

The Effect of Some Essential Oil on Fungi Isolated from the Mouth of Local Sheep

Asst. Prof. Afrah A.Habeeb

Technical institute of Al-Dewaniyah, AL-Furat AL-Awsat Technical University (ATU), Iraq.

Afrahabdulwaheed@atu.edu.iq

Abstract

The present study was aimed to determine the effect of some essential oil on the fungi isolated from the mouth of local sheep (Candidiasis), and study the relationship of some essential oil with antifungal agent (Nystatin).

The result show that A fungus which isolated and diagnosed was *Candida albicans*. Cinnamon oil inhibit the growth of *C. albicans*, and the Zone of inhibition was 31.95mm, *Nigella sativa* oil inhibit the growth in zone 22mm, Nystatin inhibit the growth 30.17mm and Garlic oil had no effect on the growth of *C. albicans*. And when mixed the oil with Nystatin the Zone of inhibition was 31mm Cinnamon oil + Nystatin, 20mm *Nigella sativa* oil + Nystatin and 15mm in Garlic oil + Nystatin. The result show that Cinnamon oil can use as antifungal alone or with Nystatin.

Key words: *Candida albicans*, Cinnamon oil, *Nigella sativa* oil, Garlic oil, Nystatin.

Introduction

Sheep are an important animal wealth, so it is necessary to take care of their health because of their direct or indirect impact on human health. One of the common disease that affect sheep is fungi (Morris et al, 2001). Candidiasis is a fungal infection due to any type of *Candida*. When it affects the mouth, in some countries it is commonly called thrush (Patil et al, 2015). It is an opportunistic as it takes advantage of the weakening of the body's immunity to become a cause of illness, and it is known as saprophytic fungi, it is widespread and is not limited to specific region (Rippon, 1988). Candidiasis is a localized mucocutaneous disease caused by species of the yeast-like fungus *Candida*, most commonly *C. albicans*. It is distributed worldwide in a variety of animals. *C. albicans* is a normal inhabitant of the nasopharynx, GI tract, and external genitalia of many species of animals and is opportunistic in causing disease (Oxford Dictionaries, 2012). Fungi is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, which is separate from the other eukaryotic life kingdoms of plants and animals (Moris et al, 2008).

Signs and symptoms of Infection in the mouth is characterized by white discolorations in the tongue, around the mouth, and throat. Irritation may also occur, causing discomfort when swallowing (Cuenca et al., 2006).

Essentially four classes of drugs exist for the treatment of fungal infections with *Candida*: polyenic agents, echinocandins, azoles, and antimetabolites. As oral infections are superficial, treatment includes

topical antifungals, such as nystatin and miconazole. Despite the number of available therapeutic options, microbial resistance to antifungals from different classes is increasing (Pianalto and Alspaugh, 2016). One of the mechanisms strongly associated with such resistance is the ability of certain microorganisms to form biofilms. Microorganisms in these communities undergo genetic changes that increase their resistance to drugs and the immune system. In the case of *Candida*, its ability to form biofilm on biotic and abiotic surfaces represents a relevant virulence factor and is thus an interesting target of study of the development of antifungal agents against candidiasis (Morace et al., 2014). Within this context, the study of the therapeutic properties of plants that may represent an alternative therapeutic resource has increased (Newman and Cragg, 2012).

Therefore, the aims of the present study were to describe the effect of some essential oil and to investigate the *in vitro* antifungal activity, and the effect on the growth of *Candida* spp.

Cinnamon oil

Cinnamon bark is a bark for, it is found as sticks or as powder (Shen et al. 2010). Cinnamon can be used as a feed additive to raise the qualities of productivity, blood and immunity to chicken (Afrah, 2019) and decrease concentration of cholesterol and LDL with increase in concentration of HDL in egg yolk and the blood of laying hens (Afrah, 2020). Cinnamon oil is prized for its lush fragrance. It's also linked to several health and beauty benefits. Studies found that cinnamon oil has: antibacterial, antifungal, antidiabetic, and antioxidant properties (El-Barotyl, 2010).

Cinnamon oil is derived from the bark or leaves of several types of trees, and comprised of compounds and phytochemicals, such as cinnamaldehyde and eugenol. It's these elements that make cinnamon beneficial for health (Doddanna et al., 2013).

Black seed oil:

Nigella sativa is a small flowering shrub with purple or white-tinged flowers that grows in Eastern Europe, the Middle East, and western Asia. While it may look unsuspecting, the shrub produces fruits that have tiny black seeds. These black seeds have been used in remedies for thousands of years (Ahmad et al., 2013). Black seed oil is high in antioxidants and may have several benefits for health. These include the treatment of asthma and various skin conditions, lowering blood sugar and cholesterol levels aiding in weight loss, and protecting brain health (Aljabre et al., 2015). Black seed oil may have other benefits for health, including: anticancer effects, reduce symptoms of rheumatoid arthritis, male infertility, antifungal (Yousefi et al., 2013).

garlic oil :

Garlic oil is the volatile oil derived from garlic. It is usually prepared using steam distillation, and can also be produced via distillation using ether. It is used in cooking and as a seasoning, a nutritional supplement, and also as an insecticide (Stanway, 2012). **garlic oil** is full of essential nutrients and contains selenium, allicin, vitamin C, vitamin B6, copper, and zinc, that helps control acne. The anti-

inflammatory properties help soothe inflamed skin (Singhal et al ,1997).Garlic oil is a pungent and unpalatable oil that is made from garlic. As a spice, garlic is extremely healthy, for it has many health benefits and is used as a diuretic, expectorant, stimulant, and diaphoretic. and the oil has many benefits, including some additional benefits like being antiparasitic, antibacterial, antifungal, and insecticidal (Capasso , 2013).

Materials and methods

Sample collection:

Twenty five swab taken from the mouth of local sheep. the swab culture in nutrient agar(sabouraud 's Dextrose Agar /Oxoid)and the agar sterilized before culturein autoclave(121^oc,p15 pound per square inch) for 15 minute.

The culturing has straining the surface of agar by swab and Three replicates of the culture were done on agar (SDA)to ensure that the fungal growth was not contaminated during the culture process ,and incubated in 37^oc for 48h.the Candida diagnosis depended on the appearance and microscopic characteristics(Kauffman , 2007).

Test of the efficiency of essential oil :

Test of the efficiency of essential oil for inhibiting growth of Candida albicans was Agar well diffusion method according to(murray et al , 1999). Preparation of fungal stuck in concentrated 5×10^8 cell/ml and transport 0.1ml to culture (SDA) and incubated in 30^oc for 24hour .after that was measured the zone of inhibition for each hole, the results were compared with that of the antifungal nystatin.

Result

Diagnosis and cultural characteristics

The current study proved that the fungus isolated from the mouth of local sheep is Candida albicans according to the cultural characteristics of colonies growing on cultural (SDA). The colonies appeared in a white to cream color and according to Figure (1).



Figure (1) growth of C.albicans on Sabourauds Dextrose Agar (SDA). The colonies of C.albicans appeared in a white to cream color.

Test of the efficiency of essential oil in inhibit the growth of *C.albicans* in culture after 48 hours.

The results of the sensitivity of *Candida* yeast to the aromatic oils used in the study showed that cinnamon oil is one of the oils most influenced by the appearance of the highest areas of inhibition. As for the black seed oil, the damping diameter reached 22 mm, while the garlic oil had no effect on the *Candida* yeast isolated from the mouth of local sheep, as shown in Table 1 and Figure 2.

Table (1) test the efficiency of essential oil in inhibiting growth of *C.albicans*

Treatment	Zone of inhibition (mm)
Cinnamon oil	31.95
Nigella sativa oil	22
Garlic oil	0
Nystatin	30.17



Figure (2) test the efficiency of essential oil in inhibiting growth of *C.albicans* on the culture media.

Test of the efficiency of essential oil and Nystatin in inhibit the growth of *C.albicans* in culture after 48 hours.

the results also show that *Candida albicans* sensitivity to essential oils by the interaction with the antibiotic Nystatin. The average diameter of inhibition in the interaction between cinnamon oil and the

antibiotic Nystatin was 31 mm. While the average diameter of inhibition for the interaction between *Nigella sativa* oil and the antibiotic Nystatin was 20 mm, as for the interaction between garlic oil and the antibiotic Nystatin, the average diameter of inhibition was 15 mm, as shown in Table 2.

Table (2) test the efficient of essential oil with antibiotic(Nystatin)in inhibiting growth of C.albicans

Treatment	diameter of inhibition (mm)
Cinnamon oil + Nystatin	31
Nigella sativa oil + Nystatin	20
Garlic oil + Nystatin	15

Discussion

the fungus isolated from the mouth of local sheep is *Candida albicans* according to the cultural characteristics of colonies growing on cultural (SDA). The colonies appeared in a white to cream color and according to Figure (1) and the studies indicate that *Candida* spp. Colonies has the same phenotypic characteristics as when grown in cultural (SDA)(Ellis et al , 2007). The characteristics (such as morphology and colour) of the colonies may allow initial diagnosis of the organism causing disease symptoms(Guarner, and Brandt ,2011).

According to research published in 2016, cinnamon oil and olive oil display antifungal and antimicrobial activity against fungal strains , which is often the cause of toenail fungus and other fungal infections(Sanjib ,2016).cinnamon oil contain cinnamaldehyde and various phenols and terpenes with antifungal, antidiarrheal , vasoactive and analgesic effects(Thomasand Kuruvilla, 2012).

Black seed oil has also been shown to have antifungal activities. In particular, it may protect against *Candida albicans*, which is a yeast that can lead to candidiasis (El-Saleh et al , 2004).

It appears that there is a relationship between the chemical constituents of oils and its antimicrobial activity. The cinnamon oil containing high amounts of phenolic compounds (18.2%), while Black seedoil was rich in nigellone, Thymoquinone , β -sesterol ,selenium and B₁,B₃,B₂²⁶ . Because both oils had a different chemical profiles, difference in antimicrobial activity could be expected. It has been reported that cinnamon (rich in eugenol and cinnamaldehyed) and essential oils possessed a wide spectrum of antimicrobial activity(Farag et al ,1989).A study has foundMice that developed *Candida Albicans* were treated with black seed extract. The researchers found a severe inhibition of the growth of *Candida albicans* fungus(Sikkema et al, 1994).

This study shown the potential use of cinnamon essential oils in ethno-medicine as a Antifungal

activity Where studies indicate. that oils and bioactive components could be besides its traditional uses. it has been proposed that lipophilicity or hydrophobicity and chemical structure of essential oils or their main compounds such as the presence of functional polar groups and aromaticity could play an important role for the antimicrobial activity(Farag et al , 1989), which enable them to partition between lipids of the bacterial or fungal cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable, which will lead to cell death(Sikkema et al,1994). Thus, as can be seen , some of the major components present in cinnamonInhibition of fungal cell wall synthesizing enzymes by trans-cinnamaldehyde (Bang et al , 2000).

They stated that the antimicrobial activity of volatile constituents was decreased in the decreasing order: phenols (highest active) alcohols , aldehydes , ketones , ethers , hydrocarbons. Generally speaking, the extract of antimicrobial mechanism of essential oils has not been completely elucidated. However, it has been proposed that lipophilicity or hydrophobicity and chemical structure of essential oils or their main compounds such as the presence of functional polar groups and aromaticity could play an important role for the antimicrobial activity. Thus, there is a major components present in cinnamon (CA and eugenol) can penetrate the membrane of the microorganisms and react with the membrane enzymes and proteins as well as phospholipids bilayer, which cause an impairment of microbial enzyme system and/or disturb genetic material functionality (Abd El-Baky and El-Baroty ,2008). Studies reported that cinnamon oil contain high amount of cinnamaldehyde (CA), which inhibited the fungal-cell-wall synthesizing systems through the reaction with sulfhydro groups present in active site of these enzymes (Bang et al ,2000)

. Therefore, the bioactivity of essential oils is dependent not only on the major compounds but also on the chemical structures of these compounds(Farag et al ,1989).

Conclusion

From this results, we can use cinnamon oil or Black seed oil as Antifungalfor treatment candida albicans ,to get better results we use cinnamon oil for its wide effect on candida albicans.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Nil

References

1. Abd El-Baky HH, El-Baroty GS (2008). Chemical and biological evaluation of the essential oil of Egyptian Moldavian balm. *Int. J. Essential Oil Therap.*, 2: 76-81.
2. Afrah A. Habeeb.(2019).The influence of cinnamon intake on some production Performance and blood Picture Parameter of broiler Chicken.*Plant Archives* .Vol.19,No.1,P:1253-1256.
3. Afrah A. Habeeb.(2020). Effect of cinnamon supplement to laying hens diet in blood and egg

cholesterol concentration .Indian Journal of public Health Research &Development.vol.11,No.3,p:1476-1478.

4. Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A. ... Anwar, F. (2013, May). A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine*, 3(5), 337–352.
5. Ahmed Jassem AL-Naely, Maytham T. Qasim, Hussein Abbas Al-Hamadawi. (2021). Transfusion of Blood Components in the Newborn Service of the Hospital. *Annals of the Romanian Society for Cell Biology*, 952–958. Retrieved from <http://annalsofrscb.ro/index.php/journal/article/view/2525>.
6. Aljabre, S. H. M., Alakloby, O. M., & Randhawa, M. A. (2015, July). Dermatological effects of *Nigella sativa*. *Journal of Dermatology & Dermatologic Surgery*, 19(2), 92–98.
7. Bang KH, Lee DW, Park HM, Rhee YH (2000). Inhibition of fungal cell wall synthesizing enzymes by trans-cinnamaldehyde. *Biosci. Biotechnol. Biochem.*, 64: 1061-1063.
8. Capasso A. *Molecules*.(2013). Antioxidant action and therapeutic efficacy of *Allium sativum* L. *Jan* 4;18(1):690-700.
9. Cuenca-Estrella, M., A. Gomez-Lopez, E. Mellado. (2006). Head-to head comparison of the activities of currently available antifungal agents against 3,378 Spanish clinical isolates of yeasts and filamentous fungi. *Antimicrob. Agents Chemother.*50:917- 921.
10. Doddanna S. J., S. Patel, M. A. Sundarrao, and R. S. Veerabhadrapa.(2013). “Antimicrobial activity of plant extracts on *Candida albicans*: an in vitro study,” *Indian Journal of Dental Research*, vol. 24, no. 4, pp. 401–405,.
11. El-Barotyl GS, et al. (2010). Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils. academicjournals.org/journal/AJBR/article-abstract/8D4DEA111465
12. Ellis,D.;Stephen ,D.;Helen,A.;Rosemary,H.and Roben,B.(2007).Description of medical fungi .Second Edition.
13. El-Saleh SC, Al-Sagair OA, Al-Khalaf MI.(2004). Thymoquinone and *Nigella sativa* oil protection against methionine-induced hyperhomocysteinemia in rats.. *Int J Cardiol* Jan;93(1):19-23.
14. Farag, R.S.; Daw, Z. Y.; Abo-Raya, S. H. (1989). Influence of some essential oils on *Aspergillus parasiticus* growth and aflatoxins production in a synthetic medium, *J. food Sci.*, 54: 74-67. Felipe CF, Kamyla SF, André L, José NSB, Manoel AN, M.
15. Guarner, J.; Brandt, M. E. (2011). "*Histopathologic Diagnosis of Fungal Infections in the 21st Century*". *Clinical Microbiology Reviews*. American Society for Microbiology. 24(2): 247–280.
16. Kauffman CA.(2007).Candidiasis. In: Goldman L, Ausiello D, eds. *Cecil Textbook of Medicine*. 23rd ed. Philadelphia, Pa: Saunders;;chap 359.
17. Morace G., F. Perdoni, and E. Borghi,.(2014). “Antifungal drug resistance in *Candida* species,” *Journal of Global Antimicrobial Resistance*, vol. 2, no. 4, pp. 254–259.
18. Moris, S.A.; Bailey, C.J. and Cartledge, J.Mep. (2008). Neonatal renal candidiasis. *J. of pediatrics and Chil. Health*, Vol. 30 No. 2:186-188.
19. Morris, M., M. Ngeleka, A. O. Adogwa, G. Lalla, G. St-Germain, and R. Higgins.(2001). Rhinocerebral zygomycosis in a sheep. *Can. Vet. J.* 42 : 227-228.
20. Mousa, H. M., & Qasim, M. T. (2015). Microbial Infection and IL-6 Urine Levels for Pregnant women in Thi-Qar Province. *World J. Pharma. Res*, 4(05), 358-365.
21. Murray ,P.R.;Baron ,E.J.;Pfaller,M.A.;Tenover,F.C.&Yolken ,R.H.,(1999).Manual of clinical Micobiology .7th ed .ASMpress.Wash-ington.
22. Newman D. J. and G. M. Cragg, (2012). “Natural products as sources of new drugs over the 30 years from 1981 to 2010,” *Journal of Natural Products*, vol. 75, no. 3, pp. 311–335,
23. Oxford Dictionaries .”Fungus”.Archived from the original on 28 July (2012).

24. Patil S, Rao RS, Majumdar B, Anil S (2015). "Clinical Appearance of Oral Candida Infection and Therapeutic Strategies". *Frontiers in Microbiology*. **6**: 1391.
25. Pianalto. K.and J. Alspaugh.(2016.), "New Horizons in Antifungal Therapy," *Journal of Fungi*, vol. 2, no. 4, p. 26,
26. Qasim, M. T., & Al-Mayali, H. K. (2019). Investigate the relation between Baicalin effect and Gene expression of LH, FSH, Testosterone in male rats treated with Gemcitabine drug. *Research Journal of Pharmacy and Technology*, 12(9), 4135-4141.
27. Qasim, M. T., & Al-Mayali, H. K. (2019, July). The immunological and protective role of Baicalin in male rats treated with chemotherapy (Gemcitabine). In *Journal of Physics: Conference Series* (Vol. 1234, No. 1, p. 012065). IOP Publishing.
28. Rippon, J.W.(1988).*Medical Mycology*.3rd.W. B. Saunders Co. Philadelphia .U.S.A.
29. Sanjib Bhattacharya, (2016). *Essential Oils in Food Preservation, Flavor and Safety*. Chapter 3 - Cultivation of Essential Oils.
30. Shen Y, Fukushima M, Ito Y, Muraki E, Hosono T, Seki T, et al.(2010).Verification of the antidiabetic effects of cinnamon (*Cinnamomumzeylanicum*) using insulin-uncontrolled type 1 diabetic rats and cultured dipocytes. *BiosciBiotechnolBiochem.* ;74:2418–25.
31. Sikkema J, De Bont JAM, Poolman B (1994). Interaction of cyclic hydrocarbons with biological membranes. *J. Biol. Chem.*, 269: 8022- 8028.
32. Singhal, R.; Kulkarni, P.R.; Rege, D.V. (1997). *Handbook of Indices of Food Quality and Authenticity*. Woodhead Publishing Series in Food Science, Technology and Nutrition Series. Taylor & Francis. p. 403.
33. Stanway, P. (2012). *The Miracle of Garlic: Practical Tips for Health & Home*. Watkins Media. p. 25.
34. Tahmasebi, S., Qasim, M. T., Krivenkova, M. V., Zekiy, A. O., Thangavelu, L., Aravindhan, S., ... & Roshangar, L. (2021). The effects of Oxygen-Ozone therapy on regulatory T-cell responses in multiple sclerosis patients. *Cell biology international*.
35. Thomas, j. and Kuruvilla K.M. , (2012). *Handbook of Herbs and Spices* (Second Edition), Volume,1
36. Yousefi, M., Barikbin, B., Kamalinejad, M., Abolhasani, E., Ebadi, A., Younespour, S., Manouchehrian, M., & Hejazi, S. (2013). Comparison of therapeutic effect of topical Nigella with Betamethasone and Eucerin in hand eczema. *Journal of the European Academy of Dermatology and Venerology*, 27(12), 1498–1504.
37. Zainab I. Mohammed, Maytham T. Qasim. (2021). Correlation of AMH and LH Levels in PCOS Patients with Pregnancy Rate. *Annals of the Romanian Society for Cell Biology*, 945–951. Retrieved from <http://annalsofrscb.ro/index.php/journal/article/view/2524>.