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Antifungal Effect of Some Natural Substances on Fluconazole-resistant *Candida* species Recovered from Mastitis

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Abstract

A wide range of essential plant oils and extracts have been shown to be fungal inhibitors and could provide attempting alternative for treating animal infections. The current study screened the in-vitro antifungal properties of propolis in addition to *Eucalyptus* and *Moringa* essential oils on some fluconazole-resistant *Candida* isolates recovered from cases of bovine and ovine mycotic mastitis through determination of their Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC). Propolis recorded MIC and MFC ranged from 44 mg/mL to 175 mg/mL and the isolates of *C. albicans* were the most sensitive. *Eucalyptus* was the most effective essential oil with MIC and MFC ranged from 2 mg/mL to 16 mg/mL and was mostly effective against *C. tropicalis*, *C. albicans* and *C. kefyr*. Regarding *Moringa* essential oil, the recorded MIC and MFC ranged from 31.25 mg/mL to 125 mg/mL and the most sensitive isolates were *C. tropicalis* and *C. kefyr*. The molecular characterization of azole-resistance genes revealed that all tested isolates harbored *CDR* gene 100%, while *MDR1* and *ERG11* genes were represented as 80% and 40%, respectively. Therefore, the antifungal effect of propolis and essential oils of *Eucalyptus* and *Moringa* against fluconazole-resistant *Candida* species were highlighted in the current study and can be employed as useful alternatives for the treatment of bovine and ovine mycotic mastitis.

KEYWORDS
Candida, *Eucalyptus*, *Moringa*, Propolis, Resistance genes

INTRODUCTION

Bovine and ovine mastitis is a condition brought on by a wide range of microorganisms that harms the dairy business severely financially by reducing milk output and driving up the cost of treatment and culling (Zaragoza *et al*., 2011).

Antibiotic therapy without identifying the mastitis-causing organisms is commonly the first choice of treatment for veterinarian and dairy farmers for diseased animals, accordingly, cases of mastitis, especially the mycotic one, occurs frequently (Dworecka-Kaszak *et al*., 2012).

The type of fungus involved, and its proportion of infectivity determine how serious mycotic mastitis is (Pachauri *et al*., 2013).

There are numerous species of yeasts and molds that cause mycotic mastitis in animals including *Candida*, *Cryptococcus, Rhodotorula, Aspergillus* and *Penicillium* species that are mostly incriminated to be associated with bovine and ovine mycotic mastitis (Bekele *et al*., 2019).

About 70% of mycotic mastitis occurrences in animals are produced by *Candida* species (Spanamberg *et al*., 2014). The most common kind of *Candida* that causes mycotic mastitis in animals is *C. albicans*, while other *Candida* species have also been involved.

Mycotic mastitis has been treated with a variety of medications but there have not yet been any trials demonstrating the effectiveness of any treatment (Roberson and Kalck, 2010).

The endogenous fungal flora has been repressed in recent years by increased use of antifungal drugs, and more resistant strains have been reported as a result of the reduction of the susceptible strains. In addition, some *Candida* species are innately resistant to specific antifungals, such as intrinsic fluconazole-resistance in *Candida krusei* (Sonmez and Erbas, 2017). Therefore, due to the limitations when treating fungus infections, such as high costs, side effects, shortages, and drug resistance or reduced susceptibility to the antifungal medications, scientists have been asked to pay more attention to conventional medicine than antifungal drugs (Bakr *et al*., 2015).

Propolis possesses several beneficial biological qualities, including antioxidant activities, antibacterial, anti-inflammatory, anti-cancer, and antifungal capabilities. It also contains components used in food, natural products, and pharmaceuticals (Kalogeropoulos *et al*., 2009). Also, propolis and its extract can be used as natural preservatives in dairy products to prevent fungal development.

It has been demonstrated that a variety of essential plant oils and aqueous plant extracts are fungal inhibitors, and thus can present attempting alternative for avoiding the contamination of foods with aflatoxins (Ponzilacqua *et al*., 2018).

Essential plant oils have little possibility in the emergence of antimicrobial resistance, in addition to not being harmful; therefore, they are categorized as GRAS (generally recognized as safe) by the United States Food and Drug Administration (Cardile *et al*., 2009). They act through denaturing the spore-germinating enzymes and interfering with essential amino acids, resulting in

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damage to the fungal enzymatic system by lowering the production of proteins and structural components in fungal cells (Basak and Guha, 2018).

Along with being powerful inhibitors of A. flavus growth, *Eucalyptus* and other plant essential oils also decrease the synthesis of aflatoxins (Kim *et al*., 2018).

The medical properties of *Moringa oleifera* include anti-inflammatory, anti-ulcerative, antioxidant, antifungal and antibacterial effects that led to widespread use of *Moringa* for the treatment of various diseases (Mehta *et al*., 2003). *Moringa* has been applied for its antibacterial properties; it has also been used for bioenhancement and as nanoparticles in medication delivery (Arora *et al*., 2013).

Hence, the current research designed to identify the in-vitro antifungal properties of propolis in addition to *Eucalyptus* and *Moringa* essential oils on fluconazole-resistant *Candida* isolates recovered from cases of bovine and ovine mycotic mastitis.

MATERIALS AND METHODS

Ethics approval and consent to participate

All animal sampling and handling for isolation of tested yeasts were ethically treated according to the Institutional Animal Care and Use Committee of Cairo University (CU-IACUC) and they approved this study with approval number (Vet CU 01122022580).

Animals

A total of 250 animals including 120 cows and 130 sheep and goats in different farms and villages in Beni-Suef and Fayoum provinces were used for milk collection during the period from January to October 2021.

Diagnosis of mastitis

The diagnosis of mycotic mastitis was based on the signs of mastitis (including swelling of the udder and changes in the color as well as the consistency of milk) and California Mastitis Test.

Yeast isolates

Fourty nine *Candida* isolates were recovered from 250 milk samples collected from cows, ewes and does that were suffered from mastitis and not responding to antibiotic therapy, all isolates were tested for fluconazole susceptibility through disc diffusion method (Wayne, 2020) Table 1. Out of them, 20 fluconazole-resistant *Candida* isolates including 5 *C. tropicalis*, 5 *C. guilliermondii*, 4 *C. parapsilosis*, 3 *C. albicans* and 3 *C. kefyr* were used in the present study. They were identified morphologically by microscopic observations of Gram stained smears, colony morphology on Sabouraud's Dextrose Agar (SDA), germ tubes formation in human serum and chlamydospores production on rice agar medium as well as biochemical identification through sugar assimilation and fermentation reactions (Pincus *et al*., 2007).

Propolis and essential oils

Propolis 70% (Sigma Aldrich) as well as the crude essential oils of *Eucalyptus* and *Moringa oleifera* (MULTIPHARMA co. for veterinary and agricultural development) were used for detection of their Minimum Inhibitory Concentrations (MIC) and Minimum Fungicidal Concentrations (MFC) through the microdilution technique, where it is a more sensitive method. The essential oils were dissolved in DMSO to prepare stock concentrations of 250 mg/mL (Palmeira-de-Oliveira *et al*., 2009).

Table 1. Number of different yeasts recovered from each animal species.

Strain	Ovine	Bovine	Total
C. guilliermondii	14	4	18
C. parapsilosis	14	2	16
C. tropicalis	4	3	7
C. albicans	1	3	4
C. kefyr	2	2	4

Determination of MIC of propolis and essential oils (Eucalyptus and Moringa) against Candida isolates

Following the guidelines of the M60 methodology, propolis and essential oils were tested for their antifungal activity on *Candida* isolates to determine their MIC and MFC (Wayne, 2020).

The inoculum, which contained $5x10^6$ CFU/mL, was made in saline solution. The inoculum was standardized at $2.5x10^3$ CFU/mL by serial dilution. One hundred µl of Sabouraud's Dextrose Broth (SDB) (Oxoid) were spread evenly throughout the wells of a sterile microplate. 100 µl of propolis and the initial concentration of the essential oils were added to the first column, and then propolis and essential oils were serially microdiluted. Ten µl of the adjusted inoculum was added following the dilution process, resulting in the following groups:

Positive control group: SDB and inoculum, for observation of yeast growth.

Negative control group: SDB and propolis or the essential oil for observation of possible contaminations in the microdilution.

Test group: propolis (initial concentration 350 mg/mL), essential oil (initial concentration 250 mg/mL) in addition to SDB and the adjusted inoculum.

The microplates were incubated at 25°C for 48 h and then visual reading was performed, through detection of the concentration that completely prevented a visible yeast growth. The tests were performed in triplicate.

Determination of MFC of propolis and essential oils (Eucalyptus and Moringa) against Candida isolates

The 96 wells were homogenized by pipette then 10 µl was inoculated onto Sabouraud's Dextrose Agar (SDA) (Oxoid) plates followed by incubation at 25°C for 48 h, formerly the lowest concentrations of propolis and essential oils that didn't allow the growth of any yeast colony on SDA were determined and considered as MFC. The tests were performed in triplicate.

Polymerase Chain Reaction (PCR) for detection of fluconazole-resistance genes of Candida isolates

Five *Candida* isolates considered as mostly associated with bovine and ovine mycotic mastitis including 3 *C. albicans* and 2 *C. tropicalis* were selected for molecular characterization by PCR for detection of 3 fluconazole-resistance genes (*CDR*, *MDR1* and *ERG11*). Primers sequences, amplified products and annealing temperatures for the targeted genes were illustrated in Table 2. The *Candida* field isolate that tested positive in the RLQP (Reference laboratory for veterinary quality control on poultry production, Dokki, Giza, Egypt) provided the positive control DNA. As a negative control, a PCR mixture without the DNA template was used.

Table 2. Primers used for amplification of fluconazole-resistance genes.

Gene	Sequence	Temp.	Product	Reference
CDR	*F* GTGGTGTTTCCGGTGGTGAAAGAAA *R* CCTGGTGTTGGATCGTTCACATTCA	57°C	503 bp	Henry *et al.* (2000)
MDR1	*F* GAGTCGTAGCTACATTGCCATTAACA *R* GGTGATTTCTAATGGTCTCCATAATGT	52°C	590 bp	
ERG11	*F* CAAGAAGATCATAACTCAAT *R* AGAACACTGAATCGAAAG	53°C	1641 bp	Wang *et al.* (2015)

CDR: *Candida* drug resistance; MDR: Multi-drug resistance; ERG: Ergosterol

RESULTS

MIC of propolis and essential oils (Eucalyptus and Moringa) against Candida isolates

Propolis and essential oils showed inhibitory activities on tested *Candida* isolates.

Propolis recorded a MIC of 44-87.5 mg/mL for *C. albicans* followed by 87.5 mg/mL for *C. guilliermondii* and *C. kefyr* and 87.5-175 mg/mL for *C. tropicalis* and *C. parapsilosis*.

Concerning *Eucalyptus*, it was the most effective essential oil with a MIC of 2-3.9 mg/mL for *C. albicans* and *C. kefyr* followed by 2-7.8, 3.9-7.8 and 3.9-16 mg/mL for *C. tropicalis*, *C. parapsilosis* and *C. guilliermondii*, respectively.

Regarding *Moringa* essential oil, it showed a MIC of 31.25-62.5 mg/mL for *C. tropicalis* and *C. kefyr* followed by 31.25-125 mg/mL for *C. parapsilosis* and 62.5-125 mg/mL for *C. albicans* and *C. guilliermondii*.

MIC and MFC ranges of propolis, and essential oils were represented in Table 3.

MFC of propolis and essential oils (Eucalyptus and Moringa) against Candida isolates

Propolis revealed a MFC of 44-87.5 mg/mL for *C. albicans* followed by 87.5 mg/mL for *C. kefyr*, 87.5-175 mg/mL for *C. guilliermondii*, 175 mg/mL for *C. parapsilosis* and 87.5-350 mg/mL for *C. tropicalis*.

Eucalyptus essential oil demonstrated fungicidal activity against tested *Candida* isolates with a MFC of 2-7.8 mg/mL for *C. tropicalis* followed by 3.9-7.8 mg/mL for *C. albicans* and *C. kefyr*, 3.9-16 mg/mL for *C. guilliermondii* and 7.8-16 mg/mL for *C. parapsilosis*.

Concerning *Moringa* essential oil, it showed a MFC of 31.25-125 mg/mL for *C. parapsilosis* and 62.5-125 mg/mL for other tested *Candida* isolates as shown in Table 3.

Fluconazole-resistance genes of Candida isolates

All tested isolates including 3 *C. albicans* and 2 *C. tropicalis* harbored *CDR* gene 100%, while 4 isolates 80% (2 *C. albicans* and 2 *C. tropicalis*) harbored *MDR1* gene and 2 isolates 40% (One *C. albicans* and one *C. tropicalis*) were positive for *ERG11* gene as shown in Table 4 and Figs 1 and 2.

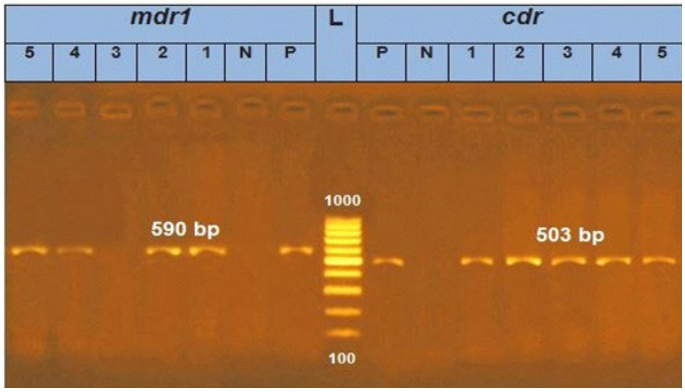

Fig. 1. PCR amplification of the *CDR* and *MDR1* genes at 503 bp and 590 bp, respectively. On the right side, lanes (1-5) showed positive amplification of *CDR* gene. On the left side, lanes (1, 2, 4, and 5) showed positive amplification of *MDR1* gene. L = 100-bp DNA molecular size ladder; P = Positive control; N = Negative control.

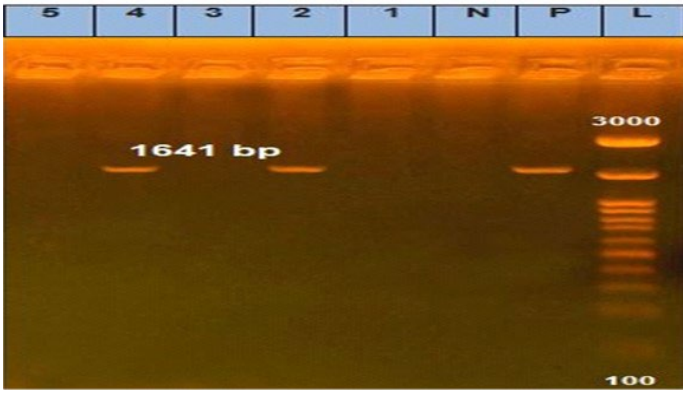

Fig. 2. PCR amplification of the *ERG11* gene at 1641 bp. Lanes (2 and 4) showed positive amplification of *ERG11* gene. L = 100-bp DNA molecular size ladder; P = Positive control; N = Negative control.

Table 3. The visual reading results of MIC and MFC of *Candida* isolates (mg/mL).

Isolate	Propolis		*Eucalyptus*		*Moringa*	
	MIC	MFC	MIC	MFC	MIC	MFC
C. albicans	44-87.5	44-87.5	2-3.9	3.9-7.8	62.5-125	62.5-125
C. tropicalis	87.5-175	87.5-350	2-7.8	2-7.8	31.25-62.5	62.5-125
C. guilliermondii	87.5	87.5-175	3.9-16	3.9-16	62.5-125	62.5-125
C. parapsilosis	87.5-175	175	3.9-7.8	7.8-16	31.25-125	31.25-125
C. kefyr	87.5	87.5	2-3.9	3.9-7.8	31.25-62.5	62.5-125

MIC: Minimum Inhibitory Concentration; MFC: Minimum Fungicidal Concentration.

Table 4. Fluconazole-resistance genes of *Candida* isolates.

Gene	1	2	3	4	5	(%)
CDR	+	+	+	+	+	100
MDR1	+	+	-	+	+	80
ERG11	-	+	-	+	-	40

1, 2, 3: *C. albicans*; 4,5: *C. tropicalis*; *CDR*: *Candida* drug resistance; MDR: Multi-drug resistance; ERG: Ergosterol.

DISCUSSION

Mycotic mastitis is mostly prevented and treated with antibiotics, however the rising antimicrobial resistance of microorganisms as well as antimicrobial residues in milk and the environment, could influence the effectiveness of traditional medications and constitute a risk to human health. Consequently, the use of natural substances as propolis, plant extracts, and essential oils may also prove to be effective treatments for the management of bovine and ovine mastitis (Lopes *et al*., 2020).

Chemical compounds found in medicinal plants have biological qualities that are thought to be beneficial to human and animal health (Paz *et al*., 2018), where all plants produce primary metabolites as carbohydrates, fatty acids, amino acids and organic acids that exert essential functions for their survival (Tian *et al*., 2018). They also create secondary metabolites, usually in lower concentrations, that are important for the plant's defenses and protect it from the majority of herbivores and microbes. The generic methods described by Pandey and Tripathi (2014) can be applied to acquire these metabolites, which could then be used to make preparations with different compositions and consistencies (Gouvea *et al*., 2017).

In this study, the data showed the antifungal activities of propolis and the tested essential oils against all tested *Candida* isolates.

The target of propolis action in fungal cells is the cell membrane, besides the induction of cell death. Additionally, propolis extract inhibits extracellular phospholipase activity, which reduces the adherence of fungal cells to epithelium. Now, it has been observed that propolis may influence the formation and integrity of the cell wall of fungi and can inhibit the morphological transformation of *C. albicans*. Furthermore, it has been declared that propolis inhibited filamentation and germination of yeasts and increased production of the superoxide anion radical. Propolis extract not only had an anti-biofilm impact but also had inhibitory effects on the growth and decreased the metabolism of *C. albicans* within the biofilms (Ożarowski *et al*., 2022).

In the current study, propolis recorded an inhibitory activity of up to '44 mg/mL', as well as other inhibitory concentrations ranged from '87.5 mg/mL' to '175 mg/mL'. Kousedghi *et al*. (2012) reported lowest MIC results of '8 mg/mL' and MFC of '16 mg/mL' for *C. albicans*, but they recorded similar results of MIC (64 mg/mL) and MBC (128 mg/mL) for Enterococcus faecalis.

The antifungal effect of *Eucalyptus* essential oil is related to its contents of eucalyptol (1,8-cineole) (Kim *et al*., 2018). Luqman *et al*. (2008) presumed the mechanisms of action of *Eucalyptus* oil as either, cell wall damage, cytoplasmic membrane disturbances, leak of cell contents, damage to membrane proteins and active transport or cell contents coagulation.

Our results showed that the *Eucalyptus* essential oil was more active towards the tested *Candida* isolates that is in agreement with previously issued reports (Ahmad and Beg, 2001; Ramezani *et al*., 2002; Wilkinson and Cavanagh, 2005).

In this study, *Eucalyptus* essential oil had an inhibitory activity of 2 mg/mL that agreed with that of Barbosa *et al*.(2018), where they recorded MIC and MFC of 2 mg/mL for *C. albicans*. Meanwhile, Agarwal *et al*. (2010) recorded a lower MIC of 0.05 µg/mL for *C. albicans*.

Other recorded inhibitory concentrations of *Eucalyptus* essential oil in this study were ranged from 3.9 mg/mL to 16 mg/mL that approved with the results of Luqman *et al*. (2008), where they recorded MIC of 5 mg/mL against *C. albicans*.

The majority of the microorganisms that are suspended during the water purification process are removed by 4-a-rhamnosyloxybenzyl isothiocyanate, also known as glucosidal mustard oil, which is contained in *Moringa* extracts (Eilert *et al*., 1981), moreover *Moringa* essential oils exhibit antifungal activity against *Trichophyton rubrum, T. mentagrophytes, Epidermophyton floccosum*, and *Microsporum canis* (Chuang *et al*., 2007). Also, both ethanolic and aqueous extracts of *Moringa* recorded activities against *Saccharomyces cerevisiae* and *C. tropicalis* (Patel *et al*., 2014).

In our study, the essential oil of *Moringa* recorded inhibitory activities against the tested isolates with a lower inhibitory activity of 31.25 mg/mL; other recorded inhibitory concentrations ranged from 62.5 mg/mL to 125 mg/mL. Similar results were recorded by Sahar *et al*. (2015) against *Streptococcus pyogens* with MIC of 125 mg/mL and methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* with MIC of 62.5-125 mg/mL. Other studies reported lower concentrations of MIC as Rocha *et al*. (2014) recorded MIC of 2.5 mg/mL.

Candida drug resistance gene (*CDR*) and multidrug resistance gene (*MDR1*) are responsible for coding of the efflux pumps in fluconazole-resistant *Candida* (Khosravi Rad *et al*., 2017). In the current study, *CDR* gene was detected in all tested isolates (100%), while *MDR1* gene was detected in 4 out of the selected 5 isolates (80%), similar results were recorded by Khosravi Rad *et al*. (2017).

A crucial enzyme in the process of ergosterol production known as lanosterol 14α demethylase is inhibited by azoles as they enter the yeast cells through enhanced diffusion. Since ergosterol is a key sterol in fungal membranes and is essential for both the fluidity and integrity of cell membranes as well as fungal cell development and proliferation. Enzyme inhibition results in both ergosterol depletion and the generation of a hazardous methylated sterol. As a result, the functions of the membrane are compromised (Monapathi *et al*., 2018). In our study, 40% of *Candida* isolates were positive for *ERG11* gene that agreed with the results of Mohamed *et al*. (2022) meaning that the *ERG11* gene is not present in all azole-resistant *Candida* isolates. Meanwhile a higher result of 68.1% was detected by Diba *et al*. (2020). In summary, propolis, together with *Eucalyptus* and *Moringa* essential oils recorded different antifungal activities against fluconazole-resistant *Candida* species, demonstrating that after further in vivo studies they can be employed as effective alternatives in the treatment of bovine and ovine mycotic mastitis.

CONCLUSION

The current study emphasized the in vitro antifungal effect of propolis as well as essential oils of *Eucalyptus* and *Moringa* on fluconazole-resistant *Candida* species revealing that after further in vivo studies, they can be used as effective alternatives in the management of bovine and ovine mycotic mastitis.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Agarwal, V., Lal, P., Pruthi, V., 2010. Effect of Plant Oils on *Candida albicans*. J. Microbiol. Immunol. Infect. 43, 447-451. https://doi.org/10.1016/S1684-1182(10)60069-2

Ahmad, I., Beg, A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant hu-

man pathogens. J. Ethnopharmacol. 74, 113-23. https://doi.org/10.1016/s0378-8741(00)00335-4

Arora, D.S., Onsare, J.G., Kuar, H., 2013. Bioprospecting of *Moringa* (*Moringa*ceae): microbiological perspective. J. Pharmacogn. Phytochem. 1, 193-215.

Bakr, E., ElTawab, A., Elshemey, T., AbdElrhman, A., 2015. Diagnostic and Therapeutic Studies on Mycotic Mastitis in Cattle. Alexandria J. Vet. Sci. 46:138. https://doi.org/10.5455/ajvs.189682

Barbosa, J.P., de Oliveira, T.R., Puppin, D.G.P.B., Teixeira, A.L., Boni, G.C., de Feiria, S.N.B., Höfling, J.F., 2018. Anti- *Candida* Activity of Essential Oils from *Eucalyptus* species. A Preliminary Study 8. Adv. Dent. & Oral Health 8, 555740. https://doi:10.19080/ADOH.2018.08.555740

Basak, S., Guha, P., 2018. A review on antifungal activity and mode of action of essential oils and their delivery as nano-sized oil droplets in food system. J. Food Sci. Technol. 55, 4701-4710. https://doi.org/10..1007/s13197-018-3394-5

Bekele, T., Lakew, M., Terefe, G., Koran, T., Abebe, O., Yimesgen, L., Tamiru, M., Demissie, T., 2019. Study on bovine mastitis with isolation of bacterial and fungal causal agents and assessing antimicrobial resistance patterns of isolated Staphylococcus species in and around Sebeta town, Ethiopia. African J. Microbiol. Res. 13, 23-32. https://doi.org/10.589/ajmr2018.8909

Cardile, V., Russo, A., Formisano, C., Rigano, D., Senatore, F., Arnold, N.A., Piozzi, F., 2009. Essential oils of Salvia bracteata and Salvia rubifolia from Lebanon: Chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. J. Ethnopharmacol. 126, 265-272. https://doi.org/10.1016/j.jep.2009.08.034

Chuang, P.H., Lee, C.W., Chou, J.Y., Murugan, M., Shieh, B.J., Chen, H.M., 2007. Antifungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. Bioresour. Technol. 98, 232-236. https://doi.org/10.1016/j.biortech.2005.11.003

Diba, K., Khademvatan, S., Jaafari, K., Abharian, S., 2020. Characterization of *ERG11* Gene in Drug-Resistant *Candida albicans* Isolated from Iranian Cases of Recurrent Vulvovaginal Candidiasis. RABMS. 6, 153-159. https://ijrabms.umsu.ac.ir/article-1-116-en.html

Dworecka-Kaszak, B., Krutkiewicz, A., Szopa, D., Kleczkowski, M., Biegańska, M., 2012. High prevalence of *Candida* yeast in milk samples from cows suffering from mastitis in Poland. Sci. World J. 2012, 196347. https://doi.org/10.1100/2012/196347

Eilert, U., Wolters, B., Nahrstedt, A., 1981. The antibiotic principle of seeds of *Moringa oleifera* and *Moringa stenopetala*. Planta. Med. 42, 55-61. https://doi.org/10.1055/s-2007-971546

Gouvea, F., Rosenthal, A., Ferreira, E.H., 2017. Plant extract and essential oils added as antimicrobials to cheeses: a review. Cienc. Rural. 47, e20160908. https://doi.org/10.1590/0103-8478cr20160908

Henry, K.W., Nickels, J.T., Edlind, T.D., 2000. Upregulation of ERG Genes in *Candida* Species by Azoles and Other Sterol Biosynthesis Inhibitors. Antimicrobial agents and chemotherapy 44, 2693-2700. https://doi.org/10.1128/aac.44.10.2693-2700.2000

Kalogeropoulos, N., Konteles, S.J., Troullidou, E., Mourtzinos, I., Karathanos, V.T., 2009. Chemical composition, antioxidant activity and antimicrobial properties of propolis extracts from Greece and Cyprus. Food Chem. 116, 452-461. https://doi.org/10.1016/j. foodchem.2009.02.060.

Khosravi Rad, K., Falahati, M., Roudbary, M., Farahyar, S., Nami, S., 2017. Overexpression of *MDR-1* and *CDR*-2 genes in fluconazole resistance of *Candida albicans* isolated from patients with vulvovaginal candidiasis. Curr. Med. Mycol. 2, 24-29. https://doi.org/10.18869/acadpub.cmm.2.4.24

Kim, H.M., Kwon, H., Kim, K., Lee, S.E., 2018. Antifungal and antiaflatoxigenic activities of 1,8-Cineole and t-Cinnamaldehyde on Aspergillus flavus. Appl. Sci. 8, 1-9. https://doi.org/10.3390/app8091655

Kousedghi, H., Ahangari, Z., Eslami, G., Ayatolahi, A., 2012. Antibacterial activity of propolis and Ca(OH) 2 against *Lactobacillus, Entrococcus faecalis, Peptostreptococcus* and *Candida albicans*. African Journal of Microbiology. (6). https://doi.org/10.5897/AJMR12.029

Lopes, T.S., Fontoura, P.S., Oliveira, A., Rizzo, F.A., Silveira, S., Streck, A.F., 2020. Use of plant extracts and essential oils in the control of bovine mastitis. Res. Vet. Sci. 131, 186-193. https://doi.org/10.1016/j.rvsc.2020.04.025

Luqman, S., Dwivedi, G.R., Darokar, M.P., Kalra, A., Khanuja, S.P.S., 2008. Antimicrobial activity of *Eucalyptus citriodora* essential oil. Int. J. Essent. Oil. Ther. 2, 69-75.

Mehta, L.K., Balaraman, R., Amin, A.H., Baffa, P.A., Gulati, O.D., 2003. Effects of fruits of M. oleifera on the lipid profile of normal and hypercholesterolaemic rabbits. J. Ethnopharmacol. 86, 191-195. https://doi.org/10.1016/s0378-8741(03)00075-8

Mohamed, E., Abo Hagar, A., Hegazy, E., 2022. Evaluation of ERG-11 Gene Expression in Azole Resistant *Candida* Isolates from Various Clinical Specimens in ICUs. Microbes Infect. Dis. 3, 166-175. https://doi.org/10.21608/2021.95727.1192

Monapathi, M.E., Bezuidenhout, C.C., Rhode, O.H.J. 2018. Efflux pumps genes of clinical origin are related to those from fluconazole-resistant *Candida albicans* isolates from environmental water. Water Sci. Technol. 77, 899-908. https://doi.org/10.2166/wst.2017.607

Ożarowski, M., Karpiński, T.M., Alam, R., Łochyńska, M., 2022. Antifungal Properties of Chemically Defined Propolis from Various Geographical Regions. Microorganisms. 10, 364. https://doi.org/10.3390/microorganisms10020364

Pachauri, S., Varshney, P., Dash, S.K., Gupta, M.K., 2013. Involvement of fungal species in bovine mastitis in and around mathura, India. Vet. World. 6, 393-395. https://doi.org/10.5455/vetworld.2013.393-395

Palmeira-de-Oliveira, A., Salgueiro, L., Palmeira-de-Oliveira, R., Martinez-de-Oliveira, J., Pina-Vaz, C., Queiroz, J.A., Rodrigues, A.G., 2009. Anti-*Candida* activity of essential oils. Mini. Rev. Med. Chem. 9, 1292-1305. https://doi.org/1019080/ADOH.2018.08.555740

Pandey, A., Tripathi, S., 2014. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J. Pharmacogn. Phytochem. 2, 115-119.

Patel, P., Pater, N., Patel, D., Desai, S., Meshram, D., 2014. Phytochemical analysis and antifungal activity of *Moringa oleifera*. Int. J. Pharm. Pharm. Sci. 6, 144-147.

Paz, J.E.W., Contreras, C.R., Munguia, A.R., Aguilar, C.N., Inungaray, M.L.C., 2018. Phenolic content and antibacterial activity of extracts of Hamelia patens obtained by different extraction methods. Braz. J. Microbiol. 49, 656-661. https://doi.org/10.1016/j.bjm.2017.03.018

Pincus, D.H., Orenga, S., Chatellier, S., 2007. Yeast identification - Past, present, and future methods. Med. Mycol. 45, 97-121. https://doi.org/10.1080/13693780601059936

Ponzilacqua, B., Corassin, C.H., Oliveira, C.A.F., 2018. Antifungal Activity and Detoxification of Aflatoxins by Plant Extracts: Potential for Food Applications. Open Food Sci. J. 10, 24-32. https://doi.org/10.2174/1874256401810010024

Ramezani, H., Singh, H.P., Batish, D.R., Kohli, R.K., 2002. Antifungal activity of volatile oil of *Eucalyptus citriodora*. Fitoterapia. 73, 261-62. https://doi.org/10.1016/j.mycmed.2015.10.009

Roberson, J.R., Kalck, K.A., 2010. Treatment of *Candida*l Mastitis in Two Holstein Cows : a Case Report and Review. Bov. Pract. 44, 52-58.

Rocha, M.F.G., Alencar, L.P., Brilhante, R.S.N., Sales, J.A., Ponte, Y.B., Rodrigues, P.H., Sampaio, C.M.S., Cordeiro, R.A., Castelo-Branco, D.S.C.M., Oliveira, F.C., Barbosa, F.G., Teixeira, C.E.C., Paiva, M.A.N., Bandeira, T.J.P.G., Moreira, J.L.B., Sidrim, J.J.C. 2014. *Moringa oleifera* inhibits growth of *Candida* spp. and *Hortaea werneckii* isolated from Macrobrachium amazonicum prawn farming with a wide margin of safety. Ciência. Rural. 44, 2197-2203. https://doi.org/10.1590/0103-8478cr20140266

Sahar, M.K., Kafi, S.K., Elbir, H., 2015. The antimicrobial activity and phytochemical characteristic of *Moringa oleifera* seeds, leaves, and flowers. World J. Pharm. Res. 4, 258-271.

Sonmez, M., Erbas, G., 2017. Isolation and Identification of *Candida* spp. from Mastitis Cattle Milk and Determination of Antifungal Susceptibilities. Inter. J. Vet. Sci. 6, 104-107.

Spanamberg, A., Fraga, C.F., Ferreiro, L., Aguinsky, M.S., Sanches, E.M.C., Roehe, C., Lautert, C., Santurio, J.M., 2014. Yeasts in the Raw Ewe's Milk. Acta. Scientiae Veterinariae. 42, 1-5.

Tian, M., Xu, X., Liu, F., Fan, X., Pan, S., 2018. Untargeted metabolomics reveals predominant alterations in primary metabolites of broccoli sprouts in response to preharvest selenium treatment. Food Res. Int. 111, 205-211. https://doi.org/10.1016/j.foodres.2018.04.020

Wang, B., Huang, L.H., Zhao, J.X., Wei, M., Fang, H., Wang, D.Y., Wang, H.F., Yin, J.G., Xiang, M., 2015. *ERG11* mutations associated with azole resistance in *Candida albicans* isolates from vulvovaginal candidosis patients. Asian Pac. J. Trop. Biomed. 5, 909-914. https://doi.org/10.1016/j.apjtb.2015.08.002

Wayne, P.A., 2020. Clinical and laboratory standards institute; Performance Standards for Antifungal Susceptibility Testing of Yeasts. 2nd edn. CLSI supplement M60.

Wilkinson, J.M., Cavanagh, H.M., 2005. Antibacterial activity of Essential oils from Australian native plants. Phytother. Res. 19, 643-646. https://doi.org/10.1002/ptr.1716

Zaragoza, C.S., Olivares, R.A., Watty, A.E., Moctezuma, Ade. L., Tanaca, L.V., 2011. Yeasts isolation from bovine mammary glands under different mastitis status in the Mexican High Plateu. Rev. Iberoam. Micol. 28, 79 82. https://doi.org/10.1016/j.riam.2011.01.002