

**FULL PAPER**

# Saponins from *Mimusops laurifolia* target some key virulence factors in *Candida albicans*

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*Mimusops laurifolia* (The Tree of Life, as it was known by the ancient Egyptians) belongs to the *Mimusops* genus, which has been used in folk medicine for a very long time. Recently, it has been concluded that a crude extract containing the saponins from the leaves of *M. laurifolia* has promising antifungal activity against *Candida albicans*. This fungus is a widely known opportunistic pathogen linked to systemic and chronic infections. As a result, the current study aims to investigate the anti-virulence potential of these saponins extract against *C. albicans* at the molecular level in an attempt to better understand how these saponins might function as an antifungal agent against *C. albicans*. Real-time PCR was used to quantify the relative gene expression levels of critical virulence factors in *C. albicans* cells treated with saponins versus untreated cells. The potential of these saponins as antifungal agents is likely to be attributed to their gene-regulatory activity, as the expression of some key genes in *C. albicans* (involved in invasion, survival, and adhesion, such as *SAP3*, *SAP5*, and *ALS3*) was strongly downregulated in saponin-exposed *Candida* cells. Generally, these data show that *M. laurifolia*-derived saponins may be efficient inhibitors and virulence modulators of *C. albicans*. Thus, the current study broadens our knowledge about saponin's potential antifungal properties. Moreover, our discovery of a strong suppressor that acts specifically on virulence-related genes paves the way to develop an oriented class of antifungal drugs.

**KEYWORDS**

Anti-virulence efficacy; agglutinin-like sequence 3; *Candida albicans*; *Mimusops laurifolia*; real-time PCR; secreted aspartyl proteinases.

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**Introduction**

*Candida albicans* is a common member of the human microbiota. It is detected normally in the mouth, genitourinary tract, and gastrointestinal tract in most healthy people [1-5]. However, when the host's immune system is impaired as a result of hospital stay, antibiotic therapy, operation, catheterization,

or use of prosthetic devices [6], *C. albicans* can result in infections, which vary from mucosal infections to serious systemic infections [7]. The emergence of pathogenic *C. albicans* depends not only on host factors, but also on fungal factors [8]. Deficiency in the immune system, epithelial injury, and microbial dysbiosis are examples of host factors [9]. To be pathogenic, microorganisms

require to evolve a strategy that allows them to colonize or infect the host successfully [10]. Consequently, most pathogenic microorganisms, including different species of *Candida*, have established a robust set of virulence determinants and particular mechanisms to help them infiltrate host tissues, develop infectious disease, and escape from host immunity. *Candida* species, particularly *C. albicans*, may express a multitude of virulence factors, depending on the type of infection (superficial or systemic), the infected area of the body, the phase of infection, and the nature of host defense [11].

In contrast to highly specialized pathogenic microorganisms that mainly express a single key virulence factor (for example, *C. tetani*), the pathogenic *C. albicans* can express numerous factors that participate in virulence and stabilization of infection, especially in immunocompromised hosts [12].

Despite the fact that *C. albicans* has a diversity of hydrolytic enzymes [13], secreted aspartyl proteinases (Saps) are the most strongly linked to virulence [11].

Several studies have extensively discussed and emphasized the significant role of Saps in the emergence of *C. albicans*-related infections. They facilitate the *C. albicans* pathogenicity through invasion of the host tissue and by destroying the cell membrane. They can also enable the pathogen to skip the defense mechanisms of the host's immunity by attacking its cells. It was further suggested that Saps are involved in the attachment of pathogenic *C. albicans* to the tissue cells, which is considered as an initiative phase for pathogenesis [14].

In spite of the fact that biofilm formation is most likely the best-known virulence factor of *C. albicans*, the expression of secreted aspartyl proteinases (SAPs) has been extensively researched as a significant virulence factor [15].

Several studies have found that: (i) the deletion of SAP genes from *C. albicans* results

in a less virulent strain than the original one, and also (ii) inhibitors of that protease result in attenuated *C. albicans* with less virulence [16].

Secreted aspartyl proteinase enzymes are typically found in the majority of *Candida* species, and they are expressed by at least ten genes (i.e. *SAP1-SAP10*) [17].

Moreover, Shi et al. reported that some genes had been known as biofilm formation determinants, and pointed out that the adhesin Als3p (agglutinin-like sequence 3) is a Hypha-specific cell wall protein [18].

It was also clarified that Als3 supports the attachment of pathogens to the endothelial cells of the human umbilical vein and the oral epithelial cells [19].

*C. albicans*' ability to attach to and colonize various types of tissues has been shown to increase its pathogenic potential [20].

Some antifungal medications can act against virulence determinants [21].

However, the rising incidence of the development of antifungal resistance after long-term treatment has evoked researchers' interest in finding new antifungal drugs [22].

As a result, continuing to investigate natural materials (such as plants) for antimicrobial action is critical in the search for new medicinal drugs [23].

According to the World Health Organisation (WHO), medicinal plants could be the best source for receiving various medications. According to Manandhar et al., numerous medicinal plants are significant sources of natural antibacterial and antifungal agents [24].

*Mimusops laurifolia* (*M. laurifolia*) has been mentioned in Egyptian historical records since the Eighteenth Dynasty and was known as the "Tree of Life". Its evergreen leaves commonly existed with Egyptian mummies and were discovered in king Tutankhamun's tomb [25]. Likewise, *M. laurifolia* is extensively dispensed throughout the Arabian Peninsula and is the most significant plant species in the Arabian

distinct due to its glamorous ethno-botanical history [26].

It is regarded as a medicinal plant and continues to be widely used by locals in southwest Saudi Arabia for a variety of medical purposes [27].

*M. laurifolia* is a member of the *Mimusops* genus, which is well-known in Indian traditional medicine as a source of fever reducers, astringents, laxatives, and stimulant agents [28,29]. It is worth mentioning that *Mimusopus elengi* is a well-known plant in traditional herbal medicine, with its leaves being used to cure various bacterial diseases [30].

Mostafa et al. have stated that saponins extracted from *M. laurifolia* leaves exhibit a promising antifungal action against *C. albicans* [31].

Therefore, we hypothesised that these saponins may exhibit this action through affecting specific virulent genes in *C. albicans* at the transcriptional level.

As far as we know, no other investigations have been done on the anti-candida activity of saponins extracted from *M. laurifolia* leaves, particularly on the molecular level. Therefore, we have been motivated to continue our previous research and conduct this pioneer research to investigate more deeply how these saponins could work as an antifungal agent against *C. albicans*.

## Experimental

### *Plant material*

The Egyptian Museum Garden in Cairo, Egypt, is the source of *Mimusopus laurifolia* (Forssk.) Friis. Prof. Abdel Halim Abdel Motjale, Chief Researcher and Head of the Flora and Phytotaxonomy Research Department, Agriculture Museum, Giza, Egypt, verified the plant's authenticity. The Plant List website (<http://www.theplantlist.org>) was used to verify the plant's name. The sample was consigned in the Faculty of Postgraduate

Studies for Advanced Sciences, Biotechnology and Life Sciences Department, Beni-Suef University, Egypt, with the spicemen voucher number (PSAS-BT01) [31].

### *Preparation and usage of crude saponins*

In brief, the collected leaves were dried and milled into a fine powder, then 80 g of the powdered leaves were mixed with 350 ml of 80% methyl alcohol and left for two days at room temperature with continuous shaking at the rate of 150 rpm. After filtration of the mixture, the maceration process of the residue was repeated twice to increase the yield. Next, the filtrates were collected and evaporated at 40 °C using a rotary evaporator. Next, the concentrate was freeze-dried using a lyophilizer to produce a yellowish-colored residue.

A volume of 100 mL of distilled water was mixed with the obtained residue before being transferred to a separating funnel and mixed with 100 ml chloroform. The separating funnel was shaken and held for a while to obtain two completely separated layers. The bottom layer containing chloroform was removed. The aqueous solution was extracted several times with chloroform to ensure complete extraction.

The remaining aqueous layer was then extracted with 100 ml of saturated n-butanol. At that time, the aqueous layer was the lower layer. This process was carried out three times. The n-butanol layers were collected and evaporated at 40 °C using a rotary evaporator to get the n-butanol fraction [32].

To improve the saponin yield, 300 mL of cold acetone was added to the n-butanol fraction dissolved in methanol [33].

Finally, the precipitate was then filtered and dried. This precipitate, which represented the crude saponins, was then kept in a sealed container in the refrigerator until required. As reported in our previous work, a saponin froth test confirmed that the obtained precipitate was rich in saponins. In

addition, saponins study using HPLC/UV, performed in accordance with the guidelines established by *Thalhamer and Himmelsbach* [34], confirmed the existence of 15 saponin components [31].

6.4  $\mu\text{g}\cdot\text{ml}^{-1}$  of saponins extract, which represent the minimum inhibitory concentration of the extracted saponins [31], was prepared and sterilized through a Millipore sterile syringe filter immediately before use.

#### *Effect of saponins extract on expression levels of C. albicans virulence genes*

To study the effect of saponins mixture extracted from *M. laurifolia* leaves on the gene expression of some virulent enzymes in *C. albicans* (*SAP3*, *SAP5*, and *ALS3*), a one-step reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay was carried out.

Briefly, *C. albicans* ATCC 60193 was obtained from the Department of Microbiology, Faculty of Pharmacy, Beni-Suef University. One colony of *C. albicans* cultured on Sabouraud dextrose agar plate was sub-cultured in 10 mL of Sabouraud dextrose liquid medium, and the culture was grown for 18 hours at 30 °C to verify the purity [35,36].

As described by earlier studies [37,38], *C. albicans* were placed in 10 mL of RPMI 1640 (with L-glutamine) to obtain a suspension of the desired final concentration of  $1 \times 10^6$  cells/ml. *C. albicans* suspension was supplemented with the saponins extract's MIC value ( $6.4 \mu\text{g}\cdot\text{ml}^{-1}$ ) [31] and incubated for 24 hours with brief shaking.

The negative control contained *C. albicans* cells incubated under identical experimental circumstances but without saponin exposure.

Next, the manufacturer's instructions of TRI Reagent® and Direct-zol™ RNA MiniPre kits were followed to isolate and purify total RNA from the centrifugated *C. albicans* cells.

A one-step kit (HERA SYBR® Green RT-qPCR kit) was used to perform reverse transcription of the total RNA to prepare cDNA templates and perform qPCR analysis. RT-qPCR was performed following the manufacturer's instructions. Primer sequences of the investigated virulence genes are presented in Table 1.

As an endogenous control, the housekeeping gene ACT1 was utilized. The relative gene expression of each targeted virulent factor was compared to the corresponding one of untreated *C. albicans* cells (expression = 1). The formula  $2^{-\Delta\Delta CT}$  was used to calculate the results. Each assay was independently conducted three times to obtain the mean value. The expression change of each investigated gene was displayed as a fold change in expression. In comparison to the control samples (untreated cells), the results show a logarithmic fold increase.

The following equation was used to determine the relative fold change: Fold change =  $2^{-\Delta\Delta CT}$ .

The mean CT (cycle threshold) value of the house keeping gene (ACT1) was subtracted from the target gene to calculate its normalized CT value, represented as  $\Delta CT$ , and then to determine the  $\Delta\Delta CT$  value, the calculated  $\Delta CT$  of the gene of interest in the control sample (untreated cells) was subtracted from the  $\Delta CT$  value of the corresponding gene in the saponins treated cells (test sample).

**TABLE 1** Primers used in quantitative PCR for the investigated genes

Target gene	Primers sequence <sup>a</sup>	Ta* (°C)	Reference of primers sequence
SAP3	CAGCTTCTGAATTTACTGCTCCATT	53	[39]
	TCCAAAAAGAAGTTGACATTGATCA		
SAP5	TGTTGACACTGGGTCTTCT	48	[40]
	ATACAACTTACCTTTAGCG		
ALS3	ACTAAAGGAAACAACGGAAATG	51	[40]
	AGAAATGATAGGCGATGAAGC		

<sup>a</sup> Primer sequences are presented in the 5' to 3' direction.

\*Ta: annealing temperature.

### Statistical analysis

Every experiment was carried out at least three times, with assay values represented as means  $\pm$  SD. GraphPad Prism software was utilized to perform the statistical analysis. All comparisons were made using the Kruskal-Wallis test, followed by the Dunn test.

### Results and discussion

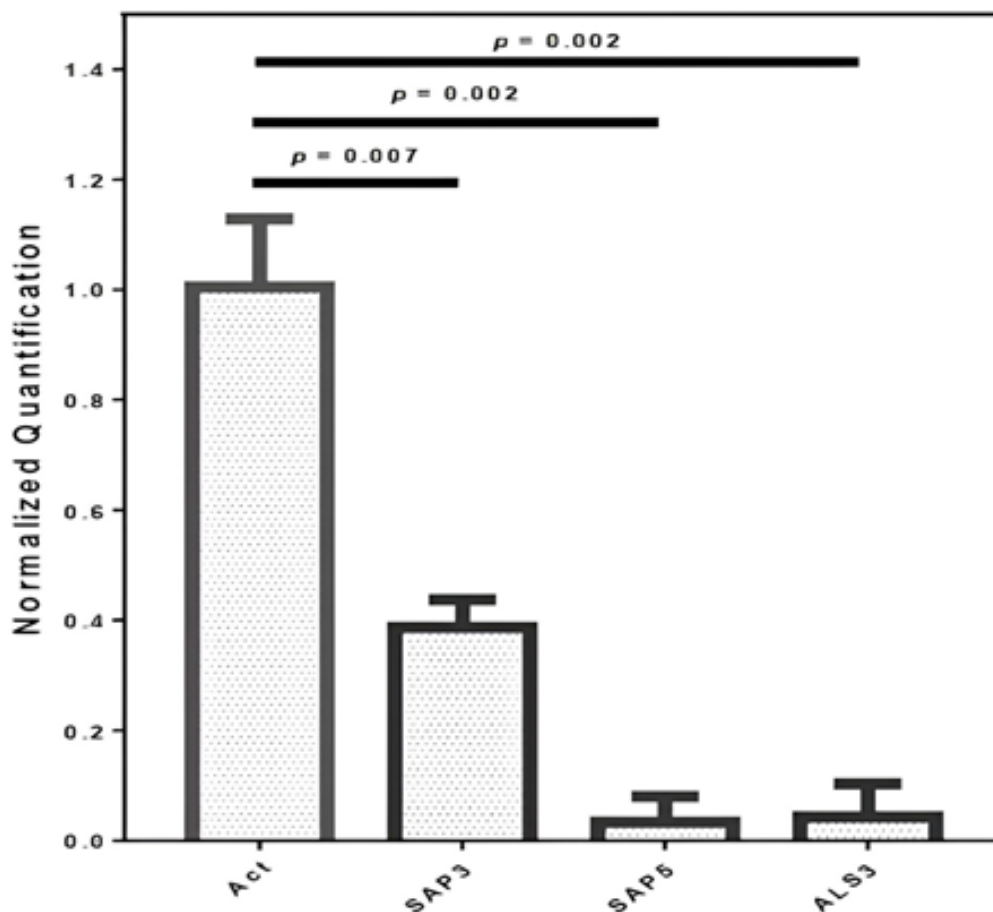
In an attempt to learn more about the anti-virulence activity of saponins extracted from *M. laurifolia* leaves and how they can inhibit *C. albicans* growth, we examined the expression profiles of certain key adhesion, invasion, and survival genes.

Real-time PCR was conducted to test the saponins mixture for possible activity against certain virulent genes in *C. albicans* ATCC 60193, in an effort to further understand its antifungal mechanism.

A total of three crucial virulence-related genes in *C. albicans* were investigated, namely, *SAP3*, *SAP5*, and *ALS3*. Following saponins treatment, the three tested genes had lower mRNA levels in the cells treated with saponins than in the control group.

Saponins significantly suppressed the expression of the *SAP3*, *SAP5*, as well as *ALS3* genes by 61% ( $p = 0.002$ ), 97% ( $p = 0.002$ ), and 96% ( $p = 0.007$ ), respectively, according to our real-time PCR (qPCR) results (Figure 1, Table 2).





**FIGURE 1** Relative fold changes of *SAP3*, *SAP5*, and *ALS3* normalized to  $\beta$ -actin in *C. albicans* ATCC 60193 treated with saponins extract from *M. laurifolia* leaves as determined by real-time PCR. *C. albicans* 60193 cells at a final concentration of  $1 \times 10^6$  cells/ml were exposed to the saponins extract at  $6.4 \mu\text{g ml}^{-1}$  and incubated for 24 hours. Following cell harvest, total RNA was isolated, reverse-transcribed to cDNA, and the expression levels of three target genes (*SAP3*, *SAP5*, and *ALS3*) in the test organism were examined using real-time PCR (see Materials and Methods). Experiments were run with at least three technical replicates of each. Mean values of the relative fold changes ( $\pm$  SD) are shown in relation to untreated (control) *C. albicans* 60193 cells.

**TABLE 2** Expression fold changes of some important virulence-related genes in *C. albicans* after treatment with crude saponins from *M. laurifolia* leaves

	<i>ACT</i>		<i>SAP3</i>	<i>SAP5</i>		<i>ALS3</i>	
	Average CT	Average CT	Fold change	Average CT	Fold change	Average CT	Fold change
<b>Control</b>	17.5	25.3	0.39	24.4	0.03	25.8	0.04
<b>Test</b>	20.6	30		36.1		36.6	

The illustrated fold changes in gene expression are relative to the control group's results (*C. albicans* ATCC 60193 not exposed to saponins mixture). In addition, the expression data were normalized using the

housekeeping gene *ACT1*. The results are displayed as the means of three separate assays (Figure 1, Table 2).

All of the genes that have been downregulated play important roles in the

pathogenicity and virulence of *C. albicans* ATCC 60193. Aspartyl protease enzymes (encoded by the *SAP3* and *SAP5* genes) are fungus-secreted enzymes that may facilitate infection through catalyzing the hydrolysis of host cell membrane components [14].

The *ALS3* gene belongs to the ALS gene family that is required for *C. albicans* adherence [41].

Candidiasis is getting more prevalent throughout the world [42].

Doubtless, virulence factors enhance a microorganism's ability to establish itself in or within a host as well as its ability to cause disease [43].

Targeting virulence factors and the genes that encode them to disarm pathogens is a promising approach for preventing and curing infectious diseases [44,45].

Rather than direct biocidal activity, one of the current paradigms in developing novel antimicrobial medicines is to target the microorganism's virulence factors [46,47].

For decades, medicinal plants have participated in improving health and treating some particular ailments [48]. It has been reported that natural products such as plants give the possibility to develop novel drugs as a result of having various chemical compounds [49].

According to 111 scientific documents released between 1969 and 2015, *C. albicans* exhibited susceptibility to 142 natural compounds [50].

*M. laurifolia* (Forssk.) Friis, of the family Sapotaceae, is a native species confined to the Red Sea Mountains and the Gulf of Aden [51].

It is considered as a medicinal plant and is still commonly used by the locals in Saudi Arabia for various therapeutic uses [27].

It was discovered that their leaves contained fifteen triterpenoidal saponins [52].

Recently, saponins have attracted more attention because of their unique chemical composition and biological properties. Triterpenoid saponins, which are found in a

variety of higher plants, have a variety of pharmacological actions, including antibacterial and antifungal activities [53].

Since saponins extract in our previous study showed a notable antifungal efficacy against *C. albicans* with MIC value of 6.4  $\mu\text{g}\cdot\text{ml}^{-1}$  and the molecular docking analysis and molecular dynamic simulation of the investigated saponins showed their affinity toward the docked enzymes, especially *SAP3* and *SAP5* [31], we hypothesized that these saponins may also affect some virulent genes in *C. albicans* at the transcriptional level. The current work demonstrated the value of using saponins to address some genes that code for virulent components. Several studies have already reported the role of genes investigated in the current study in the virulence of *C. albicans* [14-16,20,54].

Eskander *et al.* stated that the saponins extract of *M. laurifolia* leaves comprises several components (saponin 1-saponin 15) [52].

Therefore, its antifungal effect could be multitarget. In light of this, *C. albicans* ATCC 60193 was exposed in RPMI 1640 media to MIC value (6.4  $\mu\text{g}\cdot\text{ml}^{-1}$ ) of a saponins mixture extracted from the leaves of *M. laurifolia*. As expected, the findings of real-time PCR revealed that following exposure to the saponin compounds, some important genes related to survival, adhesion, and invasion were indeed differentially expressed.

This study observed a strong effect of saponins extract on the expression of *SAP3* and *SAP5* genes in *C. albicans* that was apparently suppressed by 0.61 and 0.97 folds, respectively. Aspartyl protease enzymes are important virulence factors in *C. albicans*, as they participate in tissue attack, biofilm production, adhesion, as well as phenotypic switching<sup>7</sup>.

Moreover, a study conducted by Kadry *et al.* has verified the linkage between reduced sensitivity to antifungal medications and aspartyl proteinase production, as it has been reported that all antifungal resistant isolates

were able to produce aspartyl proteinase [55].

Moreover, the saponins mixture sharply inhibited the expression level of the *ALS3* gene, which is essential for the *fungus* to develop biofilm, adhere to, and aggregate with other substances [56].

In fungal infection, attachment to host tissues belongs to the initial stages of the infection process, and inhibiting adherence restrains the colonization and infection [57]. Overall, this genetic assay demonstrated the ability of saponins extracts to attenuate *C. albicans* pathogenicity via affecting the expression of some fungal master genes.

We suppose that, this work is the first to characterize the anti-virulent activity of saponins derived from *M. laurifolia* leaves, particularly at the transcriptional level. However, our investigation's findings agree with the findings of some studies conducted to investigate the antifungal mode of action of saponins extracted from different plants.

Regarding the effect of saponins on adherence, a study conducted by Patel *et al.* showed that the extract of *Dodonaea viscosa* var. *angustifolia*, which contains several phytochemical substances including saponins, reduced the attachment of *C. albicans* to oral mucosa. Moreover, they reported that the investigated extract may reduce the biosynthesis of adhesins and subsequently reduce the attachment of *C. albicans* to epithelial tissue via affecting *ALS* genes [57].

Furthermore, as shown in research of various biological activities related to phytochemicals, flavonoids, alkaloids, and saponins are expected to have anti-SAP activity or a synergistic effect that boosts this activity [58].

## Conclusion

Our findings revealed that saponins extract of *M. laurifolia* leaves is a promising and potential anti-candida agent that affects some

basic vital processes through inhibition of some key virulence factors of *C. albicans*. Interestingly, our research revealed a virulence-selective treatment strategy. This research will pave the way for more preclinical and clinical research to inspect the potential of saponins from *M. laurifolia* as a new natural product-based anticandidal medication. Furthermore, despite the fact that resistance to available antifungals is common, our findings revealed that a saponin mixture extracted from *M. laurifolia* leaves exhibited antifungal activity against *C. albicans* by affecting several different virulence strategies of the fungal cells, implying that resistance to these compounds is supposed to be unlikely. As a result, combining saponin with other antifungal agents presently on the market could be a promising strategy for treating resistant *C. albicans* strains. Consequently, more research into this anticipated synergistic interaction as well as the potential of combination therapy may provide insight.

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## Authors' contributions

Mai Raslan, Ahmed S. Khairalla, Ahmed O. El-Gendy, Medhat abd El Fattah, and Hayam M. A. Mostafa designed the research. Mai Raslan, Ahmed O. El-Gendy, and Hayam M. A. Mostafa conducted the research. Ahmed O. El-Gendy, and Hayam M. A. Mostafa analyzed the data. All authors contributed to writing and finalizing the manuscript.

## Conflict of interest

There is no conflict of interest to declare in this article.



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**References**

- [1] N. Martins, I.C. Ferreira, L. Barros, S. Silva, M. Henriques, Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment, *Mycopathologia.*, **2014**, *177*, 223-240. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [2] N.M. Muna, A.D.W. Widodo, P.D. Endraswari, M.V. Arfijanto, Correlation Between the Bacterial and Fungal Profiles from the Clinical Specimens with the CD4 Counts and the NLR Values of HIV/AIDS Patients at Tertiary Referral Hospital in Indonesia, *J. Med. Chem. Sci.*, **2023**, *6*, 2111-2127. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [3] H.J. Naser, F.M. Abdul-Ameer, Evaluation of the Effect of Lemongrass Essential Oil on Candida Albicans Adhesion on Heat Cured Acrylic Based Soft Lining Material, *J. Med. Chem. Sci.*, **2023**, *6*, 1685-1695. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [4] D. Murdiyanto, A. Faizah, M.D. Mustikaningrum, Inhibition of Garlic Ethanol Extracts (*Allium Sativum* L.) as a Solvent in Alginate Impression Materials in the Growth of Candida Albicans, *J. Med. Chem. Sci.*, **2022**, *5*, 144-152. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [5] D.U.J. N'Guessan, S. Coulibaly, F.K.K. Kassi, P. Delaye, M. Penichon, C. Enguehard-Gueiffier, H. Allouchi, M. Ouattara, Synthesis and SAR of Imidazo[1,2-a] Pyridinyl-Phenylacrylonitrile Derivatives as Potent Anticandidosis Agents, *J. Med. Chem. Sci.*, **2021**, *4*, 554-563. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [6] S.M. Noble, B.A. Gianetti, J.N. Witchley, Candida albicans cell-type switching and functional plasticity in the mammalian host, *Nat. Rev. Microbiol.*, **2017**, *15*, 96-108. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [7] I.D. Jacobsen, D. Wilson, B. Wächtler, S. Brunke, J.R. Naglik, B. Hube, Candida albicans dimorphism as a therapeutic target, *Expert. Rev. Anti. Infect. Ther.*, **2012**, *10*, 85-93. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [8] R.A. Hall, M.C. Noverr, Fungal interactions with the human host: exploring the spectrum of symbiosis, *Curr. Opin. Microbiol.*, **2017**, *40*, 58-64. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [9] J. Ni, G.D. Wu, L. Albenberg, V.T. Tomov, Gut microbiota and IBD: causation or correlation?, *Nat. Rev. Gastroenterol. Hepatol.*, **2017**, *14*, 573-584. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [10] B.B. Finlay, S. Falkow, Common themes in microbial pathogenicity revisited, *Microbiol. Mol. Biol. Rev.*, **1997**, *61*, 136-169. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [11] J.R. Naglik, S.J. Challacombe, B. Hube, Candida albicans secreted aspartyl proteinases in virulence and pathogenesis, *Microbiol. Mol. Biol. Rev.*, **2003**, *67*, 400-428. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [12] C.A. Kumamoto, M.D. Vences, Contributions of hyphae and hypha-co-regulated genes to Candida albicans virulence, *Cell. Microbiol.*, **2005**, *7*, 1546-1554. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [13] T. Wibawa, The role of virulence factors in Candida albicans pathogenicity, *J. Med. Sci.*, **2016**, *48*, 58-68. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [14] M. Schaller, C. Borelli, H.C. Korting, B. Hube, Hydrolytic enzymes as virulence factors of Candida albicans, *Mycoses.*, **2005**, *48*, 365-377. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

- [15] R.A. Calderone, W.A. Fonzi, Virulence factors of *Candida albicans*, *Trends Microbiol.*, **2001**, *9*, 327-335. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [16] M. Vilanova, L. Teixeira, Í. Caramalho, E. Torrado, A. Marques, P. Madureira, A. Ribeiro, P. Ferreira, M. Gama, J. Demengeot, Protection against systemic candidiasis in mice immunized with secreted aspartic proteinase 2, *Immunology.*, **2004**, *111*, 334-42. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [17] W.L. Chaffin, *Candida albicans* cell wall proteins, *Microbiol. Mol. Biol. Rev.*, **2008**, *72*, 495-544. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [18] D. Shi, Y. Zhao, H. Yan, H. Fu, Y. Shen, G. Lu, H. Mei, Y. Qiu, D. Li, W. Liu, Antifungal effects of undecylenic acid on the biofilm formation of *Candida albicans*, *Int. J. Clin. Pharmacol. Ther.*, **2016**, *54*, 343-353. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [19] X. Zhao, S.H. Oh, G. Cheng, C.B. Green, J.A. Nuessen, K. Yeater, R.P. Leng, A.J. Brown, L.L. Hoyer, ALS3 and ALS8 represent a single locus that encodes a *Candida albicans* adhesin; functional comparisons between Als3p and Als1p, *Microbiology.*, **2004**, *150*, 2415-2428. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [20] N. Tsuchimori, L.L. Sharkey, W.A. Fonzi, S.W. French, J.E. Edwards Jr, S.G. Filler, Reduced virulence of HWP1-deficient mutants of *Candida albicans* and their interactions with host cells, *Infect. Immun.*, **2000**, *68*, 1997-2002. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [21] K. Ishida, J.C.P. de Mello, D.A.G. Cortez, B.P.D. Filho, T. Ueda-Nakamura, C.V. Nakamura, Influence of tannins from *Stryphnodendron adstringens* on growth and virulence factors of *Candida albicans*. *J. Antimicrob. Chemother.*, **2006**, *58*, 942-949. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [22] R. Giordani, P. Regli, J. Kaloustian, C. Mikail, L. Abou, H. Portugal, Antifungal effect of various essential oils against *Candida albicans*. Potentiation of antifungal action of amphotericin B by essential oil from *Thymus vulgaris*, *Phytother Res.*, **2004**, *18*, 990-995. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [23] G.G. Nascimento, J. Locatelli, P.C. Freitas, G.L. Silva, Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria, *Braz. J. Microbiol.*, **2000**, *31*, 247-256. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [24] S. Manandhar, S. Luitel, R.K. Dahal, In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria, *J. Trop. Med.*, **2019**, **2019**. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [25] I.B. Friis, Forests and forest trees of northeast tropical Africa: their natural habitats and distribution patterns in Ethiopia, *Djibouti and Somalia. HMSO.*, **1992**. [[Google Scholar](#)], [[Publisher](#)]
- [26] M. Hall, S. Neale, T.M. Al-Abbasi, A.G. Miller, Arabia's tallest trees: ecology, distribution and conservation status of the regionally endangered tree species *Mimusops laurifolia*, *Nord. J. Bot.*, **2010**, *28*, 240-245. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [27] A.K. Hegazy, A.A. Alatar, J. Thomas, M. Faisal, A.H. Alfarhan, K. Krzywinski, Compatibility and complementarity of indigenous and scientific knowledge of wild plants in the highlands of southwest Saudi Arabia, *J. For. Res.*, **2014**, *25*, 437-444. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [28] N.P. Sahu, K. Koike, Z. Jia, T. Nikaido, Novel triterpenoid saponins from *Mimusops elengi*, *Tetrahedron.*, **1995**, *51*, 13435-46. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [29] P.J. Shah, M.S. Gandhi, M.B. Shah, S.S. Goswami, D. Santani, Study of *Mimusops elengi* bark in experimental gastric ulcers, *J. Ethnopharmacol.*, **2003**, *89*, 305-11. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [30] M. Padhi, S. Mahapatra, Evaluation of Antibacterial Potential of Leaf extracts of *Mimusops elengi*, *Int. Res. J. Biol. Sci.*, **2013**, *2*, 46-49. [[Google Scholar](#)], [[Publisher](#)]
- [31] H.M. Mostafa, M. Taha, A.O. El-Gendy, A.S. Khairalla, M. Abd El Fattah, M. Raslan,

- Antimicrobial and antivirulence saponins of *Mimusops laurifolia* leaves, *Let. Appl. Microbiol.*, **2023**, *76*, 19:ovad071. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [32] M. Molla, N. Gameda, S.M. Abay, Investigating potential modes of actions of *Mimusops kummel* fruit extract and solvent fractions for their antidiarrheal activities in mice, *Evid. Based Complement. Altern. Med.*, **2017**, *2017*. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [33] L.S. Chua, C.H. Lau, C.Y. Chew, D.A.S. Dawood, Solvent fractionation and acetone precipitation for crude saponins from *Eurycoma longifolia* extract, *Molecules*, **2019**, *24*, 1416. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [34] B. Thalhamer, M. Himmelsbach, Characterization of quillaja bark extracts and evaluation of their purity using liquid chromatography high resolution mass spectrometry, *Phytochem. Lett.*, **2014**, *8*, 97-100. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [35] S. Theberge, A. Semlali, A. Alamri, K.P. Leung, M. Rouabhia, *C. albicans* growth, transition, biofilm formation, and gene expression modulation by antimicrobial decapeptide KSL-W, *BMC Microbiol.*, **2013**, *13*, 246. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [36] A. Semlali, K. Killer, H. Alanazi, W. Chmielewski, M. Rouabhia, Cigarette smoke condensate increases *C. albicans* adhesion, growth, biofilm formation, and EAP1, HWP1 and SAP2 gene expression, *BMC Microbiol.*, **2014**, *14*, 1-9. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [37] W. Gu, D. Guo, L. Zhang, D. Xu, S. Sun, The synergistic effect of azoles and fluoxetine against resistant *Candida albicans* strains is attributed to attenuating fungal virulence, *Antimicrob. Agents Chemother.*, **2016**, *60*, 6179-6188. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [38] M.M. Said, C. Watson, D. Grando, Garlic alters the expression of putative virulence factor genes SIR2 and ECE1 in vulvovaginal *C. albicans* isolates, *Sci. Rep.*, **2020**, *10*, 3615. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [39] H. Nailis, S. Kucharíková, M. Řičicová, P. Van Dijck, D. Deforce, H. Nelis, T. Coenye, Real-time PCR expression profiling of genes encoding potential virulence factors in *Candida albicans* biofilms: identification of model-dependent and-independent gene expression, *BMC Microbiol.*, **2010**, *10*, 1-11. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [40] H. Zhong, D.D. Hu, G.H. Hu, J. Su, S. Bi, Z.E. Zhang, Z. Wang, R.L. Zhang, Z. Xu, Y.Y. Jiang, Y. Wang, Activity of sanguinarine against *Candida albicans* biofilms, *Antimicrob. Agents Chemother.*, **2017**, *61*, 10-1128. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [41] G. Tronchin, M. Pihet, L.M. Lopes-Bezerra, J.P. Bouchara, Adherence mechanisms in human pathogenic fungi, *Sabouraudia*, **2008**, *46*, 749-772. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [42] J. Irish, D.A. Carter, T. Shokohi, S.E. Blair, Honey has an antifungal effect against *Candida* species, *Med. Mycol.*, **2006**, *44*, 289-291. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [43] L. Chen, J. Yang, J. Yu, Z. Yao, L. Sun, Y. Shen, Q. Jin, VFDB: a reference database for bacterial virulence factors, *Nucleic Acids Res.*, **2005**, *33*, D325-8. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [44] L. Cegelski, G.R. Marshall, G.R. Eldridge, S.J. Hultgren, The biology and future prospects of antivirulence therapies, *Nat. Rev. Microbiol.*, **2008**, *6*, 17-27. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [45] C.F. Low, P.P. Chong, P.V.C. Yong, C.S.Y. Lim, Z. Ahmad, F. Othman, Inhibition of hyphae formation and SIR2 expression in *Candida albicans* treated with fresh *Allium sativum* (garlic) extract, *J. Appl. Microbiol.*, **2008**, *105*, 2169-2177. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [46] K. Gauwerky, C. Borelli, H.C. Korting, Targeting virulence: a new paradigm for antifungals, *Drug Discov. Today*, **2009**, *14*, 214-222. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

- [47] A. Bink, K. Pellens, B. PA Cammue, K. Thevissen, Anti-biofilm strategies: how to eradicate *Candida* biofilms?, *The Open Mycology Journal*, **2011**, *5*, 29-38. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [48] M. Chevalier, A. Doglio, R. Rajendran, G. Ramage, I. Prêcheur, S. Ranque, Inhibition of adhesion-specific genes by *Solidago virgaurea* extract causes loss of *Candida albicans* biofilm integrity, *J. Appl. Microbiol.*, **2019**, *127*, 68-77. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [49] L.J. McGaw, N. Lall, T.M. Hlokwé, A.L. Michel, J.J.M. Meyer, J.N. Eloff, Purified compounds and extracts from *Euclea* species with antimycobacterial activity against *Mycobacterium bovis* and fast-growing mycobacteria, *Biol. Pharm. Bull.*, **2008**, *31*, 1429-1433. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [50] A. Zida, S. Bamba, A. Yacouba, R. Ouedraogo-Traore, R.T. Guiguemdé, Anti-*Candida albicans* natural products, sources of new antifungal drugs: A review, *J. Mycol. Med.*, **2017**, *27*, 1-19. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [51] I. Friis, *The taxonomy and distribution of Mimusops laurifolia (Sapotaceae)*, *Kew Bulletin.*, **1980**, *1*, 785-792. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [52] J. Eskander, C. Lavaud, S.M. Abdel-Khalik, H.S. Soliman, I.I. Mahmoud, C. Long, Saponins from the Leaves of *Mimusops laurifolia*, *J. Nat. Prod.*, **2005**, *68*, 832-841. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [53] P. Koczurkiewicz, J. Czyż, I. Podolak, K. Wójcik, A. Galanty, Z. Janeczko, M. Michalik, Multidirectional effects of triterpene saponins on cancer cells-mini-review of in vitro studies, *Acta Biochim. Pol.*, **2015**, *62*, 383-393. [[Pdf](#)], [[Google Scholar](#)], [[Publisher](#)]
- [54] M. Staniszevska, M. Bondaryk, K. Siennicka, J. Pilat, M. Schaller, W. Kurzatkowski, Role of aspartic proteinases in *Candida albicans* virulence. Part I. Substrate specificity of aspartic proteinases and *Candida albicans* pathogenesis, *Postępy Mikrobiologii*, **2012**, *51*, 127-135. [[Pdf](#)], [[Google Scholar](#)], [[Publisher](#)]
- [55] A.A. Kadry, A.M. El-Ganiny, A.M. El-Baz, Relationship between Sap prevalence and biofilm formation among resistant clinical isolates of *Candida albicans*, *Afr. Health Sci.*, **2018**, *18*, 1166-1174. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [56] L.L. Hoyer, E. Cota, *Candida albicans* agglutinin-like sequence (Als) family vignettes: a review of Als protein structure and function, *Front Microbiol.*, **2016**, *7*, 280. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [57] M. Patel, Z. Gulube, M. Dutton, The effect of *Dodonaea viscosa* var. *angustifolia* on *Candida albicans* proteinase and phospholipase production and adherence to oral epithelial cells, *J. Ethnopharmacol.*, **2009**, *124*, 562-5. [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [58] L.S. Ramírez Aristizábal, S.M. Bonilla-Castañeda, J.A. Martínez-Acosta, Evaluation of antibacterial and antisap activities of the aerial part extracts of *Palicourea guianensis* (RUBIACEAE), *Pharmacology online*, **2015**, *3*, 98-106. [[Google Scholar](#)], [[Publisher](#)]

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