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## CLOVE EXTRACT IMMORTALIZES YEAST IN EXTREME CONDITIONS FOR LIFE

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It is generally assumed that all living beings die under specific and lethal stress conditions. However, here we show that an aqueous extract of Syzygium aromaticum (clove), immortalizes yeast, the eukaryotic organism Saccharomyces cerevisiae, in fatal and combined oxidative and thermal stress conditions.

In the last 5 years, we have developed a new and rapid technique for the identification of lifespan-extending biomolecules in baker's yeast. In the investigation of the extracts effects of more than 7000 species of plants, we have found that the S. aromaticum extract has an extraordinary protective effect, preventing the death of the yeast organisms induced by extreme stress conditions. From an initial yeast suspension containing 1 g of yeast extract per 100 ml of distilled water, two suspensions are prepared in two different tubes: the Experimental suspension (ES), containing 100  $\mu$ L of yeast suspension, 100  $\mu$ L of the aqueous extract of S. aromaticum, and 100  $\mu$ L of aqueous blue methylene (1%); and the Control suspension (CS), containing 100  $\mu$ L of yeast suspension, 100  $\mu$ L of distilled water, and 100  $\mu$ L of aqueous blue methylene (1%). After 1 minute of incubation, the two suspensions are microwaved at 900 W for 30 seconds. Live (colorless) and dead (dark blue) yeast organisms are visualized using a microscope. As expected, all yeast organisms in the CS exposed to the combined stresses are dead. However, surprisingly all yeast organisms in the ES survive (Fig. 1). Yeast viability was also accessed by Colony Forming Units (CFUs), confirming the results.

We also have found that, using the same procedure, resveratrol reduces yeast mortality in 10%, and some medicinal plants, such as Camellia sinensis (green tea) and Ginkgo biloba (ginkgo tree), have a positive effect between 5 and 15%. Notably, our study showed that by using the clove extract, yeast can reach a life span seven times of those grown under standard conditions.

Real-Time Relative RT-PCR confirmed a significative overexpression of various stress-related yeast genes with the presence of clove extract: RIM15 (21.3-fold); SOD2 (20.5-fold); SIR2 (17.7-fold); MSN2 (13.3-fold); HST2 (12-fold); MSN4 (12.7-fold); GIS1 (8-fold); PNC1 (7.7-fold); HSP90 (7.1-fold); SOD1 (7-fold); HSP70 (3.3-fold) and HSP12 (3-fold).

In conclusion, our findings suggest that the extract of S. aromaticum have powerful biomolecules that can reduce molecular instability and, therefore, could have an important role in the prevention and therapy of ageing-associated diseases.