

Article

## Study of the effect of Synephrine against some contaminating fungi

Esraa Muffic Obead<sup>1</sup>, and Rusul Mohamed Jasim<sup>2</sup>

<sup>1</sup> Faculty of Science, University of Baghdad, Iraq

<sup>2</sup> Faculty of Science, University of Baghdad, Iraq

\* Correspondence: za77ra5@gmail.com

Available from: <http://dx.doi.org/10.21931/RB/CSS/2023.08.04.50>

### ABSTRACT

This study aims to identify fungi (*Aspergillus flavus*, *Arthroderma insingulare*, *Alternaria alternata*, *Penicillium chrysogenum*, *Penicillium expansum*, *Candida krusei*, *Candida famata*). Those were identified according to morphological and microscopic examination. The yeast is identified by Vitek. Synephrine can be used as an antifungal. It was extracted from the leaves of *Citrus aurantium*. The diameter of *Aspergillus flavus* (6.767), *Arthroderma insingular* (6.467), *Alternaria aitenata* (6.733), *Penicillium expansum* (6.700), *Penicillium chryso-genum* (6.900), *Candida famata* (1.133), *Candida krusei* (1.233).

**Keywords:** Synephrine, contaminated fungi, Exposure

---

### INTRODUCTION

Exposure effects to fungi are dependent on the species present, the metabolic products, concentration and duration of exposure, and also individual susceptibility<sup>1</sup>. P-synephrine is the primary protoalkaloid in *Citrus aurantium* (bitter orange) and other *Citrus* species<sup>2</sup>. Chemically and structurally, p-synephrine, p-octopamine (nor-synephrine) and m-synephrine (phenylephrine) are similar to ephedrine<sup>3</sup>. leaves, the synephrine content varies between 0.006% and 0.087%<sup>4</sup>. The safety of p-synephrine and bitter orange extract has recently been reviewed, and based on the available studies in animals, humans and cell culture systems, it has been concluded that when taken orally and in recommended amounts, both are safe<sup>5</sup>.

### MATERIALS AND METHODS

#### *Collection of plant samples*

This study included the use of leaves of *Citrus aurantium* collected from a local herbarium market in Baghdad city, Iraq. The plant was identified in herbarium, Department of Biology/College of Science/University of Baghdad. The plant leaves were dried and ground into powder from a mechanical grinder. It was kept at 4 C until further investigation.

### *Isolation and purification synephrine*

The extract was adjusted to pH 8.5 with ammonium hydroxide and passed through a Dowex 50-X4 ion exchange column. The column was first treated with 1 N sodium hydroxide, followed by 4% hydrochloric acid, again with sodium hydroxide, and finally with water until the pH of the effluent was between 8 and 9. After the passage of the extract, the column was washed with water and then with methanol. The I-synephrine was then eluted with 2 N ammonium hydroxide in methanol. The eluate was dried in a rotary vacuum evaporator at 50°. Z-Synephrine crystals formed during the final stages of evaporation. These were dissolved in a small volume of hot methanol and recrystallized at 4°. This material could also be crystallized from water or 80% acetone and water<sup>6</sup>.

### *Microscopic and macroscopic examination:*

In this study, fungi were diagnosed according to <sup>7</sup>. This identification depends on the colony characteristic and Microscopic morphology (microconidia and macroconidia: their size, shape, arrangement, and hyphal structures). The yeast identification was done by VITEK2.

### *Estimation of antifungal activity for aqueous extract of C.aurantum against fungi:*

Mold.

We mixed the aqueous extract with the media at a ratio (1:10) (Extract: media) and used the D.W. as a control. We took a pure colony by needle and cultured it in a middle petri dish. We incubated at 37°C for 7 days. Studied the effect of aqueous extract on mold by measuring the diameter of the growth zone.

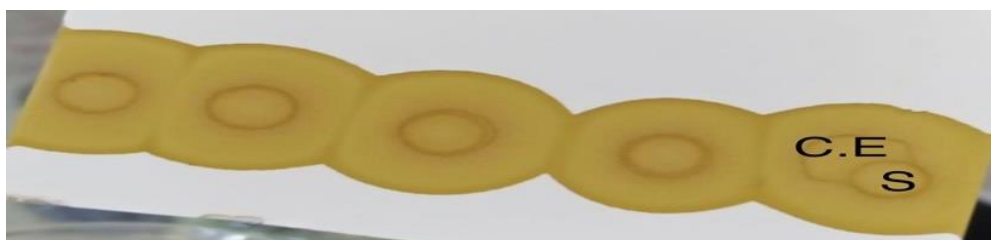
YEAST

Made pore in media and put (100) µl from the aqueous extract in every pore and used D.W. as control.

## **RESULTS**

### *Detection of Synephrine Thin layer chromatography (TLC)*

Drops of solvent were applied to the plate containing the sample and sorbent layer. Thus, the procedure was termed drop chromatography. <sup>8</sup> TLC of the Citrus aurantium extract obtained from dried leaves confirms the presence of Synephrine in all extraction portions compared with standards. As in Figure ( 1 ).



**Figure 1. TLC of leaves extract of *Citrus aurantium* obtained by extraction method using silica gel GF254 as adsorbent and (S1) as a mobile phase. ( C.E: *Citrus aurantium* extract, S: Synephrine standard)**

### *Fungi caused contamination*

The results showed the emergence of five types of molds and two types of *Candida* yeast, as shown in Figure (2)

1. *Aspergillus Flavus*

2. *Arthroderma*

Morphological Description: colonies are usually flat to downy with a suede-like to granular texture resembling *T. mentagrophytes*.

3. *Alternaria Alternata*

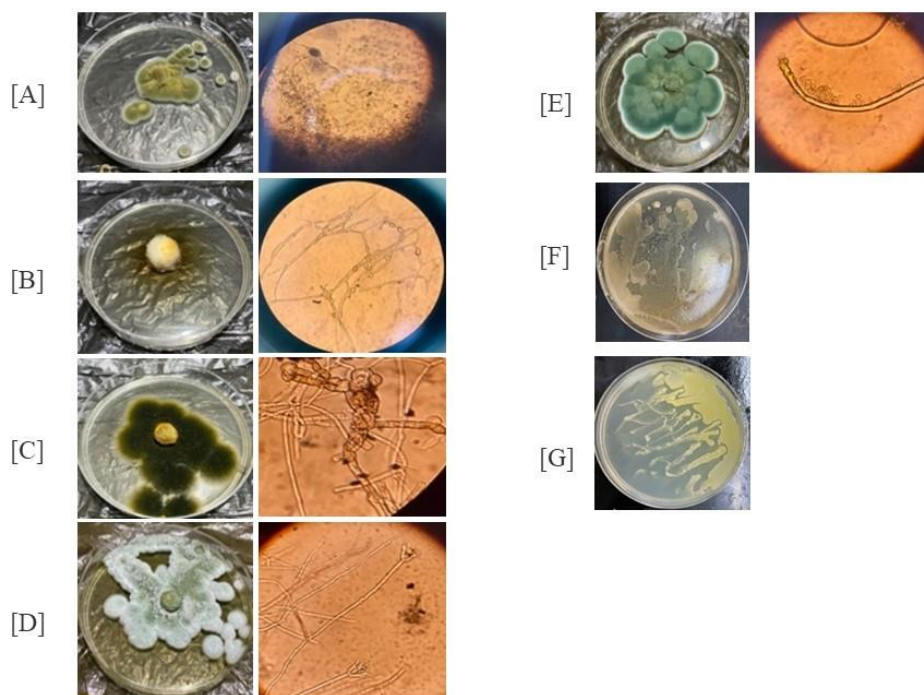
Morphological studies have shown that typical colonies of *A. alternata* are lettuce-green to olive green in color and usually have a prominent (2–5mm) white margin when growing on potato dextrose agar<sup>11</sup>.

4. *Penicillium*

*Penicillium* is one of the largest and most important genera of microscopic fungi, with over 400 described species distributed worldwide. Its name comes from the Latin "penicillus," which refers to the brush-like appearance of the conidiophores that resemble a painter's brush.<sup>12</sup>

5. *Candida*

Pathogenic fungi in the genus *Candida* can cause both superficial and serious systemic disease and are now recognized as major agents of hospital-acquired infection<sup>13</sup>



**Figure 2. Contaminating fungi. A. *Aspergillus flavus*, B. *Arthroderma insingular*, C. *Alternaria aalternata*, D. *Penicillium chrysogenum*, E. *Penicillium expansum*, F. *Candida krusei*, G. *Candida famata***

### *Evaluated of inhibitory activities of Synephrine(In vitro)*

The effect of aqueous extract of Synephrine on isolated fungi was studied. It was given the highest inhibitory rate comparable to control when used D.W. by the small diameter of the colony resulting<sup>14</sup>.

<b>Fungi</b>	<b>Control</b>	<b>Synephrine</b>
<b>Mold</b>		
<i>Aspergillus flavus</i>	9.767	6.767
<i>Arthroderma insingulare</i>	9.767	6.467
<i>Alternaria alternata</i>	9.767	6.733
<i>Penicillium chrysogenum</i>	9.767	6.900
<i>Penicillium expansum</i>	9.767	6.700
<b>Yeast</b>		
<i>Candida krusei</i>	0.800	1.233
<i>Candida famata</i>	0.767	1.133

**Table 1. Evaluated the inhibitory activities of Synephrine**

## DISCUSSION

*Aspergillus flavus* is a ubiquitous and cosmopolitan filamentous fungus known to proliferate in various environmental conditions<sup>9</sup>.

The surface color may range from white to cream, buff to yellow, or greenish-yellow<sup>10</sup>.

The results are consistent with Tripathy, who proved in 2017 that the extract of the *Citrus aurantium* gives inhibition results on microorganisms<sup>15</sup>.

## CONCLUSIONS

The yeast is identified by Vitek. Synephrine can be used as an antifungal.

## References

- 1 Viegas, C., Veríssimo, C., Rosado, L., & Santos, C. S. (2010). Air fungal contamination in two elementary schools in Lisbon, Portugal. *WIT Transactions on Ecology and the Environment*, 136, 305-312.

- 2 Stohs, S. J., & Ratames, N. A. (2017). Effects of p-synephrine in combination with caffeine: a review. *Nutrition and Dietary Supplements*, 9, 87-96.
- 3 Stohs, S. J., Shara, M., & Ray, S. D. (2020). p-Synephrine, ephedrine, p-octopamine and m-synephrine: Comparative mechanistic, physiological and pharmacological properties. *Phytotherapy Research*, 34(8), 1838-1846.
- 4 Rossato, L. G., Costa, V. M., Limberger, R. P., de Lourdes Bastos, M., & Remião, F. (2011). Synephrine: from trace concentrations to massive consumption in weight-loss. *Food and chemical toxicology*, 49(1), 8-16.
- 5 Stohs, S. J., Preuss, H. G., Keith, S. C., Keith, P. L., Miller, H., & Kaats, G. R. (2011). Effects of p-synephrine alone and in combination with selected bioflavonoids on resting metabolism, blood pressure, heart rate and self-reported mood changes. *International journal of medical sciences*, 8(4), 295.
- 6 Stewart, I., Newhall, W. F., & Edwards, G. J. (1964). The isolation and identification of l-synephrine in the leaves and fruit of citrus. *Journal of Biological Chemistry*, 239(3), 930-932.
- 7 Tille, P. M., & Forbes, B. A. (2014). *Bailey & Scott's diagnostic microbiology*. St. Louis, Missouri.
- 8 Sherman, J., & Fried, B. (Eds.). (2003). *Handbook of thin-layer chromatography*. CRC press.
- 9 Thathana, M. G., Murage, H., Abia, A. L. K., & Pillay, M. (2017). Morphological characterization and determination of aflatoxin-production potentials of *Aspergillus flavus* isolated from maize and soil in Kenya. *Agriculture*, 7(10), 80.
- 10 Kidd, S., Halliday, C. L., Alexiou, H., & Ellis, D. (2016). *Descriptions of medical fungi* (Vol. 3). David Ellis.
- 11 Troncoso-Rojas, R., & Tiznado-Hernández, M. E. (2014). *Alternaria alternata* (black rot, black spot). In *Postharvest decay* (pp. 147-187). Academic Press.
- 12 Yin, G., Zhang, Y., Pennerman, K. K., Wu, G., Hua, S. S. T., Yu, J., ... & Bennett, J. W. (2017). Characterization of blue mold *Penicillium* species isolated from stored fruits using multiple highly conserved loci. *Journal of Fungi*, 3(1), 12.
- 13 Douglas, L. J. (2003). *Candida* biofilms and their role in infection. *Trends in microbiology*, 11(1), 30-36.
- 14 Zohra, H. F., Rachida, A., Malika, M., Benali, S., Samir, A. A., & Meriem, B. (2015). Chemical composition and antifungal activity of essential oils of Algerian citrus. *African Journal of Biotechnology*, 14(12), 1048-1055.
- 15 Tripathy, P. P., & George, O. D. (2017). Evaluation of antimicrobial activity of ZITRITIDE, a natural and organic antimicrobial fogging solution with special reference for infection prevention and control in hospital environments and all other clean room facilities. *Int J Curr Microbiol App Sci*, 6, 1822-37.

Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023

Citation: Obead, E.M.; Jasim, R.M. Study of the effect of Synephrine against some contaminating fungi. *Revista Bionatura* 2023;8 (2) 63. <http://dx.doi.org/10.21931/RB/CSS/2023.08.04.50>