**Objectives**:

*Nakaseomyces glabratus* is considered a high priority of attention according to WHO, and also is an important yeast species due to its high rate of intrinsic/acquired resistance against fluconazole. This study aimed at the possible mechanisms of action of thymol, as the promising new antifungal agent, in *N. glabratus*.

**Materials & Methods:**

Thirty previously identified *N. glabratus* isolates were selected for investigation of the thymol susceptibility pattern. The antifungal susceptibility test was performed according to the Clinical and Laboratory Standards protocol published as M27-A2 document. Likely changes in the expression pattern of genes involved in the ergosterol biosynthesis pathway were assessed by Real-time PCR assay. The ultrastructure characteristics of thymol-treated yeasts and also the possible interactive proteins, as targets for thymol binding, were performed by transmission electron microscopy (TEM) and reverse molecular docking, respectively.

**Results**:

Minimum inhibitory concentrations ranged between 32-128 µg/mL which were statistically significant between the fluconazole-susceptible and fluconazole-resistant yeast group (P<0.05). TEM observation results showed that thymol led to peripheral vacuole formation which refers to plasma membrane damage and cell membrane separation from the cell wall. Thymol exhibits antifungal activity against *N. glabratus* by regulating multiple signaling pathways including ergosterol biosynthesis (*ERG1*) and *HOG* (high-osmolarity glycerol) MAPK (mitogen-activated protein kinase) pathways. In consistence with the yielded gene expression patterns, docking evaluation findings also revealed the high affinity of thymol with proteins related to the *ERG1* gene. Accordingly, thymol's high affinity to chitin synthase and calcineurin subunit B was noteworthy.

**Conclusions**:

: Thymol might employ its antifungal effect by involving different pathways comprising ergosterol biosynthesis inhibition but not identical to the azole drugs. It is highly suggested that thymol ruins cell membrane function by decreasing the ergosterol/or chitin content. However, studying more ergosterol biosynthesis-related genes and also the yeast apoptotic responses is highly recommended for future investigations.