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Curcumin and Fluconazole to Resolve Fluconazole-resistant *Candida albicans* Infection in HIV Patient

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ABSTRACT

In the last decade, there has been a significant increase in antifungal resistance against C. albicans. Curcumin is a choice of a novel therapy that is currently developing because curcumin is an agent that can affect epigenetics. Epigenetic factors significantly affect the expression of drug efflux transporter proteins, one of which is Cdr1p. This protein is encoded by CDR1. This research aimed to show documentation that curcumin overcomes *Candida albicans* resistance through *Candida* drug resistance protein (*Cdr1p*) expression and *CDR1* methylation. *C. albicans* from HIV patient's oropharyngeal isolates were observed by molecular (SDS PAGE, DNA sequence analysis), colony growth, and MICs (Minimum Inhibition Concentrations) of Fluconazole and Curcumin combination. The MICs of compounds for *C. albicans* strains were determined using the tube dilution test by the CLSI microdilution reference method. The endpoint may be read spectrophotometrically. Colony growth was assessed by staking 0.001 ml of dilution into an agar plate. Yeast DNA isolation using Zymo Research Quick DNA Fumgal Miniprep Kit No. D6005, followed by Bisulfite treatment, Polymerase Chain Reaction (PCR), and CDR1 sequences. The most significant decrease in MICs in the *C. albicans* colony occurs for a combination of Fluconazole and Curcumin with a value of 6.25+ 12.5 µg/ml. *Cdr1p* expression for the curcumin group and the combination of fluconazole plus curcumin group was much lower than other groups, but CDR1 silencing was higher. Thus, combining curcumin with fluconazole has synergized to resolve fluconazole resistance through *CDR1* silencing and decreasing *Cdr1p* expression.

Keywords: *Candida albicans*, Candida drug resistance protein, Curcumin, Fluconazole.

Introduction

Antifungal resistance against Candida albicans has significantly increased during the past ten years. Resistance to antifungal drugs is an emerging concern worldwide in both space and time, including novel resistant variants of previously susceptible pathogens (for example, the ubiquitous mould Aspergillus fumigatus) as well as entirely new emerging species that are resistant to multiple antifungal drugs (for example, the yeast Candida auris6). The increasing public health burden is now officially recognized with the listing of both of these pathogens on the urgent antimicrobial resistance (AMR) threat list published by the US CDC in 2019. Fluconazole is fungistatic rather than fungicidal, so treatment allows acquired resistance to develop in the presence of this antifungal. This resistance carries important implications for mortality, morbidity, and community health service cost.[1] The increasing resistance has made *C. albicans* one of the microorganisms with difficult management.[2]

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Resistant Candida is commonly detected in hospitalized patients. About 7% of bloodstream infections are resistant to antifungals, which can affect the mouth, skin, and vagina, resulting in more than 3.6 million U.S. healthcare visits each year and $3 billion in estimated direct medical costs. The challenge in dealing with drug resistance against *C. albicans* infection was harder since using an antifungal agent such as azole was limited by its toxicity which causes great liver damage, with lots of side effects such as hypersensitivity and allergic reactions and also a narrow range of therapeutic for this category.[3] It is very crucial to find a new therapy strategy (novel therapeutic) against this resistance. Fungal diseases kill more than 1.5 million and affect over a billion people. However, they are still a neglected topic by public health authorities, even though most deaths from fungal diseases are avoidable. Serious fungal infections occur because of other health problems, including asthma, AIDS, cancer, organ transplantation, and corticosteroid therapies. Early accurate diagnosis allows prompt antifungal therapy; however, this is often delayed or unavailable leading to death, serious chronic illness, or blindness. Recent global estimates have found ~700,000 cases of invasive candidiasis occur annually. Curcumin has become one of the optional alternatives to overcome antifungal resistance nowadays since curcumin has been used since ancient times until date. Communities have been familiar with and know about this herbal ingredient.[4] Curcumin has become a developing choice because the wider community has widely used curcumin. People are already familiar and familiar with this herbal ingredient. Natural plant products have been used as the foundation of several medical treatments in humans.

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Although modern aspects of Western medicine has become the forefront of clinical practice today. Natural plant products continue to be used as remedies in alternative medicine throughout the world. It is estimated that 80% of individuals in developing countries depend primarily on natural products to meet their healthcare needs. Even in the United States, it has been found that approximately one in three Americans uses natural medicinal products daily.1 It has been estimated that of the 877 small-molecule drugs introduced worldwide between 1981 and 2002, approximately 61% can be traced back to their origins in natural products. Natural products are not only effective but are relatively non-toxic and have therapeutic doses well below their toxic levels. Curcumin is one such molecule that has shown promise since time immemorial. Nonetheless, there exists a significant barrier to the utilization of these natural plant products in modern healthcare due to the stigmatization of these "natural" remedies.[5] Studies have shown that curcumin has synergized with the azole category as an antifungal agent. Another study also showed that curcumin can modulate the expression of resistance proteins in *C. albicans*.[6] It is expected that the use of curcumin to resolve antifungal resistance would be accepted. Antifungal resistance has four mechanisms, which are drug metabolism change, a mutation in protein target coding gene, prevention of drug intake, and drug efflux of cells through increased expression of drug efflux pumps (drug efflux pumps/transporter). *C. albicans* has its resistance mechanism by increasing drug elimination pump expression.[7-10] This mechanism was the main mechanism of *C. albicans* in facing resistance. Epigenetic factors are factors outside the gene that can affect the expression of drug efflux transporter proteins. Drug efflux transporter is increased in resistant C. albicans. Epigenetic factors affect the protein expression of drug efflux transporter, one of which was Cdr1p. This protein was coded by CDR1. Epigenetic factors could take the form of the effect of Histon acetylation or DNA methylation. The effect of this epigenetic factor would affect Cdr1p expression. A flavonoid (such as curcumin) was assumed to be the agent that could affect epigenetic factors. The study of Link et al. (2013) showed that curcumin modulates DNA methylation in colon cancer cells. However, there was little evidence that was able to show whether curcumin becomes an inhibitor for CDR1 methylation in *C. albicans* with Fluconazole-resistant strain, or whether this inhibition ability of curcumin would lower drug efflux transporter of Cdr1p and thus solving the problem of antifungal resistance (particularly fluconazole). This study aimed to provide evidence that curcumin reduced C. albicans resistance through CDR methylation and Cdr1p expression.

Materials and Methods

Yeast strain and culture conditions

The strain used in this study was obtained from the Institute of Microbiology Cipto Mangunkusumo Hospital, Jakarta, Indonesia Strain was isolated from HIV patients with oropharyngeal Candidiasis. Species determination was performed by standard protocols. Yeast was grown on yeast agarose gel (Sigma Aldrich, Cat. No. Y1500). Fluconazole-resistant *C. albicans* clinical isolates were identified using the microscope CLSI standard by Microbiology Laboratory. Strains collection were isolated sequentially from buccal mucose of HIV patients using a cotton swab and cultured on candida and fluconazole media. Only one strain (C103) was classified as Fluconazole resistant according to the Clinical and Laboratory Standards Institute (CLSI) with interpretive breakpoint criteria (Fluconazole MIC >64µg/ml).

Chemical and antifungal agents

Fluconazole (obtained from TCI Laboratories, Ltd., Cat. No. F0677), curcumin (obtained from TCI Laboratories, Ltd., Cat. No. C0434. *C. albicans* bacteria were cultured into yeast agarose gel containing antifungal agents with the following doses of treatment: (1) Fluconazole (0, 6.25, 12.5, 25, 50,100, 200 ppm), (2) curcumin (0, 12.5, 25, 50, 100, 200, 400 ppm), and (3) combination of fluconazole and curcumin (0, 6.25+12.5, 12.5+25, 50+100, 100+200, 200+400 ppm). All treatments were conducted in four replications.

Drug susceptibility assays for identification of curcumin and fluconazole-resistant yeast

Minimum inhibitory concentrations (MICs) of compounds for the *C. albicans* strain were determined using a tube dilution test in accordance with the CLSI microdilution reference method (CLSI guidelines document M27-A3). The endpoint may be read spectrophotometrically. MICs for fluconazole were the minimum concentrations giving >80% growth inhibition compared to the no-drug control. Colony growth was assessed by streaking 0.01 ml dilution into an agarose plate. MICs were determined if the subculturing colony was lower in diameter and density of colonies than previous doses or treatments. Minimum bactericidal concentrations (MBCs) were determined if no subculturing colony was growing in the medium.[11]

Cdr1p protein expression assessment

Whole protein was extracted using Qproteome Bacterial Protein Prep Kit's protocol (Qiagen, Cat No./ID: 37900). SDS-PAGE assessed cdr1p protein expression among the group according to the Laemmli method with 8% (wt/vol) acrylamide separating gels (Bio-Rad, Cat. No. 161-0125). Separated polypeptides were visualized using Coomassie blue R250 and electroblotted (100 V, 1.5 h, 4°C). Cdr1p protein could be determined if every sample had a 160 kDa band.[12]

Analysis of CDR1 gene methylation

DNA yeast isolation was using a DNA isolation kit of Zymo Research, Quick DNA Fungal Minipreparation (Zymo, Cat. No. D6005). The methylation pattern can be observed using the MethylCode Bisulfate Conversion Kit (Invitrogen, Cat. No. D5001-1). Bisulfate would convert methylated cytosine into thymine, while non-methylated cytosine did not change or react with sodium bisulfate. CDR1 gene promoter amplification was done using GoTaq Green Master Mix (Promega) for 1x reaction with a total volume of 25µl and continued with sequences of Rtt109 promoter region analysis. Amplicon or PCR products were sequenced to determine methylated DNA position. The sequence of the CDR1 gene from treatments and control was compared with the *C. albicans* CDR1 gene with accession number X77589 provided by Prasad et al.[13]

Data analysis

Data collected were analyzed by using analysis of variance (ANOVA) p-value < 0.05 was used to determine the significance of the result or to determine the significance of the difference between the control and doses group.

Results and Discussion

C. albicans Growth

Increasing the concentration of fluconazole reduced *C. albicans* growth. Candida growth was measured by the number of colonies that increased on the culture media. An increasing dose of curcumin and a combination of fluconazole and curcumin also shows a similar result, which reduces *C. albicans* growth. The most significant decrease of *C. albicans* colony occurs since the first dose of combination fluconazole and curcumin (6.25 µg/ml of fluconazole + 12.5 µg/ml of curcumin resulting 3.3.104 *C. albicans* colony, etc). In combination with both agents, *C. albicans* colony did not exist at the sixth dose (100 µg/ml of fluconazole + 200 µg/ml of curcumin). At the same dose, if given separately, curcumin (200 µg/ml) or fluconazole (100 µg/ml) would still result in quite a large *C. albicans* colony. From the table above, the largest colony decrease occurs in a combination of fluconazole and curcumin (Table 1). The fluconazole-resistant *C. albicans* strain (which is called C103 strain) growth may be assessed by streaking 0.01 ml from the appropriate serially tube dilution test on an agar plate. After incubation for 24 hours, the colonies may be enumerated. The result is referred back to the whole plate and the original undiluted sample to approximate the bacterial load as colony forming units (CFU) per 1 ml sample. The visually different colonies may be enumerated separately and then subcultured for subsequent Candida identification. Hence, the total and partial Candida load of any sample may be identified (Figure 1).

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Table 1: Growth of *C. albicans* fluconazole-resistant strain

Group	Dose (µg/ml)	Cell numbers ± SD (10^3 cells/ml)
Fluconazole	0	300.00 ± 0.00
	6.25	130.25 ± 9.71*
	12.5	121.50 ± 10.25*
	25	105.50 ± 18.69*
	50	99.00 ± 2.83*
	100	0.75 ± 0.5*
	200	0.00 ± 0.00*
Curcumin	0	300.00 ± 0.00
	12.5	236.75 ± 7.68*
	25	174.50 ± 12.58*
	50	155.25 ± 11.95*
	100	14.50 ± 4.12*
	200	3.00 ± 0.82*
	400	0.00 ± 0.00*
Fluconazole + curcumin	0	300.00 ± 0.00
	6.25 + 12.5	33.00 ± 8.29*
	12.5 + 25	26.75 ± 3.86*
	25 + 50	12.25 ± 2.22*
	50 + 100	3.50 ± 1.00*
	100 + 200	0.00 ± 0.00*
	200 + 400	0.00 ± 0.00*

* The cell numbers value showed a significantly different between treatment and control.

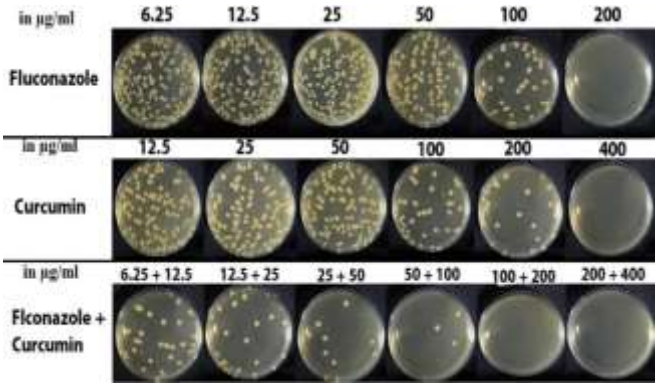

Figure 1: Growth of *C. albicans* fluconazole-resistant strain colonies on medium containing antifungal agent.

Table 2: Minimum inhibition concentrations (MICs) and minimum bactericidal concentrations (MBCs) of fluconazole and curcumin against *C. albicans* fluconazole-resistant strain

Groups	MIC (µg/mL)	MBC (µg/mL)
Fluconazole	100	200
Curcumin	200	400
Fluconazole + curcumin	6.25 + 12.5	100 + 200

Effect of Curcumin and Fluconazole on C. albicans Fluconazole-resistant colonies Growth

Minimum inhibition concentrations (MICs) and minimum bactericidal concentrations (MBCs) were obtained based on tube dilution test regarding *C. albicans* colony growth. It indicates that MIC of fluconazole was 100 µg/ml and curcumin was 100 µg/ml. MIC of the combination was lower than fluconazole or curcumin only. The result of MBC was similar to MIC. Combination of fluconazole and curcumin resulting lower MBC compare to fluconazole-only or curcumin-only MBCs (Table 2).

Expression of Cdr1p Protein

This qualitative study was obtained through SDS-PAGE of various groups (control, fluconazole, curcumin, combination of fluconazole + curcumin with MIC dosage) exposed to *C. albicans* fluconazole-resistant strain. According to the SDS-PAGE result, the far right would be a marker. The second lane would be controlled. The third lane would be the fluconazole group. The fourth lane is the curcumin group. The fifth lane is a combination of fluconazole and curcumin groups. The cdr1p band was shown with a black arrow. The control and fluconazole groups have the thickest band, followed by the curcumin and combination groups. It shows that Cdr1p expression in curcumin and the combination of fluconazole + curcumin groups was the thinnest (Figure 2). The Control group showed demethylation, while fluconazole, curcumin, and combination groups showed methylation in position 1057. Methylation through bisulfate treatment toward cytosine would change into thymine. The alignment result shows that there was a change in cytosine at 1057 position into thymine which occurs in curcumin, and a combination of fluconazole + curcumin (blue box in alignment result). Cytosine at this position lies in the promoter region and CpG (near G base). This nitrogen base change will inactivate CDR1. Activation of the CDR will produce a drug efflux transporter protein that plays an important role in the resistance mechanism. On the other hand, the inactivation of CDR1 will decrease resistance (Figure 3). Curcumin is a new choice for an experimental therapy that is currently being investigated since it is a substance that can affect epigenetics. Cdr1p and other drug efflux transporter proteins are significantly altered by epigenetic factors. It is encoded by CDR1. In general, DNA methylation or histone modification is used by epigenetic mechanisms to control gene expression. Environmental elements that encourage human epigenetic alterations are comparable to those that increase cancer risk, such as nutrition, lifestyle, and exposure to toxins. Numerous ingredients in food, such as selenium or polyphenols (antioxidants found in fruits and vegetables), specifically affect DNA methylation. Others, like the abundant butyric acid in cheeses and the sulforaphane in broccoli, can alter histones by preventing histone deacetylase.[14] This study showed that fluconazole, curcumin, and a combination of fluconazole and curcumin could inhibit fluconazole-resistant *C. albicans* growth along with the increasing dose used. The results showed that the curcumin exposure group and the fluconazole + curcumin combination resulted in methylation at CDR1. DNA methylation is a process by which a methyl group is added to a DNA molecule. Methylation can change the activity of a DNA segment without changing the DNA sequence. When in a gene promoter, DNA methylation usually acts to suppress gene transcription. DNA methylation is essential for genomic inactivation, so it is closely related to the expression or absence of drug resistance. DNA methylation caused by curcumin will result in gene silencing. In the Candida-resistant strains that were sampled in this study, CDR1 silencing occurred so that Cdr1p expression decreased. This decrease in Cdr1p expression will cause the efflux drug transporter not to work so that Fluconazole that enters Candida will not be released into the extracellular Candida. In the end, Fluconazole will work with intracellular Candida that is interfering with Candida membrane biosynthesis so that it can work again as a fungistatic. The fluconazole dose that could inhibit *C. albicans* growth was supposed to be a toxic dose (above 64 µg/ml). This occurs since the strain used in this study was a fluconazole-resistant *C. albicans* with MIC (Minimal Inhibition Concentration) above 64 µg/ml. Although high-dose fluconazole inhibited the growth of the *C. albicans* fluconazole-resistant strain, this dose was difficult to apply to

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patients due to its high-level side effects and toxicity. Fluconazole was mostly given to patients with a high risk of candidemia, such as individuals with low body immunity, for example, HIV-infected patients.[9] The development of antifungal resistance was no longer a new phenomenon. Antifungals would stimulate response to eliminate the disadvantageous effect of the drug and continue to grow through upregulation of the multidrug transporter gene belonging to the ABC family, or major facilitator superfamily (MFS), leading to the occurrence of the phenomenon of Candida resistance. For clinical isolates of C. albicans, it is known that the ABC transporters of C. albicans Cdr1p and CaCdr2p, as well as the MFS transporter CaMdr1p, are the main MDR transporters that contribute to Fluconazole resistance.[15] Antifungal stress through the signaling pathway would induce a stress response and affect fungal virulence, including the resistance mechanism developed by this fluconazole-resistant *C. albicans* strain.[10] Curcumin in this study was focused on its ability to resolve *C. albicans* resistance. This study showed that fluconazole, curcumin, and the combination of fluconazole + curcumin inhibited the growth of fluconazole-resistant C. albicans strains as the test dose increased. Curcumin in this study was focused on its ability to resolve C. albicans resistance. This study showed that fluconazole, curcumin, and the combination of fluconazole + curcumin inhibited the growth of fluconazole-resistant C. albicans strains as the test dose increased. The specificity of this study is the use of fluconazole-resistant candida strains, and it is proven that the addition of curcumin reduces the need for fluconazole doses to inhibit the growth of these resistant strains. The specificity of this study is the use of fluconazole-resistant candida strains, and it is proven that the addition of curcumin reduces the need for fluconazole doses to inhibit the growth of these resistant strains. However, curcumin itself can act as an antifungal. This might explain the result in which curcumin also inhibits the *C. albicans* fluconazole-resistant strain. There were many mechanisms of curcumin acting as an antifungal. The reported curcumin work target mechanism was: 1) curcumin causing cell death through reactive oxygen species (ROS), which induce apoptosis, 2) ergosterol metabolism disruption, which is a membrane component, 3) curcumin work to *C. albicans* cells wall through calcineurin-mediated signal and 4) filamentation disturbance or hypha development.[6,13,16,17]

A combination of fluconazole and curcumin shows the most interesting result in this study. It was the most significant inhibition for *C. albicans* fluconazole-resistant strain growth. Several studies do show that fluconazole and curcumin have a synergy to work as an antifungal. However, the strain used in this study was a fluconazole-resistant strain. Thus it is very likely that the significant decrease in growth of the fluconazole-resistant *C. albicans* strain mainly came from the ability of curcumin to resolve resistance issues in the fluconazole-resistant *C. albicans* strain used in this study and not came from the ability of curcumin as an antifungal. Due to the ability of curcumin to resolve this resistance issue, fluconazole was able to enter *C. albicans* and works as an effective antifungal, just like when there is no resistance in *C. Albicans*. The results showed that the curcumin exposure group and the fluconazole plus curcumin combination resulted in methylation at CDR1. DNA methylation is a process by which a methyl group is added to a DNA molecule. Methylation can change the activity of a DNA segment without changing the DNA sequence. When in a gene promoter, DNA methylation usually acts to suppress gene transcription. DNA methylation is essential for genomic inactivation, so it is closely related to the expression or absence of drug resistance. DNA methylation caused by curcumin will result in gene silencing. In the Candida-resistant strains sampled in this study, CDR1 silencing occurred so that Cdr1p expression decreased. This decrease in Cdr1p expression will cause the drug efflux transporter not to work so that Fluconazole that enters Candida will not be released into the extracellular Candida. In the end, Fluconazole will work intracellularly with Candida, which interferes with the biosynthesis of the Candida membrane so that it can work again as a fungistatic.

The synergy of curcumin and the antifungal drug was studied previously. A combination of voriconazole and terbinafine was synergistic with *C. albicans* isolates obtained from HIV-infected patients.[18-19]

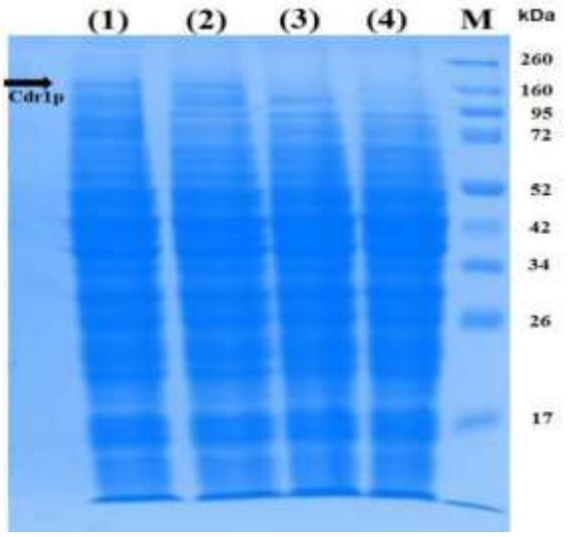

Figure 2: Profile of C. albicans protein in 8 % separating acrylamide gel. Cdr1p protein for each sample is shown with a black arrow with a molecular weight is 160 kDa. 3.4. CDR1 GeneMethylation.

Many herbal products, such as a combination of fluconazole and allicin, showed good synergistic antifungal effects, *in vivo* and *in vitro*.[20-21] Three pure curcuminoids isolated from pure curcumin showed antifungal activities, CUR-I (diferuloylmethane), CUR-II (demethoxycurcumin), and CUR-III (bisdemethoxycurcumin). Studies showed that pure CUR-I, the main component of pure curcumin, can be exploited further by combining it with azole or polyenes.[3] Battling *C. albicans* infection, particularly one caused by a resistant strain, was becoming a challenge. Efforts have been made, including (i) looking for a new antifungal agent, (ii) developing a new formulation, and (iii) increasing an antifungal agent's efficacy through combination therapy. The result of this study can be used as the basis to develop a combination therapy of curcumin and fluconazole for fluconazole-resistant *C. albicans* strain infection. The result of this study showed that curcumin has a potential role in resolving *C. albicans* resistance issue. The result of this study was in line with Sharma et al.[6] In that study, curcumin has a potential role in modulating the efflux activity of CaCdrq1p and comparing it with the flux activity of CaCdr2p and Pdr5p of *Saccharomyces cerevisiae*. The study result showed that curcumin act as an efflux rhodamine 6G (R6G) specific modulator that is mediated by CaCdr1p, CaCdr2p, and ScPdr5p to modulate the work of these transporters. The effect of curcuminoid on fungal ABC transporter has modulated its works.

This study showed that curcumin and a combination of fluconazole and curcumin caused CDR1 methylation. DNA methylation is a process where a methyl group is added to DNA molecules. Methylation could change the activity of a DNA segment without changing its sequence. When located in the gene promoter, DNA methylation works to silence gene transcription. DNA methylation was essential for genomic inactivation. Thus it was highly related to drug resistance. DNA methylation caused by curcumin would result in gene silencing. CDR1 silencing occurs in resistant *C. albicans* strain, so Cdr1p expression would decrease. This was in line with the result of the SDS PAGE analysis, whereas curcumin and the combination of fluconazole + curcumin showed lowered Cdr1p expression. This also proved that curcumin influences epigenetics, such as DNA methylation. Although curcumin could repress the gene, this effect is reversible. Methylation toward cytosine will form 5-methylcytosine. Spontaneous deamination of 5-methylcytosine would convert it into thymine. This would cause inappropriate T: G. Correction mechanism would correct it by changing G with A, changing the original pair C: G into A: T. This would cause mutation. This wrong base would not be corrected during DNA replication, thus creating mutant-derived cells, or in other words, this mutation would be permanent.

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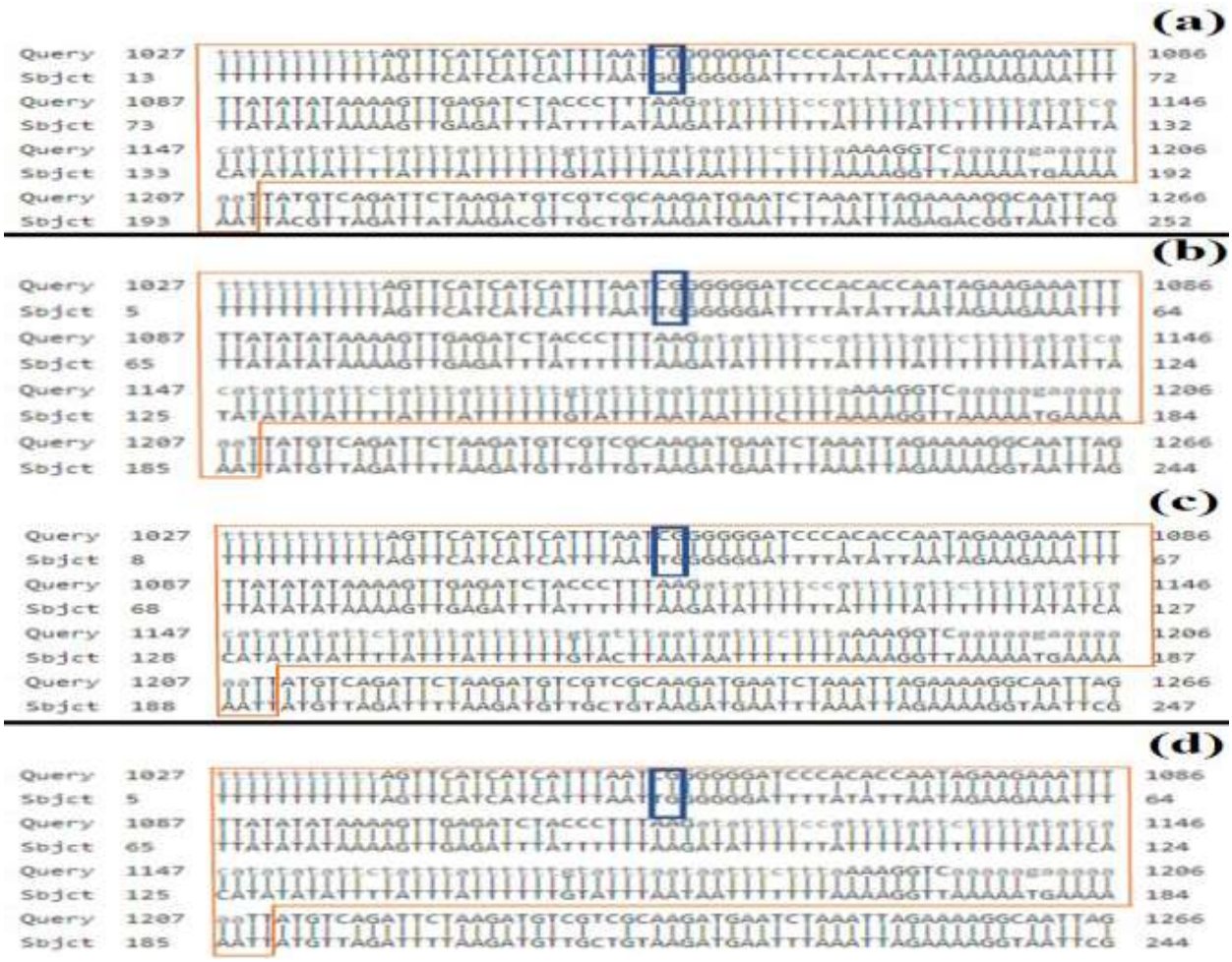

Figure 3: Promoter area of CDR1 gene of *C. albicans* fluconazole-resistant strain. (a) Control; (b) fluconazole; (c) curcumin; (d) combination of fluconazole and curcumin. Legends: ▭: Promoter area observed; ▭: Methylation position observed. The query is shown as *C. albicans* from the NCBI database (Accession number X77589), and the subject is *C. albicans* in this present study for each treatment.

DNA methylation was found in three different sequences: CG (or CpG island), CHG, or CHH (in which H is A, T, or C). DNA methylation would almost be found in CpH around 60-80%. CpG islands were commonly defined as a region with 1) a length is more than 200 bp, 2) G+C content is more than 50%, and 3) a CpG ratio larger than 0.6. About 50% of CpG island lies in the gene promoter region, while the other 25% lies in the gene body.[22] DNA methylation could affect gene transcription in two ways. First, DNA methylation itself could inhibit transcription protein binding to the gene, and second (more importantly), methylated DNA can be bound by a protein known as methyl-CpG-binding domain proteins (MBD). MBD protein would recruit additional proteins into the locus, such as histone deacetylases and other chromatin remodeling proteins, which could modify histone to become inactive or mediate transcriptional silencing from hypermethylated genes. Fungal has low-level cytosine methylation (0.1 to 0.5%).[23] The result of this study was in line with a study conducted by Link et al.[24] Link's study showed that curcumin modulates DNA methylation in colorectal cancer cells. Curcumin-induced DNA methylation change occurs in a time-dependent manner. Treatment with curcumin would cause methylation change in certain partially methylated locations, and not in fully methylated CpG locations. The result of this study was not in line with the study done by Boyanapalli and Kong in which curcumin lowered DNA methylation. Curcumin lowered DNMT1 expression in AML cell lines, either *in vitro* or *in vivo*, and primary AML cells *ex vivo*. Furthermore, curcumin and other epigenetic modifiers (such as 5-azacytidine (5AZA) and decitabine) have been shown to change the DNMT inhibitor effect toward DNA methylation to reactivate silent genes in leukemia cells.

Conclusion

The combination of fluconazole and curcumin had synergized effect to reduce *C. albicans* colonies' growth based on MICs and MBCs value. A combination of fluconazole and curcumin could reduce the expression of cdr1p protein effectively. They also caused CDR1 gene methylation, which was indicated by changes of cytosine into thymine, as well as curcumin and fluconazole only.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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