Malaysian Journal of Microbiology, Vol 18(5) 2022, pp. 482-489
DOI: http://dx.doi.org/10.21161/mjm.221436

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Antifungal and antibiofilm activities of selected plant extracts

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Received 9 February 2022; Received in revised form 22 August 2022; Accepted 24 August 2022

ABSTRACT

Aims: *Candida albicans* is a regular member of the human microbiota but also one of the most frequent pathogens with a strong biofilm-forming capacity and prominent resistance to antimycotic drugs. The aim of this investigation was to evaluate the anti-*C. albicans* biofilm activity of ethanolic and methanolic leaf extracts of spinach, Swiss chard and garden orache.
Methodology and results: Antifungal activity was established by determining the minimum inhibitory (MIC) and minimum fungicidal concentrations (MFC) by the broth microdilution method. The antibiofilm activity was tested by the tissue culture plate method, followed by the determination of the biofilm inhibition. Results showed that all extracts exhibit antifungal activity, with the MIC value of 62.50 µg/mL. This is in accordance with the results of antibiofilm activity, where extracts showed the ability to decrease the biofilm-forming capacity at sub-inhibitory concentrations. Overall antibiofilm effect of spinach extracts were narrow, but biofilm inhibition activity was observed at 31.25 µg/mL of ethanolic extract. Considering the dilution range, garden orache extracts had the broadest antibiofilm activity, with a biofilm inhibition of 20.96-38.10% and 12.11-12.97% for ethanolic and methanolic extracts, respectively. Swiss chard ethanolic extract inhibited biofilm from 14.52% to 31.39% and methanolic from 37.66% to 44.70%.
Conclusion, significance and impact of study: Study revealed that investigated plant extracts have antifungal and antibiofilm potential against *C. albicans*, which could be important in light of its emerging resistance to synthetic drugs, as well as the possible toxicity of antimycotics.

Keywords: Anti-biofilm activity, *Candida albicans* biofilm, minimum fungicidal concentration, plant extracts

INTRODUCTION

Candida species represent commensal yeasts that can become pathogenic and cause severe infections with high mortality rates (Brighenti *et al*., 2017; Cavalheiro and Teixeira, 2018). *Candida albicans* is a regular member of the healthy human microbiota, asymptomatically colonizing particular body niches (Gulati and Nobile, 2016). However, under specific circumstances, it can proliferate rapidly and cause possibly fatal infections. According to Magill *et al*. (2014), *Candida* species are the primary cause of nosocomial fungal infections and *C. albicans* is involved in about 15% of all sepsis cases and cause around 40% of bloodstream infections in clinical environments (Nobile and Johnson, 2015). It is estimated that about 80% of all microorganisms live in the form of biofilm (d'Enfert and Janbon, 2016) and *Candida* species are no exception. Moreover, *C. albicans* is considered one of the major biofilm producers within the genus (Chandra *et al*., 2001). This species is the most frequent pathogen responsible for *Candida* infections that are increasingly difficult to treat. Furthermore, the biofilm-forming ability, resistance to many antifungal drugs and expression of virulence factors contribute to those issues (Uppuluri *et al*., 2009; Cavalheiro and Teixeira, 2018). Since every *Candida* species differs in many aspects of biofilm formation, including the morphology, properties of the extracellular matrix and antifungal resistance capacity, treatment of such infections is challenging, as well as the design of therapeutic and prophylactic strategies (Golia *et*

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(5) 2022, pp. 482-489
DOI: http://dx.doi.org/10.21161/mjm.221436

al., 2012). Furthermore, such aggregates are more resistant to antifungal therapy and possess the ability to avoid the host immune response (Douglas, 2003) in comparison to the planktonic cells (Majumdar *et al*., 2016). There are some crucial steps in the formation of highly organized biofilms of *C. albicans*, starting from adhesion, followed by germ tubes formation and hyphal elongation, accumulation of extracellular matrix (ECM) material (mixture of biopolymers, including proteins, carbohydrates, lipids and nucleic acids) and ultimately, dispersion of cells from the biofilm (Eix and Nett, 2020; Ourabah *et al*., 2020). Dispersed cells are elongated and exhibit greater pathogenicity, higher filamentation, adhesion and biofilm formation capacity (Uppuluri *et al*., 2009) and their transcriptional profile differs from biofilm and planktonic *C. albicans* (Eix and Nett, 2020). The formation of *C. albicans* biofilms inevitably means higher resistance to antifungal agents (Cavalheiro and Teixeira, 2018). This eventually results in higher mortality rates caused by infection with this fungus (Brighenti *et al*., 2017). Considering also the toxicity of some antifungal drugs (Elisabeth *et al*., 2016), the need to identify new effective, tolerable and affordable compounds against *C. albicans* is highlighted.

Plant-based medicines are used by 70-95% of the population in the developing world. Nearly 30,000 plant species are noted for medicinal uses, from which about 5000 are cited in medicinal regulatory publications (Chassagne *et al*., 2021). In this sense, active compounds of natural origin with pronounced antifungal and antibiofilm activity may be important. So far, there are some findings regarding the activity of plant products against *C. albicans* biofilms (Torey and Sasidharan, 2011; Elisabeth *et al*., 2016; Brighenti *et al*., 2017; Soliman *et al*., 2017; Ourabah *et al*., 2020). This investigation tested the antifungal and antibiofilm potential of three well-known, edible plants from the Amaranthaceae family: spinach (*Spinacia oleracea* L.), Swiss chard (*Beta vulgaris* L. subsp. *vulgaris*) and garden orache (*Atriplex hortensis* L.). Family Amaranthaceae encompass about 165 genera and 2040 species of flowering plants (Christenhusz and Byng, 2016) and many of them are traditionally used as ornamental plants, vegetables, pseudocereals, as well as medicinal plants since they possess various phytochemicals with bioactive properties (Müller and Borsch, 2005). Spinach is an annual leafy vegetable cultivated and consumed worldwide due to its nutritional value and many other health-related beneficial properties related to the presence of bioactive compounds (Subhash *et al*., 2010). Swiss chard is an herbaceous biennial leafy vegetable with many cultivars and varieties. Even though Swiss chard is best known as an edible plant with a high amount of nutrients (Sacan and Yanardag, 2010), this plant is proven for different biological activities (Mzoughi *et al*., 2019). Garden orache is widely used as a vegetable and therapeutic agent in traditional medicine (Bylka *et al*., 2001).

The aim of this investigation was *in vitro* evaluation of the antifungal and antibiofilm activity of ethanolic and methanolic leaf extracts of selected plants against *C. albicans*. According to the available literature and our best knowledge, there are so far no data about the anti-*C. albicans* biofilm activity of plants included in this study. Investigated plants are chosen since they are available, well-known edible plants and there is no data regarding their antibiofilm activity against *C. albicans*. All plants are earlier mentioned in terms of biological activity, but not in this manner. Bearing in mind all the difficulties of creating an efficient, affordable and non-toxic antifungal drug, natural compounds derived from plants could be interesting. The null hypothesis of the investigation is that examined plant extracts would cause significant changes in *C. albicans* biofilm formations. The alternative hypothesis is that biofilm formations of *C. albicans* would not be significantly changed by the extract's activity.

MATERIALS AND METHODS

Plant material

Samples of spinach, Swiss chard and garden orache were collected from the sunny field locality in Dragodol (44°32'59''N; 18°39'34''E) in the Tuzla region, Bosnia and Herzegovina, in September 2021. Plant material identity verification was done in the Laboratory for Plant Systematics, University of Sarajevo-Faculty of Science. Upon washing, leaves were cut and dried in the shade at 30 °C for seven days. Dried samples were then crushed and homogenized.

Extraction procedures

In order to obtain ethanolic and methanolic extracts, maceration (Schilcher *et al*., 2005) and Soxhlet extraction methods (Luque de Castro and Priego-Capote, 2009) were applied. Extraction was performed by using 4 g of each sample, where 80 mL of methanol (Sigma Aldrich) was used for maceration and ethanol (Sigma Aldrich) for the Soxhlet method. Other used reagents were of high analytical grade. All extracts were filtered and concentrated on a rotating vacuum evaporator. Obtained extracts were stored in dark at 4-6 °C.

Candida albicans (Robin) Berkhout (ATCC 10231)

The strain used in this investigation was *Candida albicans* (Robin) Berkhout (ATCC 10231) obtained from the American Type Culture Collection, ATCC (Manassas, Virginia, USA). In the ampoules with lyophilized pellet, 1.0 mL of sterile distilled water is added to form a suspension. Suspension is then transferred back into the test tube with distilled water. The test tube is left for 2 h at room temperature and after mixing, several drops are used to inoculate the growth medium. The inoculum was incubated according to the supplier's recommendation, and signs of viability were observed after 1-2 days of incubation. *Candida albicans* ATCC 10231 is a clinical strain isolated during the 1960s from a patient with bronchomycosis. This strain was used in early infection model studies and still is frequently used for

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 18(5) 2022, pp. 482-489
DOI: http://dx.doi.org/10.21161/mjm.221436

pharmacological purposes (Thewes *et al*., 2008), agricultural research, antimicrobial resistance research, drug development, food testing, media testing, quality control etc.

Determination of the minimum inhibitory and minimum fungicidal concentration

Overnight cultures of yeast cells were adjusted to the turbidity of 0.5 McFarland standard (corresponding to 1-5 × 10^6 CFU/mL) in Sabouraud Glucose Broth, SGB (Sigma-Aldrich). The suspension was diluted with SGB to prepare the final inoculum suspension (1 × 10^3 CFU/mL). After that, each well was inoculated with 100 µL of yeast suspension and an equal volume of two-fold dilutions of extracts (ranging from 1000 to 1.95 µg/mL), which resulted in 5.0 × 10^2 cells/mL (NCCLS, 2002). Pure Dimethyl Sulfoxide, DMSO (Sigma-Aldrich) was used as a solvent in which pure extracts were dissolved to the final concentration of 1000 µg/mL. SGB inoculated by fungal culture was used as a positive control, while the SGB medium with DMSO at the same concentration used for dissolving the extracts was taken as the negative control. Microtiter plates were incubated overnight at 35 ± 1 °C in the standard incubator with natural convection (Binder BD 53E2). After incubation, results were read on a microplate reader (Biochrom EZ Read 400) at 595 nm. These experiments were performed in quadruplets. Upon reading the MIC results, the content of the well described as the MIC and the content from the two surrounding wells were replated on a sterile Sabouraud Glucose Agar, SGA (Sigma Aldrich) in order to evaluate the minimum fungicidal concentration (MFC). Plates were incubated overnight at 37 °C. This experiment was performed in triplicate.

Determination of antibiofilm activity

Changes in *C. albicans* biofilm formation in the presence of examined plant extracts were analyzed by using the tissue culture plate (TCP) method in 96 well plates (Merritt *et al*., 2005). As the dilution medium, tryptic soy broth, TSB (Sigma Aldrich) was used. The primary concentration of extracts (1000 µg/mL) was two-fold diluted in TSB up to the end concentration of 1.95 µg/mL. The final volume of 100 µL of such dilutions was added to each well, followed by inoculation with 10 µL of the investigated fungal strain. Inoculum was prepared as described above. The biofilm formation was determined through the adherence of fungal cells only in the presence of TSB. After overnight incubation, the content of the plates was decanted, while plates were washed in Phosphate Buffered Saline, PBS (Sigma Aldrich) and stained with 0.1% solution of crystal violet for 10 min. After that, 96% ethanol was added to each well and the results were read on the microplate reader (Biochrom EZ Read 400) at 595 nm. The experiment was carried out in quadruplets and the results are presented as the mean value ± STDEV. Biofilm forming category of investigated strain was determined according to Stepanović *et al*. (2007) and by using the Biofilm Classifier Software ver 1.1 (Avdić *et al*., 2019). The optical density cut-off value (ODc) was calculated as three standard deviations above the mean OD of the negative control, while the biofilm categories were determined as follows: OD ≤ ODc: Non-adherent (NA), ODc < OD ≤ 2×ODc: Weakly adherent (W), 2×ODc < OD ≤ 4×ODc: Moderately adherent (M) and 4×ODc < OD: Strongly adherent (S). The percentage of biofilm inhibition caused by the activity of examined extracts was calculated according to (Jadhav *et al*., 2013):

% = ($OD_{595\ nm}$ of experimental well with the extract/$OD_{595\ nm}$ of control well without the extract) × 100%

Statistical analysis

Descriptive statistical parameters (mean values and standard deviation) and the percentage of biofilm inhibition were calculated using Microsoft Office 2019 Excel (Microsoft Corporation, USA). The student's t-test at the significance level of $p<0.05$ is performed to compare the mean absorbance values between controls and the highest sample concentration. Analyses were performed by using software STATISTICA 10; StatSoft.Inc.

RESULTS

Determination of the minimum inhibitory concentration

All investigated plant extracts performed inhibitory activity against the tested strain of *C. albicans*. Generated results showed that ethanolic and methanolic extract of spinach, Swiss chard and garden orache inhibit the growth of *C. albicans* at the same concentration of 62.50 µg/mL, which is the MIC value. The exact values of MIC were obtained over four replications of the protocol. Obtained OD values after the microplate reading (± STDEV) are presented in Figure 1. The minimum fungicidal concentration of the extracts was not recorded since there was a presence of fungal growth after replating the yeast on sterile SGA.

Assessment of the antibiofilm activity

The examined strain of *C. albicans* formed moderately adherent biofilm in all replications of the positive control. The obtained mean absorbance values of biofilm according to the activity of examined ethanolic and methanolic extracts against *C. albicans* and the values detected for the positive and negative control are presented in Table 1.

Spinach ethanolic extract destroyed biofilm only at the first subinhibitory concentration (31.25 µg/mL), while with lower dilutions, the biofilm formation remained moderately adherent. Spinach methanolic extract did not cause changes in the biofilm-forming category of *C. albicans*, but there is a recorded decrease in the mean absorbance values in comparison to the positive control (Figure 2).

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

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Table 1: Recorded mean absorbance values of *C. albicans* biofilm formations.

Plant	Solvent	Negative control	Positive control	Subinhibitory concentrations of extracts (µg/mL)				
				31.25	15.63	7.81	3.90	1.95
Spinach	EtOH	0.067 ± 0.011	0.203 ± 0.038	0.100 ± 0.020	0.230 ± 0.018	0.311 ± 0.045	0.356 ± 0.031	0.303 ± 0.111
	MeOH	0.059 ± 0.002	0.240 ± 0.058	0.164 ± 0.016	0.206 ± 0.040	0.238 ± 0.037	0.234 ± 0.032	0.254 ± 0.058
Swiss chard	EtOH	0.059 ± 0.004	0.154 ± 0.059	0.114 ± 0.044	0.120 ± 0.068	0.111 ± 0.031	0.142 ± 0.079	0.179 ± 0.136
	MeOH	0.057 ± 0.002	0.183 ± 0.051	0.096 ± 0.014	0.108 ± 0.010	0.223 ± 0.173	0.156 ± 0.057	0.204 ± 0.070
Garden orache	EtOH	0.051 ± 0.003	0.116 ± 0.010	0.087 ± 0.020	0.096 ± 0.041	0.088 ± 0.037	0.099 ± 0.030	0.088 ± 0.035
	MeOH	0.061 ± 0.003	0.141 ± 0.008	0.132 ± 0.027	0.125 ± 0.028	0.167 ± 0.039	0.163 ± 0.029	0.186 ± 0.022

Recorded ODc values: Spinach, EtOH=0.100; MeOH=0.065. Swiss chard, EtOH=0.070; MeOH=0.063. Garden orache, EtOH=0.059; MeOH=0.068. EtOH=Ethanolic extract; MeOH=Methanolic extract; Negative control: SGB with DMSO; Positive control: SGB with fungal inoculum.

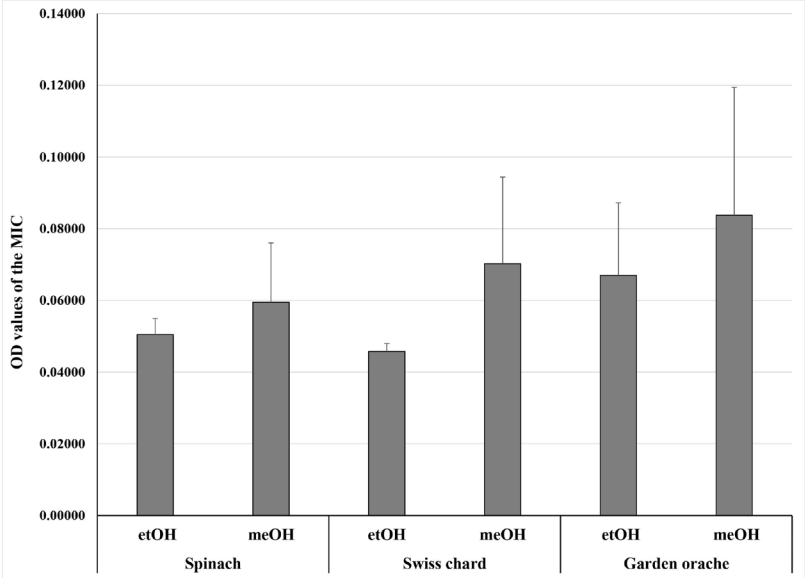

Figure 1: OD values recorded in the determination of MIC (± STDEV).

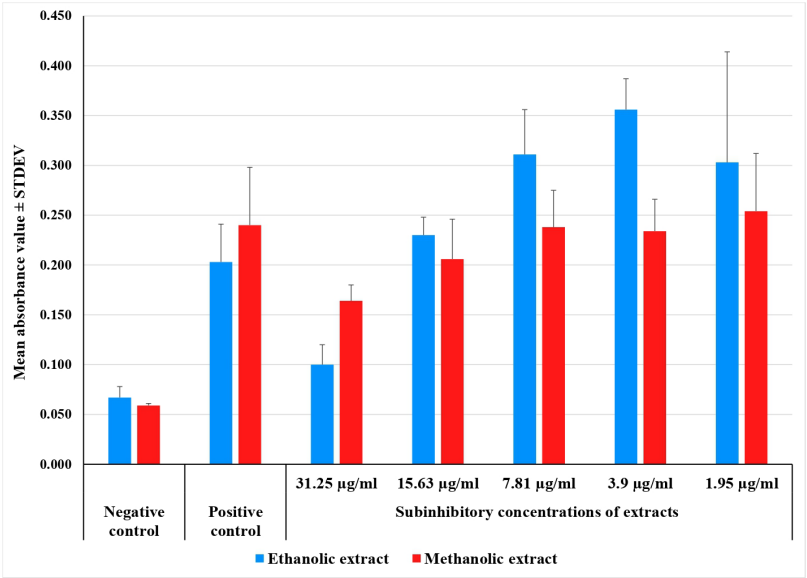

Figure 2: Dynamics in *C. albicans* biofilm formation after application of the spinach extracts. (NA: Non-adherent; M: Moderately adherent. Error bars represent the standard deviation between obtained mean absorbance values).

ISSN (print): 1823-8262, ISSN (online): 2231-7538

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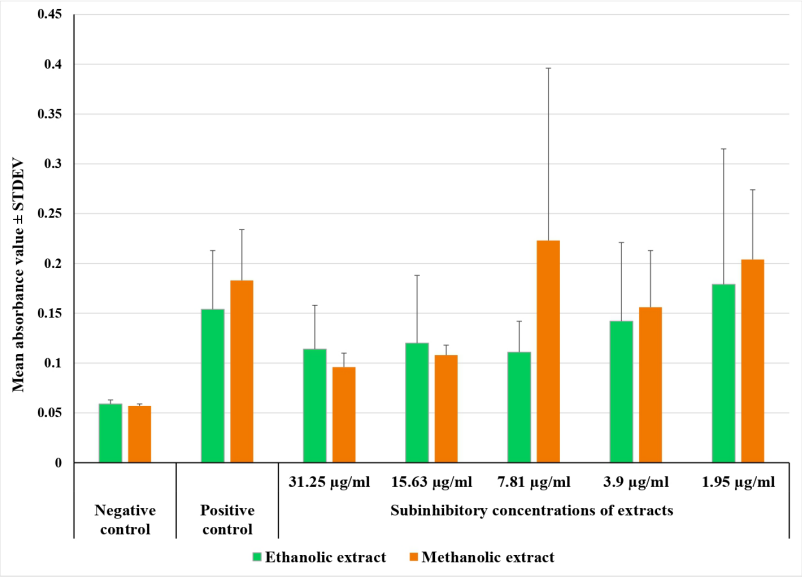

Figure 3: Changes in *C. albicans* biofilm formation due to the activity of Swiss chard extracts (NA: Non-adherent; W: Weakly adherent; M: Moderately adherent. Error bars represent the standard deviation between obtained mean absorbance values).

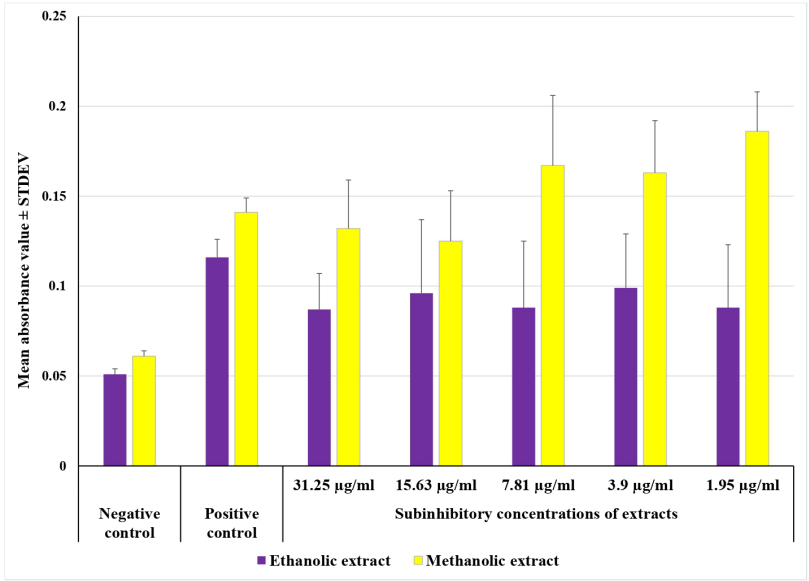

Figure 4: Determined biofilm-forming category of *C. albicans* after application of garden orache extracts (NA: Non-adherent; W: Weakly adherent; M: Moderately adherent. Error bars represent the standard deviation between obtained mean absorbance values).

The ethanolic extract made from Swiss chard leaves led to the formation of weakly adherent *C. albicans* biofilm in the range of 31.25-7.81 µg/mL, while methanolic extract acted like this at concentrations of 31.25 and 15.63 µg/mL (Figure 3).

Finally, garden orache extracts also showed the capacity to change the biofilm-forming category of *C. albicans*, especially the ethanolic extract, where all subinhibitory concentrations caused a decrease in the biofilm-forming category through the occurrence of weakly adherent biofilm. Methanolic extract of this plant lowers the moderately adherent to weakly adherent biofilm in concentrations of 31.25 and 15.63 µg/mL (Figure 4).

Overall results regarding the percentage of inhibition of *C. albicans* biofilm formations through the activity of examined plant extracts suggest that garden orache possesses the broadest antibiofilm activity against this fungus. All subinhibitory concentrations of garden orache ethanolic extract inhibited *C. albicans* biofilm formation in the amount of 20.96-38.10%, while garden orache methanolic extract demonstrated antibiofilm activity with an inhibition percentage of 12.11-12.97% (Figure 5). On the other hand, Swiss chard ethanolic extract caused inhibition of *C. albicans* biofilm from 14.52% (7.81 µg/mL) to 31.39% (31.25 µg/mL) and methanolic extract of the same plant did so in the amount of 37.66-44.70% (Figure 5). Spinach extracts investigated in this study performed narrow antibiofilm activity against *C. albicans*, but the first inhibitory concentration of spinach ethanolic extract was the only one to prevent biofilm adherence, with a recorded inhibition percentage of 49.20% (Figure 5). Although there are some variations in the antibiofilm activity of investigated extracts, statistical analysis showed that observed differences are not statistically significant.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

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DOI: http://dx.doi.org/10.21161/mjm.221436

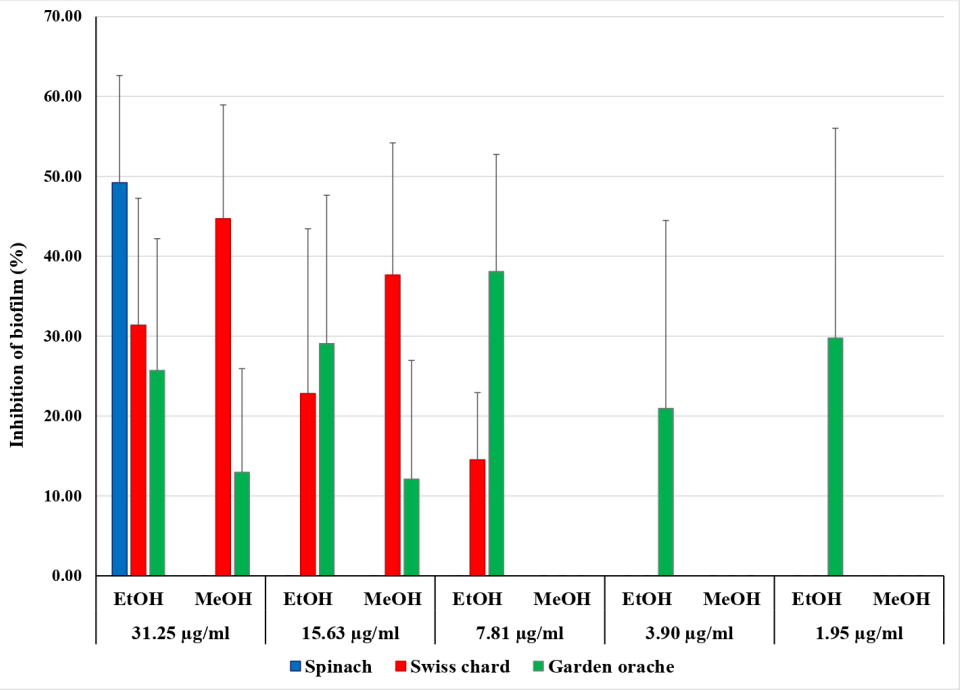

Figure 5: Performed inhibition of *C. albicans* biofilm under activity of investigated plant extracts.

DISCUSSION

There are four major classes of antifungal drugs: azoles, polyenes, echinocandins and nucleoside analogues, but *C. albicans* biofilms are resistant to the majority of these agents, mainly due to the changes in efflux pumps mechanisms, presence of ECM and the existence of metabolically inactive persistent cells (Gulati and Nobile, 2016). Results of the conducted investigation suggest that plant products could be discussed in terms of antifungal and antibiofilm activity against *C. albicans*. Previously reported studies regarding antibiofilm activity of plants against *C. albicans* are limited and proven for: *Cassia spectabilis* (DC) Irwin et Barn (Fabaceae) (Torey and Sasidharan, 2011); *Buchenavia tomentosa* Eichler (Combretaceae) (Brighenti *et al*., 2017); *Eugenia uniflora* L. (Myrtaceae), *Terminalia mantaly* H. Perrier (Combretaceae) (Elisabeth *et al*., 2016); *Lawsonia inermis* L. (Lythraceae), *Portulaca oleracea* L. (Portulacaceae) (Soliman *et al*., 2017); *Clematis flammula* L. (Ranunculaceae) and *Fraxinus angustifolia* Vahl (Oleaceae) (Ourabah *et al*., 2020). This study tested three edible plants from the Amaranthaceae family, mainly known for their nutritive values. Both ethanolic and methanolic extracts of spinach, Swiss chard and garden orache inhibited growth of *C. albicans*. These research findings are important as *C. albicans* represents a multidrug-resistant pathogen, as was mentioned before. Earlier studies regarding the antimicrobial activity of different spinach preparations go in favour of flavonoids, terpenes, fatty acids and peptides as the main chemical compounds responsible for these effects (Nasim *et al*., 2012; Galani *et al*., 2017; Adapa *et al*., 2018; Olasupo *et al*., 2018). Spinach extracts investigated in this study had low antibiofilm activity against *C. albicans* and only the highest subinhibitory concentration of ethanolic extract has weakened the biofilm to non-adherent. Swiss chard is previously proven for many biological activities (Sacan and Yanardag, 2010). This investigation showed that Swiss chard extracts can reduce the biofilm-forming category of *C. albicans* in specific subinhibitory concentrations. According to Mzoughi *et al*. (2019), this plant is rich in phytochemicals and antioxidant compounds, such as flavonoids, phenolic acids, pigments and volatile compounds, and its ethanolic extract should be considered to use in phytotherapy. These findings are in accordance with our study, where ethanolic extract showed slightly broader antibiofilm activity in comparison to the methanolic extract. Garden orache is not widely discussed as an antimicrobial agent, but there are some data regarding its pharmacological activity, as well as for some other species of *Atriplex* genus (Zine *et al*., 2021). This study revealed that garden orache possesses the broadest antibiofilm activity against *C. albicans* in comparison to other investigated plants, particularly the ethanolic extract that caused a decrease of the biofilm category in all subinhibitory concentrations. *Atriplex* species mainly inhabit saline environments (Zohra *et al*., 2019) and halophytes generally exhibit various bioactive properties (Ksouri *et al*., 2012). Species from this genus are rich in flavonoids and Bylka *et al*. (2001) reported two new flavonoid sulfates from *A. hortensis* leaves: kaempferol 3-O-sulphate-7-O-arabinopyranoside and quercetin 3-O-sulphate-7-O-arabinopyranoside. The

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

majority of investigated extracts and their different dilution performed the capacity to decrease the biofilm formation of *C. albicans*. However, with one exception, there was no observed complete removal of the biofilm nor fungicidal effect recorded after completing the broth microdilution method. As was already mentioned, *C. albicans* biofilms are dependent on the ECM, which among other things, prevents the diffusion of antimycotic drugs (Cavalheiro and Teixeira, 2018). Developing novel therapeutics for *C. albicans* biofilms is challenging, where besides the physical protection provided by ECM, cells in the biofilm are intrinsically resistant to antifungal agents (Gulati and Nobile, 2016). Our investigation has also shown that some mean absorbance values detected in the biofilm assay are higher in comparison to the positive control (Table 1), suggesting that biofilm could be stronger after the plant extract application. Similar observations were noted earlier (Abedini *et al*., 2020) and could be related to the microbe's response to the stressful environment.

CONCLUSION

Overall results go in favor of the alternative hypothesis since the values between control and experimental groups are similar. Considering the resistance of *C. albicans* and its biofilms to synthetic antifungal drugs, the toxicity of those agents and the increasing rate of severe infections caused by this fungus, the finding of new antifungal and antibiofilm compounds should be encouraged. Results showed that tested extracts made from three edible and commercially available plants exhibit antifungal and antibiofilm potential against the tested strain of *C. albicans*. Further investigation should be carried out to define the precise chemical profile of these plant products and determine specific molecular mechanisms involved in antibiofilm activity. This information, combined with the determination of the particular concentration of the plant extract with antibiofilm activity, could be important in the light of the broader application of antifungal and antibiofilm products of plant origin.

REFERENCES

Abedini, A., Colin, M., Hubert, J., Charpentier, E., Angelis, A., Bounasri, H., Bertaux, B., Kotland, A., Reffuveille, F., Nuzillard, J., Renault, J. and Gangloff, S. C. (2020). Abundant extractable metabolites from temperate tree barks: The specific antimicrobial activity of *Prunus avium* extracts. *Antibiotics* **9(3), 111.**

Adapa, S. B., Sushanth, V. H., Prashant, G. M. and Imranula, M. (2018). *In vitro* antimicrobial activity of *Spinacia oleracea* against *Streptococcus mutans* and *Lactobacillus acidophilus*. *Journal of Indian Association of Public Health Dentistry* **16(3), 251-255.**

Avdić, M., Džuzić, N., Hasanić, O., Spahić, A., Smajlović-Skenderagić, L., Badnjević, A. and Hukić, M. (2019). Development of a novel biofilm classification tool and comparative analysis of results interpretation methodologies for the evaluation of biofilm forming capacity of bacteria using tissue culture plate method. *Medicinski Glasnik* **16(1), 7-12.**

Brighenti, F. L., Salvador, M. J., Gontijo, A. V. L., Delbem, A. C. B., Delbem, Á. C. B., Soares, C. P., de Oliveira, M. A. C., Girondi, C. M. and Koga-Ito, C. Y. (2017). Plant extracts: Initial screening, identification of bioactive compounds and effect against *Candida albicans* biofilms. *Future Microbiology* **12(1), 15-27.**

Bylka, W., Stobiecki, M. and Frański, R. (2001). Sulphated flavonoid glycosides from leaves of *Atriplex hortensis*. *Acta Physiologiae Plantarum* **23(3), 285-290.**

Cavalheiro, M. and Teixeira, M. C. (2018). *Candida* biofilms: Threats, challenges, and promising strategies. *Frontiers in Medicine* **5, 28.**

Chandra, J., Kuhn, D. M., Mukherjee, P. K., Hoyer, L. L., McCormick, T. and Ghannoum, M. A. (2001). Biofilm formation by the fungal pathogen *Candida albicans*: Development, architecture, and drug resistance. *Journal of Bacteriology* **183, 5385-5394.**

Chassagne, F., Samarakoon, T., Porras, G., Lyles, J. T., Dettweiler, M., Marquez, L., Salam, A. M., Shabih, S., Farrokhi, D. R. and Quave, C. L. (2021). A systematic review of plants with antibacterial activities: A taxonomic and phylogenetic perspective. *Frontiers in Pharmacology* **11, 586548.**

Christenhusz, M. J. M. and Byng, J. W. (2016). The number of known plants species in the world and its annual increase. *Phytotaxa* **261(3), 201-217.**

d'Enfert, C. and Janbon, G. (2016). Biofilm formation in *Candida glabrata*: What have we learnt from functional genomics approaches? *FEMS Yeast Research* **16(1), fov111.**

Douglas, L. J. (2003). *Candida* biofilms and their role in infection. *Trends in Microbiology* **11(1), 30-36.**

Eix, E. F. and Nett, J. E. (2020). How biofilm growth affects *Candida* - host interactions. *Frontiers in Microbiology* **11, 1437.**

Elisabeth, Z. M., Lopez, V. C., Soto, S. M. and Fabrice, F. B. (2016). Anti-*candida* biofilm properties of Cameroonian plant extracts. *Journal of Medicinal Plants Research* **10(35), 603-611.**

Galani, J. H. Y., Patel, J. S., Patel, N. J. and Talati, J. G. (2017). Storage of fruits and vegetables in refrigerator increases their phenolic acids but decreases the total phenolics, anthocyanins and vitamin C with subsequent loss of their antioxidant capacity. *Antioxidants* **6(3), 59.**

Golia, S., Hittinahalli, V., Sangeetha, K. T. and Vasudha, C. L. (2012). Study of biofilm formation as a virulence marker in *Candida* species isolated from various clinical specimens. *Journal of Evolution of Medical and Dental Sciences* **1(6), 1239-1246.**

Gulati, M. and Nobile, C. J. (2016). *Candida albicans* biofilms: Development, regulation, and molecular mechanisms. *Microbes and Infection* **18(5), 310-321.**

Jadhav, S., Shah, R., Bhave, M. and Palombo, E. A. (2013). Inhibitory activity of yarrow essential oil on

Listeria planktonic cells and biofilms. *Food Control* **29(1), 125-130.**

Ksouri, R., Ksouri, W. M., Jallali, I., Debez, A., Magné, C., Hiroko, I. and Abdelly, C. (2012). Medicinal halophytes: Potent source of health promoting biomolecules with medical, nutraceutical and food applications. *Critical Reviews in Biotechnology* **32(4), 289-326.**

Luque de Castro, M. D. and Priego-Capote, F. (2009). Soxhlet extraction: Past and present panacea. *Journal of Chromatography A* **1217(16), 2383-2389.**

Magill, S. S., Edwards, J. R., Bamberg, W., Beldavs, Z. G., Dumyati, G., Kainer, M. A., Lynfield, R., Maloney, M., McAllister-Hollod, L., Nadle, J., Ray, S. M., Thompson, D. L., Wilson, L. E. and Fridkin, S. K. (2014). Multistate point-prevalence survey of health care-associated infections. *The New England Journal of Medicine* **370(13), 1198-1208.**

Majumdar, T., Mullick, J. B., Bir, R., Roy, J. and Sil, S. K. (2016). Determination of virulence factors and biofilm formation among isolates of vulvovaginal candidiasis. *Journal of Medical Sciences* **36(2), 53-58.**

Merritt, J. H., Kadouri, D. E. and O'Toole, G. A. (2005). Growing and analyzing static biofilms. *Current Protocols in Microbiology* **1, 1B.1.**

Müller, K. F. and Borsch, T. (2005). Phylogenetics of Amaranthaceae based on matK/trnK sequence data-evidence from parsimony, likelihood, and bayesian analyses. *Annals of the Missouri Botanical Garden* **92(1), 66-102.**

Mzoughi, Z., Chahdoura, H., Chakroun, Y., Cámara, M., Fernández-Ruiz, V., Morales, P., Mosbah, H., Flamini, G., Snoussi, M. and Majdoub, H. (2019). Wild edible Swiss chard leaves (*Beta vulgaris* L. var. *cicla*): Nutritional, phytochemical composition and biological activities. *Food Research International* **119, 612-621.**

Nasim, F., Andleeb, S., Iqbal, M., Ghous, T., Khan, A. N. and Akhtar, K. (2012). Evaluation of antimicrobial activity of extracts of fresh and spoiled *Spinacia oleracea* against some mammalian pathogens. *African Journal of Microbiology Research* **6(29), 5847-5851.**

NCCLS, National Committee for Clinical Laboratory Standards. (2002). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Second Edition. NCCLS document M27-A2. NCCLS, Pennsylvania, USA.

Nobile, C. J. and Johnson, A. D. (2015). *Candida albicans* biofilms and human disease. *Annual Review of Microbiology* **69, 71-92.**

Olasupo, A. D., Aborisade, A. B. and Olagoke, O. V. (2018). Phytochemical analysis and antibacterial activities of spinach leaf. *American Journal of Phytomedicine and Clinical Therapeutics* **6(2), 8.**

Ourabah, A., Atmani-Kilani, D., Debbache-Benaida, N., Kolesova, O., Azib, L., Yous, F., Benloukil, M., Botta, B., Atmani, D. and Simoneti, G. (2020). Anti-*Candida albicans* biofilm activity of extracts from two selected indigenous Algerian plants: *Clematis flammula* and *Fraxinus angustifolia*. *Journal of Herbal Medicine* **20, 100319.**

Sacan, O. and Yanardag, R. (2010). Antioxidant and antiacetylcholinesterase activities of chard (*Beta vulgaris* L. var. *cicla*). *Food and Chemical Toxicology* **48(5), 1275-1280.**

Schilcher, H., Imming, P. and Goeters, S. (2005). Pharmacology and toxicology. *In*: Chamomile Industrial Profiles. Franke, R. and Schilcher, H. (eds.). CRC Press, United States. **pp. 245.**

Soliman, S. S. M., Semreen, M. H., El-Keblawy, A. A., Abdullah, A., Uppuluri, P. and Ibrahim, A. S. (2017). Assessment of herbal drugs for promising anti-*Candida* activity. *BMC Complementary Medicine and Therapies* **17, 257.**

Stepanović, S., Vuković, D., Hola, V., Di Bonaventura, G., Đukić, S., Cirković, I. and Ruzicka, F. (2007). Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *APMIS* **115(8), 891-899.**

Subhash, G. P., Virbhadrappa, S. R. and Vasant, O. K. (2010). *Spinacia oleracea* Linn: A pharmacognostic and pharmacological overview. *International Journal of Research in Ayurveda and Pharmacy* **1(1), 78-84.**

Thewes, S., Moran, G. P., Magee, B. B., Schaller, M., Sullivan, D. J. and Hube, B. (2008). Phenotypic screening, transcriptional profiling, and comparative genomic analysis of an invasive and non-invasive strain of *Candida albicans*. *BMC Microbiology* **8, 187.**

Torey, A. and Sasidharan, S. (2011). Anti-*Candida albicans* biofilm activity by *Cassia spectabilis* standardized methanol extract: An ultrastructural study. *Europaean Review for Medical and Pharmacological Sciences* **15(8), 875-882.**

Uppuluri, P., Pierce, C. G. and López-Ribot, J. L. (2009). *Candida albicans* biofilm formation and its clinical consequences. *Future Microbiology* **4(10), 1235-1237.**

Zine, H., Ibrahimi, M., Loqman, S., Papazoglou, E. G., Ouhaddou, S., Elgadi, S., Ouhdouch, Y., Hakkou, R., El Adnani, M. and Ouhammou, A. (2021). Chemical composition, antioxidant, and antibacterial activities of essential oil of *Atriplex semibaccata* R.Br. aerial parts: First assessment against multidrug-resistant bacteria. *Agronomy* **11(2), 362.**

Zohra, T., Ovais, M., Khalil, A. T., Qasim, M., Ayaz, M., Shinwari, Z. K., Ahmad, S. and Zahoor, M. (2019). Bio-guided profiling and HPLC-DAD finger printing of *Atriplex lasiantha* Boiss. *BMC Complementary and Alternative Medicine* **19, 4.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538