

Reversing Antifungal Drug Resistance using Natural Plant Products

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Fungal infections have increased tremendously in the past few decades [1] contributing to morbidity and mortality. The genus *Candida* which includes around 200 human pathogenic species [2] is basically a commensal that causes both superficial and systemic infections [3] mainly in immuno compromised hosts like AIDS and cancer patients. *Candida albicans*, the fourth most common cause of hospital acquired infections, can manifest as a pathogen with the help of germ tube induction and biofilm formation [4]. It is the predominant cause of virtually all types of Candidiasis but other emerging *Candida* species like *C. glabrata*, *C. krusei*, *C. Tropicalis* and *C. parapsilosis* are also serious nosocomial threats [5,6]. The conventional drugs available to treat fungal infections are toxic having undesirable side effects and are limited in number largely due to the eukaryotic nature of fungal cells. Till date, it is still difficult to identify unique antifungal targets not shared with human hosts. The excessive use of these drugs often leads to drug resistance maybe due to their fungistatic nature [7,8]. The incidence of antifungal resistance in clinically important strains is increasing at an alarming rate. Since azole fungicides are used in agriculture, drug resistance is also a matter of concern in opportunistic plant pathogens [9]. There are several mechanisms that can give rise to azole resistance in fungi. Two main classes of drug efflux pumps are responsible for development of antifungal resistance – the ABC superfamily that uses energy of ATP hydrolysis to drive the efflux of drugs and the major facilitator superfamily (MFS) that utilizes the electrochemical gradient of protons across the plasma membrane to efflux substrates [10]. Over-expression of the efflux pump genes MDR1, FLU1, CDR1 and CDR2 [11] and genetic alterations in the ERG11 gene encoding lanosterol 14a-demethylase [12] are the main mechanisms of azole resistance in *Candida*. Plant essential oils and their constituents are an important source compounds that are scaffolds for innumerable drugs. Several of these plant products have significant antimicrobial properties [2,13,14]. Thymol and carvacrol are two potent antifungal agents found abundantly in essential oils of plants like thyme and organo [15]. They have excellent antifungal properties with low MIC values and negligible cytotoxicity. Hoechst 33342 and R6G are two fluorescent dyes that accumulate into cells and are then pumped out by the ABC superfamily as they are substrates for these efflux pumps [16]. Our results demonstrated that the mean intracellular concentration of these dyes in various *Candida* spp exposed to thymol and carvacrol were significantly higher than in the untreated controls [17]. These two natural compounds inhibit drug efflux pumps in *Candida*, decreasing efflux activity and hence increasing intracellular dye accumulation. Gene expression is altered to a large extent when fungal cells are grown in the presence of antifungal compounds. Azole treated *Candida* cells cause up-regulation of several drug resistance genes [18]. When fluconazole resistant *Candida* cells were exposed to thymol and carvacrol at their respective MIC values for 2 h, significant changes in gene expression was observed in *C. albicans*, *C. glabrata* and *C. krusei*. The expression of CDR and MDR genes which encode Cdr1p and Mdr1p efflux pumps decreased and hence contributed to fluconazole resistance in pathogenic fungi. Interestingly, it was observed that the resistant strains of *C. albicans* overexpress only CDR1, the multidrug resistance gene and not the major facilitator gene MDR1, whereas the strains of *C. glabrata* and *C. krusei* over express both CDR1 and MDR1 [19]. These results were in concurrence with the observed changes in the accumulation and efflux of R6G and Hoescht 33342 dyes by *Candida* cells treated with thymol and carvacrol. Although some

results show upregulation of these genes in the presence of thymol [20-22], we can say that these natural antifungals may not specifically impair expression of the transporters but their fungicidal activity may include inhibition of the plasma membrane proton efflux pump and disruption of membrane integrity [13,15]. Inhibition of drug efflux pumps using natural plant products seems to be excellent strategy for overcoming or rather reversing drug resistance in pathogenic fungal species. Natural compounds like thymol and carvacrol can be administered along with conventional antifungal drugs to improve the efficacy and reduce the dose administered as they have a synergistic effect [13,23-25]. Fluconazole is the most prescribed azole for fungal infections specially Candidiasis [26,27]. When given along with natural antifungals it will overcome its incompetence by reversing resistance in fungal pathogens. Hence we can say that thymol and carvacrol augments the efficacy of fluconazole by chemo-sensitizing fungal cells and decreasing the activity of Cdr1 and Mdr1 efflux pumps. These and other natural products may find use in antifungal chemotherapy alone or in combination with conventional drugs.

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