as: Ali et al.: In vitro antifungal activity of hydroxychavicol isolated from Piper betle L. Annals of Clinical Microbiology and Antimicrobials 2010 9:7.