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**Title:**Phytochemical and anticandidal Investigations of *Curcuma longa* rhizome extract

## Abstract

**Introduction:** The potential for developing antifungals from plants appears rewarding, as it will lead to the development of less toxic phytomedicines. Turmeric (*Curcuma longa* L.) is a medicinal plant having underground rhizomes. The rhizomes from this plant are reported to have significant anti-inflammatory, antibacterial, antiparasitic, antithelmintic, and anticancer activities.Turmeric is a rich source of bioactive secondary metabolites of wide variety that are reported to have significant *in vitro* antifungal properties.

*Candida* is the most common human fungal pathogen and the fourth leading cause of nosocomial bloodstream infections.*Candida albicans* is the predominant causative organism of virtually all types of candidiasis, but other species, are now posing serious nosocomial threats. Of the two prominent classes of antifungals, polyenes cause serious host toxicity, whereas azoles are fungistatic and their prolonged use contributes to the development of drug resistance.In this study, we have performed the photochemical analysis and investigated the anticandidal potential of methanolic rhizome extract of *C. longa* (MRECL), ethanolic rhizome extract of *C. longa* (ERECL), and petroleum ether rhizome extract of *C. longa* (PRECL). Finally, the toxicity studies have been performed against human red blood cells.

**Keywords*:*** *Candida*, *C. longa*, Rhizome, Phytochemicals, Hydrolytic enzymes, Microscopy.

**Methods:**Extraction and phytochemical tests were carried out using the standardarized methods. The presence of different chemical compounds in the extracts was done by performing GC-MS analysis by using Shimadzu 2010 gas chromatograph. To understand the effect of test extracts on growth and pathogenicity of *Candida* we have investigated with the following objectives: a). Determination of minimum inhibitory concentration (MIC) and study of growth at different concentrations. b). Effect of test extracts in solid media (disc diffusion assay & spot assay). c). Proteinase and Phospholipase secretion. d). Microscopic analysis (Confocal imaging, SEM & TEM). Finally, the toxicity studies have been performed against human red blood cells.

**Results and discussion:** Preliminary phytochemical analysis of MRECL showed the presence of alkaloids, steroids/ terpenoids, glycosides, tannins, and saponins. ERECL showed the presence of alkaloids, tannins, diterpenes, amino acids and cardiac glycosides. Finally, PRECLshowed the presence of flavonoids, glycosides, alkaloids and tannins. GC-MS

analysis of MRECL, ERECL, and PRECLshowed the presence of 39, 34, and 29 different compounds.

In antifungal screening the MIC of MRECL against different *Candida* isolates ranged 300-1200 µg/ml, ERECL ranged 600-2400 µg/ml and that of PRECL ranged 1200-4800 µg/ml. In disc diffusion assay results obtained demonstrated that the ability to kill *Candida* species is concentration dependent. PRECL showed the mean sensitivity index of 0.52 ± 0.06 mm/mg, ERECL 0.68 ± 0.11 mm/mg and MRECL showed 0.81 ± 0.11 mm/mg of clearance.In growth curve studies, results obtained on growth pattern in presence of these extracts show extension of lag phase as compared to control. After 24 h growth, at MIC/2 concentration, PRECL, ERECL and MRECL showed the average reduction in optical density by 36.2%, 53.2% and 64.5%. In spot assay, at MIC/4, MIC/2 and MIC of test extracts, colony growth was visibly reduced at increased dilutions. Results of spot assay demonstrate effectiveness of tested extracts on solid media. All three extracts are found to be effective against both fluconazole sensitive and more importantly against fluconazole resistant isolates. Inhibition of proteinase secretion by MIC/4 and MIC/2 of PRECL was 14.6% and 31.3%. Similar values for ERECL and MRECL were 27.6%, 42.6% and 38.6%, 56.8% respectively. Inhibition of phospholipase secretion by MIC/4 and MIC/2 of PRECL, ERECL and MRECL was 18.3%, 32.3%; 32.2%, 42.8% and 38.6%, 48.7% respectively.In confocal microscopy, permeation to PI, following incubation with MIC of test extractscaused disruption in cell membrane that results in imbibtion of dye. SEM analysis of treated cells shows that the extracts caused an irreparable damage to the cell wall that is sharply defined by prominent rupturing of cells. TEM micrograph of treated cells exhibited notable alterations or damage in the cell membrane and the cell wall. If mechanism of action of three extracts is taken as same, it would indicate that killing originates from loss of membrane integrity.PRECL, ERECL and MRECL at their highest MIC (against any strain) of 4800 µg/ml, 2400 µg/ml and 1200 µg/ml caused 7%, 6% and 9% hemolysis of fresh human RBC as compared to 30.0% haemolysis caused by fluconazole (32 µg/ml).

**Conclusion:**This work reveals that naturally occurring rhizome extracts from *C. longa* used in this study are found to be effective anticandidal agents. MRECL is found to be most active extract. Immediate part of this antifungal activity may be originating from damage of membrane and cell wall as visible in SEM and TEM results. The extracts are also found to substantially inhibit hydrolytic enzyme secretion even at sub-MIC concentrations.These results taken together with limited toxicity of test extractstowards human red blood cells make them eligible for further development as antifungals.