Journal of Applied and Natural Science
15(2), 741 - 747 (2023)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Antifungal synergic activity of essential olive oil and alcoholic turmeric extracts against isolates from the dried grapes raisins

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Article Info
https://doi.org/10.31018/jans.v15i2.4516
Received: March 7, 2023
Revised: May 28, 2023
Accepted: June 3, 2023

How to Cite
Mula Abed, F. N. A. *et al.* (2023). Antifungal synergic activity of essential olive oil and alcoholic turmeric extracts against isolates from the dried grapes raisins. *Journal of Applied and Natural Science*, 15(2), 741 - 747. https://doi.org/10.31018/jans.v15i2.4516

Abstract
Fungi are responsible for a wide variety of harm to humans, including food spoilage and infections. Using chemicals to restrict fungal development or infections has negative repercussions, such as human health dangers from the chemical applications and rising antifungal-drug resistance, so this study aimed to use medicinal plants and their extracts as an alternative method to restrict fungal growth. Ten isolates of the genus Aspergillus were identified from the fruits of dried grapes (raisins) of all kinds (Iraqi black raisins, Iranian yellow raisins, and brown raisins) at the species level using three – differential media: Czapek Yeast Extract Agar(CYA), Malt Extract Agar(MEA), and 25% Glycerol nitrate agar (G25N) incubated in 5, 25 and 37 ºC. *Aspergillus niger* was the most common isolated species. The number of A. niger isolates reached seven from all types of dried grapes, while A. flavus recorded three isolates from black raisins and brown raisins. Aspergillus Flavus Parasiticus Agar (AFPA) was used to detect the ability of A. flavus isolates to produce aflatoxin at 25-30 ºC for one week. Alcoholic extract of turmeric showed a significant inhibitory effect on the colony diameter of both *A. flavus* and *A. niger* isolated from the fruit of Iraqi black raisins with an inhibition rate of 86.6% and 68.8 %, respectively, at 4 mg/ ml concentration. The mixture of turmeric and essential olive oil gave a distinct inhibitory effect, reaching a 100% inhibition rate from the lowest to highest concentration for both *A. niger* and *A. flavus* .

Keywords: *Aspergillus,* Essential olive oil, Raisins, Turmeric

INTRODUCTION

Dried grapes (dry and oil raisins) are a healthy food, as well as one of the important ingredients in biscuits, cake, and other food. Glucose and fructose in grapes can be concentrated by drying them with sun heat (Keskin, *et al*., 2022 and Gul *et al*., 2016).

United States and Turkey are the two main producers of raisins today. Other countries that produce raisins include Australia, South Africa, Chile, and a few in the eastern Mediterranean basin and the Middle East. The raisins are picked from kinds of grapes with a high sugar concentration, with seeds and coherent pith, or from the Sultana grapes which do not have seeds. All kinds of white grapes are the best grape used in raisins production because of their thin peel and delicious flavor (Soylemezoglu *et al.* , 2016)

Presently the use of herbals in medicine has taken a big place because herbals are the unique resource for the drug before the output of the chemicals has become known to everyone. From the 1930s till the 1960s, the use of medical plants dropped while the chemicals beat the medical plants, but under the logo of Return to Nature, the use of medical plants increased again (Taylor, 2015).

A very small percentage of known medical plants are used in medical treatment (Ekwenye and Elegalam, 2005). Currently, medicinal plants are widely used as drugs because of their availability in native communities, natural origin, less troublesome, cheap, and easier

in administer, as well as herbal medicine, perhaps useful as an alternative treatment in case of many drug resistance and side effects (Abubakar and Haque, 2020).

A medicinal plant contains therapeutic purposes substances in one or more of its organs and which are distinguished from rest plants (Sofowora *et al.,* 2013).Olives and their derived products, including extra virgin olive oil, can resist pathogen attack, by affecting the interaction between the host and the pathogen, due to polyphenols' presence, which can also offer antimicrobial activity (Nazzaro *et al*., 2019).

Turmeric is an annual plant regarding leaves, and herbaceous roots (Castellote, 2013), with large, yellow color tubers growing near the earth's surface and available in the markets as tubers or yellow powder (Dovigo, 2013). Turmeric is used to treat many diseases, such as cough, diabetic wounds, coryza, rheumatism, ulcer, hepatic disorders, anorexia and sinusitis (Asnaashari *et al.*, 2018).

The medicinal characteristics of turmeric belong to curcumin or diferuloylmethane (1 , I – bis (4 – hydroxyl – 3 –methoxyphenyl) – 1 , 6 – heptadiene – 3 , 5 – dione). It is a yellow polyphenolic pigment that occurs in the rhizome of turmeric (Sharifi-Rad ,2020). Turmeric (*Curcuma longa*) is a spice and herbal medicine whose active ingredient is curcumin, an active component with antifungal properties (Neda and Shiva, 2016). The present work set out to investigate the synergistic antifungal efficacy of ethanolic turmeric extract and essential olive oil against raisin-specific fungi isolated from dried grapes raisins marketed in Iraq

MATERIALS AND METHODS

Sample collection

The medical plants' samples (soft powder of turmeric *C. longa*, and Spanish essential olive oil) were obtained from the local markets in Mosul/ Iraq and packed the soft turmeric powder in a plastic bag until use.

Resources of fungal isolates

Ten isolates of *Aspergillus* spp. were isolated from dried grapes fruits (Iraqi black raisins, Iranian yellow raisins, and brown raisins) which were identified according to keys from Pitt and Hocking (2009).

Identification of *Aspergillus* spp. isolates

Czapek Yeast Extract Agar (CYA), Malt Extract Agar (MEA) and 25 % Glycerol Nitrate Agar (G25%N) plates were inoculated with *Aspergillus* isolates to identify them to the species level. They were incubated at 25 and 37°C for 7 days. All plates were viewed for macroscopic characteristics using the identification keys from Pitt and Hocking (2009).

Preparation of spore suspension

Spores suspension was prepared by adding 10 ml of sterile distilled water to vials in size of 20 ml containing purified fungal colonies grown on PDA. The fungal colonies were scarped with a hooked glass rod and passed in a sterile piece of gauze. The concentration of the Spores suspension was controlled to $13*10^6$ spore/ml using the hemocytometer (William *et al*., 1976 a, b).

Detection of toxigenic *Aspergillus* spp. by using *Aspergillus flavus* and *parasiticus* agar media

Aspergillus flavus and parasiticus agar media (AFPA) was used, a separate media, to detect the toxigenic strains that produced aflatoxin and related to *A. parasiticus and A. flavus*. The toxigenic strains gave yellow-orange color and brilliant on the lower side of AFPA after 48 hours from the occupation at 30 ºC (Agarwal and Sicelair, 1997).

Preparation of alcoholic crude extracts:

Alcoholic crude extract was prepared according to Grand and others (1988), which was modified from the basic method (Verpoorte and others (1982). 20 gm of the plant was crushed in 200 ml of 95 % ethanol inside the ice bath. After good shaking, it was left in the refrigerator for 24 h, then filtered through many layers of gauze and Buchner funnel, rotary vacuum evaporator (Electrothermal) was used to evaporate extract solvent from filtrate at a temperature less than 40 ^{0}C, and then dried using Lyophilizer (Germany Edwards). 1g from the crude extract was dissolved in 5 ml of dimethyl sufoxide (DMSO), and then was purified by pasteurization at 64 ºC for ten minutes (Al- Nuaman, 1998). The 1, 2, 3, and 4 mg/ml concentrations were prepared and incorporated in the Sabouraud agar (SGA) culture medium to get minimum inhibitory concentration (MIC). The control plate consisted of the media (SGA) without any addition (Rios *et al.*,1987).

Screening of the percentage inhibition of diameter growth

The percentage inhibition of diameter growth (PIDG) was calculated according to the equation below:

PIDG (%) = (Diameter of control– Diameter of sample)/ Diameter of control X100% Eq.1

Preparation of the mixture of turmeric and essential olive oil

To prepare a mixture of turmeric and essential olive oil at a concentration of 40mg/ml, 10g of turmeric powder was mixed with 25 ml of essential olive oil. This standard concentration was used to prepare the other concentrations (4,8,12,16) mg/ml used in this study (AL-Refai, 2006), according to the equation as below:

N1V1= N2V2 Eq. 2

RESULTS AND DISCUSSION

Fungal species isolated from dried grapes (Raisins)

*Ten isolates of the genus Aspergillus from t*hree kinds of dried grapes *i.e., raisins (Iraqi black raisins, Iranian yellow raisins and brown raisins) were identified at the species level* according to morphological features of colonies on Czpek yeast agar (CYA) , Malt extract agar (MEA), and Glycerol Nitrate Agar (G25%N) at three different temperatures of 5, 25 and 37 ^{0}C as described by Pitt and Hockig (2009), are mentioned in Table 1 and Fig. 1.

The data in Table 1 shows that *A. niger* was the most common species isolated from kinds of raisins, where include four isolates from Iraqi black raisins and two isolates from oil brown raisins and one isolate from Iranian yellow raisins, same results have been reported by Chebil *et al*. (2020) and Ramadan *et al.* (2022) who referred that the percentage of *Aspergillus niger* aggregate isolates detected in dried grapes samples from Kelibia, Sfax, and Rafraf areas of Tunisia markets ranged from 70% to 85%.

Table 1. Fungal species isolated from *raisins*

Kinds of *Aspergillus*	Isolation Resource	Isolate No.
Aspergillus niger	Black raisins(Iraqi)	(1),(2),(3),(4)
A. flavus		(5),(6)
A.niger	(Oil) Brown Raisins	(8),(7)
A. flavus		(9)
A.niger	Yellow Raisins (Iranian)	(10)

Detection of the toxigenic *A. flavus* strains using *A. flavus* and *parasiticus* agar media

The *A. flavus* strains that showed brilliant orange-yellow on the lower side of the *A. flavus* and *parasiticus* agar (AFPA) plates were considered toxigenic strains. A total of 3 isolates of *A. flavus* were isolated from Iraqi black raisins and oil brown raisins . The results indicated that out of the 3 strains of *A. flavus*, only 2 strains showed positive reaction to toxigenicity, which were *A. flavus* (5) and *A. flavus*(6) that isolated from black raisins (Fig. 2) and agreed with (Zhang *et al.*, 2020, Kushiro *et al.*, 2018). They referred that the reverse side of *A. flavus* colony on AFPA was bright orange. In contrast, the isolates of *A. oryzae* and *A. sojae* showed brown colors on the same medium, indicating that they were non-toxigenic.

Antifungal activity of alcoholic turmeric extract against *A. nigar and A. flavus* colonies

The antifungal response of turmeric alcoholic extract against *A. nigar* (1) *and A. flavus* (5) colonies are summarized in Tables 2, 3; Fig. 1.

There was an apparent effect on the growth rate of two fungal species isolates *A.nigar* (1) *and A. flavus* (5), detected by measuring the colonies diameter. The maximum effect of alcoholic turmeric extract was achieved at a concentration 16 mg/ml for these two fungal species. The inhibition percentage reached 86.6% and 68.8% for *A. flavus*(5) *and A. nigar*(1), respectively. The inhibitory effect of alcoholic turmeric extract increased with concentration, as shown in Tables 1, and 2. The inhibitory effect of turmeric extract is due to the active substance, such as the prominent phenolic compound, the reason for antimicrobial efficiency of turmeric was due to the presence of curcumin which was represented as an antimicrobial agent (Marchi *et al*., 2019).

The present results agreed with Chen *et al*. (2018) who reported that the *C. longa* possesses inhibition activity against fungi. They studied the antifungal behaviours of *Curcuma longa* alcoholic extract against *Fusarium tricinctum*, *F. graminearum*, *F. chlamydosporum*, *F. culmorum*, *F. oxysporum, Alternaria alternate*, *Rhizopus oryzae*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Cladosporium cladosporioides*, and *Colletotrichum higginsianum*, as well as evaluated the antifungal activity of the compounds derivative from *Curcuma longa* such as curcumin, curdione, curcumenol, β-elemene, isocurcumenol, curcumol and germacrone. They referred to the antifungal activity, including the disruption of fungal cell membranes and inhibition of the synthesis of ergosterol, succinate dehydrogenase (SDH), respiration and NADH oxidase. The results also agree with Sultan *et al*. (2018), who reported that alcoholic turmeric extract inhibited Candida albicans' growth.

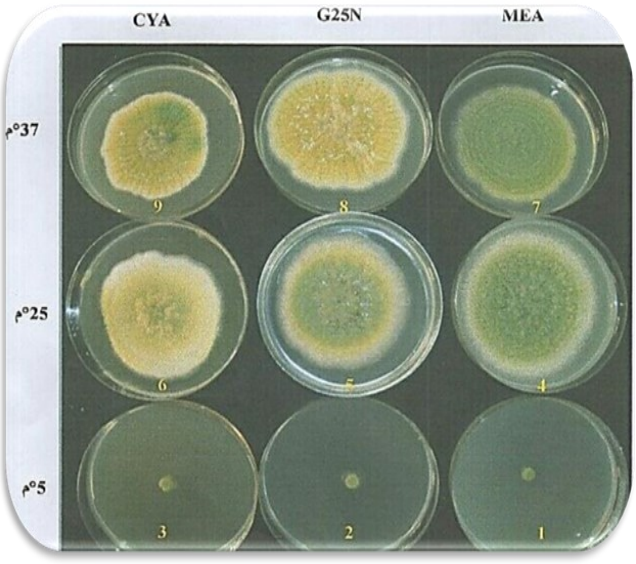

Fig. 1. *Colonies of A. flavous on three differential media* (G25N, MEA, CYA) at 5, 25, 37 ^{0}*C*

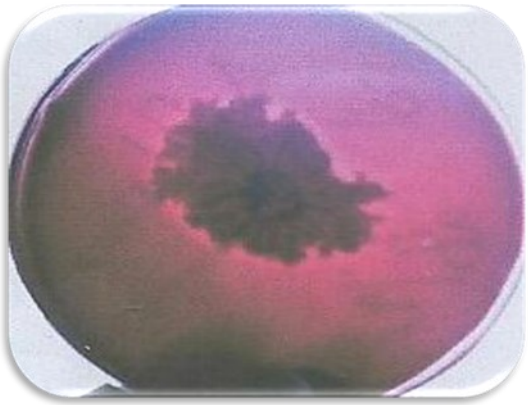

Fig. 2. *Brilliant orange yellow pigment produced by toxigenic A. flavus strains on AFPA media*

Antifungal activity of mixture solution from turmeric powder and essential Olive Oil on the growth of *A. nigar and A. flavus* colonies

As shown from the data in Table 4 and 5, the inhibitory activity of the mixture made from Turmeric powder and essential olive oil against *A. nigar(Isolate1) and A. flavus (Isolate 5)* was 100% and there was no fungal growth at all concentrations of the mixture as shown in Fig. 3 and 4.

There was an obvious difference between the antifungal activity of alcoholic turmeric extract and the mixture of turmeric powder and essential olive oil against the growth of *A. nigar (*Isolate 1*) and A. flavus* (Isolate 5*)*. The maximum effect of alcoholic turmeric extract reached 86.6% for A. *flavus* (Isolate 5) and 68.8% for *A. nigar* (Isolate 1) at a concentration of 16 mg/ml. This significant effect may be due to the direct use of turmeric powder without extraction and the synergistic activity of the essential olive oil in combination with turmeric powder against fungal growth. Plant essential oils act as antifungal substances due to their lipophilic nature, which disrupt the plasma membrane and damage the cellular component responsible for vital function in the cell has been confirmed by studies on the effect of essential oil against *Aspergillus flavus* (Chang *et al*, 2022) . The synergy between the essential oils' main components and other constituents results in their antimicrobial activity (Chouhan *et al*., 2017). The activity mechanism of antifungal compounds in the plant extract on the fungal suppression detects alterations in the hyphal morphology and become withered at tips, while the

Table 2. Effect of turmeric alcoholic extract on the growth of *A. flavus* (Isolate 5)

Isolate no. *A.flavus*	**Concentration of alcoholic extract (mg/ml)**	**Inhibition percentage %**	**Colony diameter (cm)**
Cont.	0	0	9
Aspergillus flavus (5)	16	86.6	1.2
	12	82.7	1.55
	8	81.5	1.66
	4	74	2.33

Table 3. Effect of turmeric alcoholic extract on the growth of *A. niger* (Isolate 1)

Isolate no. *A.niger*	**Concentration of alcoholic extract (mg/ml)**	**Inhibition percentage %**	**Colony diameter (cm)**
Cont.	0	0	9
Aspergillus niger (1)	16	68.8	2.8
	12	63.3	3.3
	8	57.77	3.8
	4	44.4	5

Table 4. Effect of mixture solution from Turmeric powder and essential Olive oil on the growth of *A. flavus* (Isolate 5)

Isolate no. *A.flavus*	**Concentration of mixture solution from Turmeric powder and essential Olive Oil (mg/ml)**	**Inhibition percentage %**	**Colony diameter (cm)**
Cont.	0	0	9
A. flavus (5)	16	100%	0
	12	100%	0
	8	100%	0
	4	100%	0

Table 5. Effect of mixture solution from Turmeric powder and essential olive oil on the growth of *A. niger* (Isolate 1)

Isolate No. *A.niger*	**Concentration of mixture solution from Turmeric powder and essential Olive Oil (mg/ml)**	**Inhibition percentage %**	**Colony diameter (cm)**
Concentration	0	0	9
	16	100%	0
	12	100%	0
A. niger(1)	8	100%	0
	4	100%	0

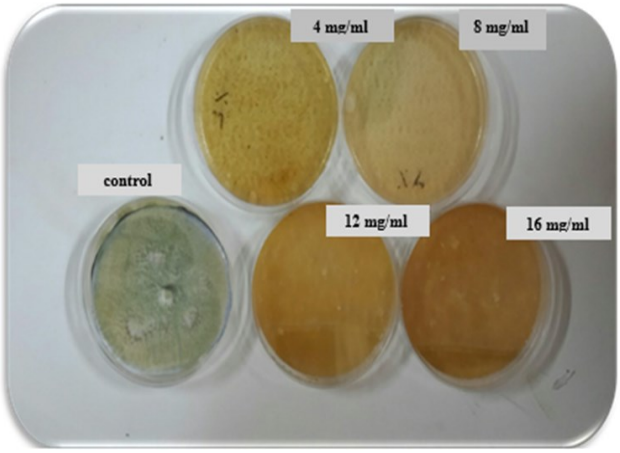

Fig. 3. *Effect of mixture solution from Turmeric powder and essential olive oil on the growth of A. flavus (5)*

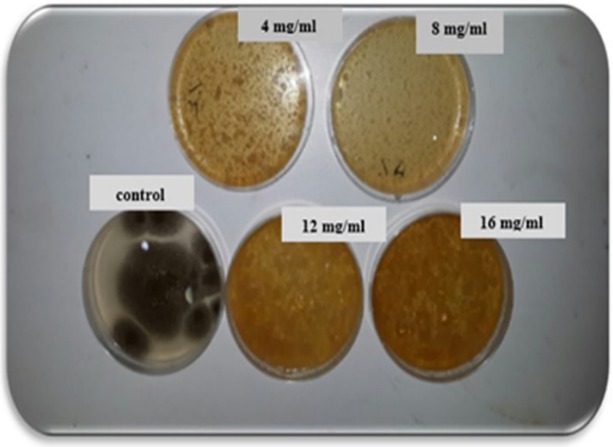

Fig. 4. *Effect of mixture solution from Turmeric powder and essential olive*

mycelia twist and fold with a jagged edge. Conidia become shrinkage, and plant extract contains secondary metabolites, which act as antifungal material to limit fungal growth (Yusoff *et al.,* 2020). The secondary metabolites restrict fungal growth by cell membrane derangement and inhibit the many actions of the cell, such as cell wall cell and protein synthesis, and cell division (Kursa *et al.*, 2022 . Al-Otibi *et al.*, 2023)

Upendra *et al.* (2011) studied the addition of turmeric powder in plant tissue cultures at 0.8 and 1.0g/L, which inhibited fungal contaminations. Moghadamtousi *et al*. (2014) referred to Upendra *et al.(*2011)'s study of the addition of turmeric powder in plant tissue cultures at 0.8 and 1.0g/L resulted in the inhibition of fungal contaminations. Murugesh *et al*. (2019) evaluated that the minimum inhibitory concentration (MIC) of ethanolic extract of turmeric against *Candida albicans* was determined to be 800 µl, whereas the minimum fungicidal concentration was found to be 1600 µl . *Chen et al. (2018)* determined inhibitory effects of the compounds derived from *Curcuma longa* such as curdione, curcumenol, germacrone, curzerene, β-elemene, isocurcumenol, curcumin, and curcumol on *F. graminearum*, fungal cell membranes were disrupted and also inhibited all ergosterol production, respiration, succinate dehydrogenase (SDH), and NADH oxidase contributing to the antifungal action.

Conclusion

The present study concluded that alcoholic turmeric extract showed a significant inhibitory effect on both fungal species *A. flavus and A. niger* with an inhibition rate of 86.,6% and 68.8 %, respectively, at 4 mg/ ml concentration. The mixture of turmeric and essential olive oil gave a distinct inhibitory rate for both fungal species *A. niger and A. flavus,* reaching a 100% for all concentrations. In combination with essential olive oil, turmeric powder led to an improved inhibition rate.

ACKNOWLEDGEMENTS

The authors are very grateful to the University of Mosul / College of Science and Northern Technical University, Mosul Technical Institute, for their facilities, which helped to improve the quality of this work.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Al-Refai, F. N. (2006). Isolation and identification of fungi from cosmetic using some plant extracts as preservative agents (Doctoral dissertation, Ph. D. Thesis. College of

Science. Mosul Univ. Iraq).
2. Abubakar , A.R. & Haque , M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes . *J Pharm Bioallied Sci.*, 1– 10 . doi: 10.4103/jpbs.JPBS_175_19.
3. Agarwal, V.K. & Sinclair, J.B. 1997. Principles of Seed Pathology, 2. ed. Boca Raton: CRC, 538p .doi https://doi.org/10.1201/9781482275650
4. Al-Otibi, F. ; Moria, G. A., Alharbi, R.I., Yassin, M.Y. & Al -Askar , A.A. (2023) . The Antifungal Properties of Tamarix aphylla Extract against Some Plant Pathogenic Fungi . *Microorganisms* 11, 127. https://doi.org/10.3390/microorganisms11010127 .
5. Al- Nauman, A.Y. & Sharif H. 1998, Molecular effect for some plant extracts on growth and Metabolism of some positive and negative germs for Gram pigment, Ph.D. Dissertation, college of science, Mosul University, Iraq.
6. Asnaashari, S., Dastmalchi, S., & Javadzadeh, Y. (2018). Gastroprotective effects of herbal medicines (roots). *International Journal of Food Properties*, *21*(1), 902-920. DOI: 10.1080/10942912.2018.1473876.
7. Coronado-Castellote, L., & Jiménez-Soriano, Y. (2013). Clinical and microbiological diagnosis of oral candidiasis. *Journal of clinical and experimental dentistry*, *5*(5), e279. doi: 10.4317/jced.51242
8. Chang, Y., Harmon, P. F., Treadwell, D. D., Carrillo, D., Sarkhosh, A., & Brecht, J. K. (2022). Biocontrol potential of essential oils in organic horticulture systems: From farm to fork. *Frontiers in Nutrition*, *8*, 1275. doi: 10.3389/fnut.2021.805138.
9. Chebil, S., Rjiba-Bahri, W., Oueslati, S., Ben Ismail, H., Ben-Amar, A., & Natskoulis, P. (2020). Ochratoxigenic fungi and Ochratoxin A determination in dried grapes marketed in Tunisia. *Annals of Microbiology*, *70*, 1-9. https://doi.org/10.1186/s13213-020-01584-7.
10. Chen, C., Long, L., Zhang, F., Chen, Q., Chen, C., Yu, X., ... & Long, Z. (2018). Antifungal activity, main active components and mechanism of Curcuma longa extract against Fusarium graminearum. *PloS one*, *13*(3), e0194284. doi: 10.1371/journal.pone.0194284
11. Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial activity of some essential oils—present status and future perspectives. *Medicines*, *4*(3), 58. doi:10.3390/medicines4030058.
12. Dovigo, L. N., Carmello, J. C., de Souza Costa, C. A., Vergani, C. E., Brunetti, I. L., Bagnato, V. S., & Pavarina, A. C. (2013). Curcumin-mediated photodynamic inactivation of Candida albicans in a murine model of oral candidiasis. *Sabouraudia*, *51*(3), 243-251. doi: 10.3109/13693786.2012.714081
13. Ekwenye, U. N., & Elegalam, N. N. (2005). Antibacterial activity of ginger (Zingiber officinale Roscoe) and garlic (Allium sativum L.) extracts on Escherichia coli and Salmonella typhi. *International Journal of Molecular Medicine and Advance Sciences*, *1*(4), 411-416. https://medwelljournals.com/abstract/?doi=ijmmas.2005.411.417
14. Fillinger, S., & Elad, Y. (Eds.). (2016). *Botrytis-the fungus, the pathogen and its management in agricultural systems* (pp. 189-216). Cham, Switzerland: Springer International Publishing. dio:10.1007/978-3-319-23371-0
15. Le Grand, A., Wondergem, P. A., Verpoorte, R., & Pousset, J. L. (1988). Anti-infectious phytotherapies of the tree-savannah of Senegal (West-Africa) II. Antimicrobial activity of 33 species. *Journal of ethnopharmacology*, *22*(1), 25-31. doi: 10.1016/0378-8741(88)90227-9
16. Osman, G. U. L., Mortas, M., Dervisoglu, M., Mehtap, E. R., Atmaca, M., & Atalar, İ. (2016). Furfural contents and some physical and chemical properties of raisins. *Akademik Gıda*, *14*(3), 235-241.
17. Keskin, N., Kaya, O., Ates, F., Turan, M., & Gutiérrez-Gamboa, G. (2022). Drying grapes after the application of different dipping solutions: effects on hormones, minerals, vitamins, and antioxidant enzymes in Gök Üzüm (Vitis vinifera L.) raisins. *Plants*, *11*(4), 529. https://doi.org/10.3390/plants11040529
18. Kursa, W., Jamiołkowska, A., Wyrostek, J., & Kowalski, R. (2022). Antifungal Effect of Plant Extracts on the Growth of the Cereal Pathogen Fusarium spp.—An In Vitro Study. *Agronomy*, *12*(12), 3204. https://doi.org/10.3390/agronomy12123204.
19. Kushiro, M., Hatabayashi, H., Yabe, K., & Loladze, A. (2018). Detection of aflatoxigenic and atoxigenic Mexican Aspergillus strains by the dichlorvos–ammonia (DV–AM) method. *Toxins*, *10*(7), 263. doi:10.3390/toxins10070263
20. Marchi, L., Dornellas, F., Polonio, J., Pamphile, J., Monteiro, A., Goncalves, O., & Perdoncini, M. (2019). Antifungal activity of Curcuma longa L.(Zingiberaceae) against degrading Filamentous Fungi. *Chemical Engineering Transactions*, *75*, 319-324. DOI:10.3303/CET1975054.
21. Zorofchian Moghadamtousi, S., Abdul Kadir, H., Hassandarvish, P., Tajik, H., Abubakar, S., & Zandi, K. (2014). A review on antibacterial, antiviral, and antifungal activity of curcumin. *BioMed research international*, *2014*. http://dx.doi.org/10.1155/2014/186864
22. Murugesh, J., Annigeri, R. G., Mangala, G. K., Mythily, P. H., & Chandrakala, J. (2019). Evaluation of the antifungal efficacy of different concentrations of Curcuma longa on Candida albicans: An in vitro study. *Journal of Oral and Maxillofacial Pathology: JOMFP*, *23*(2), 305. doi: 10.4103/jomfp.JOMFP_200_18
23. Nazzaro, F., Fratianni, F., Cozzolino, R., Martignetti, A., Malorni, L., De Feo, V., ... & d'Acierno, A. (2019). Antibacterial activity of three extra virgin olive oils of the Campania region, Southern Italy, related to their polyphenol content and composition. *Microorganisms*, *7*(9), 321. doi: 10.3390/microorganisms7090321
24. Neda, B., & Shiva, Z. (2016). Inhibitory Effect of Curcumin on Candida-albicans compared with Nystatin: an in-vitro Study. *Journal of Dental Materials & Techniques*, *5*(4).
25. Norajit, K., Laohakunjit, N., & Kerdchoechuen, O. (2007). Antibacterial effect of five Zingiberaceae essential oils. *Molecules*, *12*(8), 2047-2060. doi: 10.3390/12082047
26. Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage* (Vol. 519, p. 388). New York: Springer.
27. Ramadan, E. A., Ramadan, N. A., & Mohammed, A. A. H. (2022). aflatoxigenic fungi in nuts and dried fruits in mosul and duhok city. *Mil. Med. Sci. Lett.,* 91(3), 224-234. DOI: 10.31482/mmsl.2021.049.
28. Rios, J. L., Recio, M. C., & Villar, A. (1987). Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. *Journal of ethnopharmacology*, *21*(2), 139-152. doi: 10.1016/0378-8741(87)90124-3
29. Sharifi-Rad, J., Rayess, Y. E., Rizk, A. A., Sadaka, C., Zgheib, R., Zam, W., ... & Martins, N. (2020). Turmeric

and its major compound curcumin on health: bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. *Frontiers in Pharmacology*, *11*, 01021.

30. Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary and Alternative Medicines*, *10*(5), 210-229. doi: 10.4314/ajtcam.v10i5.2
31. Söylemezoğlu, G., Atak, A., Boz, Y., Ünal, A., & Sağlam, M. (2016). Viticulture in Turkey. *Chronica Horticulturae*, *56* (2), 27-31.
32. Sultan, S. M., Saady, A. M., & Irzoqy, M. E. (2018). A Comparative Study of the Effect of Alcoholic Extract of Turmeric Plant in Inhibiting the Growth of Candida Albicans. *International Journal of Engineering and Technology*, *7*(4.37), 12-16.
33. Taylor, D. (2016). The Pharmaceutical Industry and the Future of Drug Development. *Pharmaceuticals in the Environment: Volume 41*, 41, 1. Doi: https://doi.org/10.1039/9781782622345-00001
34. Upendra, R. S., Khandelwal, P., & Reddy, A. M. (2011). Turmeric powder (Curcuma longa Linn.) as an antifungal agent in plant tissue culture studies. *International Journal of Engineering Science*, *3*(11), 7899-7904.
35. Verpoorte, R., Siwon, J., Van Essen, G. F. A., Tieken, M., & Svendsen, A. B. (1982). Studies on Indonesian medicinal plants. VII. Alkaloids of Arcangelisia flava. *Journal of Natural Products*, *45*(5), 582-584. https://doi.org/10.1021/np50023a011
36. Wold, W. S., & Suzuki, I. (1976). The citric acid fermentation by Aspergillus niger: regulation by zinc of growth and acidogenesis. *Canadian Journal of Microbiology*, *22*(8), 1083-1092. https://doi.org/10.1139/m76-159
37. Yusoff, S. F., Haron, F. F., Tengku Muda Mohamed, M., Asib, N., Sakimin, S. Z., Abu Kassim, F. & Ismail, S. I. (2020). Antifungal activity and phytochemical screening of Vernonia amygdalina extract against Botrytis cinerea causing gray mold disease on tomato fruits. *Biology*, *9*(9), 286. doi:10.3390/biology9090286
38. Wold, W. S., & Suzuki, I. (1976). Regulation by zinc and adenosine 3′, 5′-cyclic monophosphate of growth and citric acid accumulation in Aspergillus niger. *Canadian Journal of Microbiology*, *22*(8), 1093-1101. https://doi.org/10.1139/m76-160
39. Zhang, W., Chang, X., Wu, Z., Dou, J., Yin, Y., Sun, C., & Wu, W. (2020). Rapid isolation of non-aflatoxigenic Aspergillus flavus strains. *World Mycotoxin Journal*, *13*(2), 277-286. DOI 10.3920/WMJ2019.2490