

***In vitro* anticandidal activity and gas chromatography-mass spectrometry (GC-MS) screening of *Vitex agnus-castus* leaves extracts**

Ibtisam Mohammed Ababutain ^{Corresp., 1}, **Azzah Alghamdi** ¹

¹ Department of Biology, College of Science, P.O. Box 1982, Dammam 31441, Saudi Arabia. Basic & Applied Scientific Research Center (BASRC), Imam Abdulrahman Bin Faisal University, P.O. Box 1982, 31441 Dammam Saudi Arabia., Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Corresponding Author: Ibtisam Mohammed Ababutain
Email address: iababutain@iau.edu.sa

Background Regarding the increasing in drug resistance of candida infections, it is necessary to search for alternative medication. Therefore, the present study estimates the anticandidal activity of *Vitex agnus-castus* (VA-C) leaves' extracts. **Methods** Agar well diffusion method was used to screened the anticandidal activity of three different VA-C leaves extracts (ethanol, methanol, and water), against three *Candida* species (*Candida tropicalis*, *Candida albicans* and *Candida ciferrii*). Minimum Inhibitory Concentration (MIC) was estimated using the two- fold dilution method and *determination of Minimum Fungicidal Concentration (MFC)* was determined by using the classic pour plate technique. The ratio MFC/MIC was calculated to estimate the microbicidal or microbiostatic activities. Gas Chromatography-Mass Spectrometer was used to screen the phytochemical of VA-C leaves extracts (ethanol, methanol, and water). **Results** All VA-C extracts ethanol, methanol, and water were significantly inhibited the growth of the test *Candida* species. and the inhibition activity depends on the solvent type and *Candida* species. The results showed that *C. tropicalis* was the most inhibited by all the extracts follow by *C. albicans* then *C. ciferrii*. The MICs values were between 12.5 µg/ml to 25 µg/ml, and MFCs values were between 25 µg/ml to 100 µg/ml. The ratios of MFC/MIC were found one to two-folds and that was considered as candidacidal activity. One hundred and one phytochemical compound were identified by the GC-MS assay for the VC-A leaves extracts. These phytochemical compounds belong to different bioactive chemical group such as polyphenols, fatty acids, terpenes, terpenoid, steroids, aldehydes, alcohol and esters, and most of these compounds have an anticandidal activity. **Conclusions** Based on the high activity of the VC-A leaves extracts, against all test *Candida* species at low concentrations, it is possible to use this plant extracts in manufacturing of alternative anticandidal drugs.

In vitro anticandidal activity and gas chromatography-mass spectrometry (GC-MS) screening of *Vitex agnus-castus* leaves extracts

Ibtisam Mohammed Ababutain^{1,2,*}, Azzah Ibrahim Alghamdi^{1,2}

¹Department of Biology, College of Science, Imam Abdulrahman Bin Faisal University, P.O. Box 1982, 31441, Dammam, Saudi Arabia

²Basic & Applied Scientific Research Center (BASRC), Imam Abdulrahman Bin Faisal University, P.O. Box 1982, 31441 Dammam Saudi Arabia

*Corresponding author: Ibtisam Mohammed Ababutain (iababutain@iau.edu.sa; Dr.King2007@hotmail.com);

ABSTRACT

Background

Regarding the increasing in drug resistance of candida infections, it is necessary to search for alternative medication. Therefore, the present study estimates the anticandidal activity of *Vitex agnus-castus* (VA-C) leaves' extracts.

Methods

Agar well diffusion method was used to screened the anticandidal activity of three different VA-C leaves extracts (ethanol, methanol, and water), against three *Candida* species (*Candida tropicalis*, *Candida albicans* and *Candida ciferrii*). Minimum Inhibitory Concentration (MIC) was estimated using the two- fold dilution method and *determination of Minimum Fungicidal Concentration (MFC)* was determined by using the classic pour plate technique. The ratio MFC/MIC was calculated to estimate the microbicidal or microbiostatic activities. Gas Chromatography-Mass Spectrometer was used to screen the phytochemical of VA-C leaves extracts (ethanol, methanol, and water).

Results

All VA-C extracts ethanol, methanol, and water were significantly inhibited the growth of the test *Candida* species. and the inhibition activity depends on the solvent type and *Candida* species. The results showed that *C. tropicalis* was the most inhibited by all the extracts follow by *C. albicans* then *C. ciferrii*. The MICs values were between 12.5 µg/ml to 25 µg/ml, and MFCs values were between 25 µg/ml to 100 µg/ml. The ratios of MFC/MIC were found one to two-folds and that was considered as candidacidal activity. One hundred and one phytochemical compound were identified by the GC-MS assay for the VC-A leaves extracts. These phytochemical compounds belong to different bioactive chemical group such as polyphenols, fatty acids, terpenes, terpenoid, steroids, aldehydes, alcohol and esters, and most of these compounds have an anticandidal activity.

Conclusions

Based on the high activity of the VC-A leaves extracts, against all test *Candida* species at low concentrations, it is possible to use this plant extracts in manufacturing of alternative anticandidal drugs.

Introduction

Recently, the incidence of severe infections caused by *Candida* species has increased and became a risk to human health. Due to their virulence, ability to survive in extreme environments, and resistance to antifungal agents (Paramythiotou et al., 2014). *Candida* species are able to cause a wide variety of infections ranging from mild to severe infections such as candidemia_which is associated with a mortality rate up to 38% in immunosuppressed patients i.e. organ transplantation patients, patients under chemotherapy, HIV- infected and diabetics (Koehler et al., 2019; de Oliveira Santos et al., 2018). Moreover, it has been found that the rate of fungal infections reaches 20% in the intensive care unit, including candidiasis. Usually antifungals like azoles, echinocandins, fluoropyrimidines and polyenes are used to treat this infection, despite the challenges of determining the appropriate dose for treatment and avoiding _ side effects at the same time (Chatelon et al., 2019). Candidiasis is one of the most common fungal diseases in the world, and it has many different types such as cutaneous candidiasis, mucosal candidiasis, onychomycosis and systemic candidiasis, in fact healthy individuals are also susceptible to candidiasis (de Oliveira Santos et al., 2018). Genus *Candida* is deuteromycetes fungi and belongs to the Cryptococcaceae family, with several species up to 200 among these, thirty species are most often isolated in humans' infections including *Candida albicans*, *Candida tropicalis*, *Candida dubliniensis*, *Candida parapsilosis*, *Candida glabrata*, *Candida lusitanae*, *Candida kefyr* and *Candida krusei* (Rodrigues et.al., 2019; Kim et al., 2016; Brandt and Lockhart, 2012; Miceli et al., 2011).

Despite the diversity of antifungals, it is difficult to obtain an ideal treatment because of its limited use and its side effects. In addition to the increase of resistance to these types of medications as a result of extensive and indiscriminate use (de Oliveira Santos et al., 2018). Accordingly, research continues to explore therapeutic alternatives such as the use of plants' essential oils or their extracts, which have proven their ability to treat several diseases, because they contain phytochemicals components with physiological and therapeutic effect on humans, limited toxicity and low therapeutic costs (Abdulrasheed et al., 2019; Sardi et al., 2013). World Health Organization reports indicate that up to 25% of modern medicines used in the United States of America are plant origin also in Africa and Asia 80% of its population still uses medicinal herbs in a number of primary health care centers (WHO, 2002). Moreover, antimicrobial potential for more than 1340 plant has been well documented (Yilar et al., 2016). *Vitex* is one of the largest genera in the family Verbenaceae that comprised of 250 genera found worldwide (Ganapaty and Vidyadhar, 2005). Therapeutic applications of *Vitex agnus-castus* (VA-C) and its safety use as a medicinal plant is well stated (Niroumand et al., 2018; Neves and da Camara 2016; Rani and Sharma, 2013). Most of previous studies emphasize on the antibacterial activity of VA-C essential oil of its' seed and fruit (Eryigit et al., 2015; Dervishi-Shengjergji et al., 2014; Ghannadi et al., 2012) some studies investigated the antimicrobial activity of VA-C essential oil of its' leaves (Katirae et al., 2015; Ulukanli et al., 2015) and few studies demonstrated the antifungal activity of seeds oil (Asdadi et al., 2014). Also, another study examined the antifungal potential of VA-C leaves essential oils against plant pathogens (Yilar et al., 2016). The antibacterial activity of VA-C leaves extract was identified by few studies (Ababutain and Alghamdi, 2018; Kalhor et al., 2014; Arokiyaraj et al., 2009) and antimicrobial activity of VA-C leaves extract was estimated by other researches (Kalhor et al., 2014; Maltaş et al., 2010) both studies used only a few number of bacteria and one yeast which was *Candida albicans*. Recently, Keikha et al. (2018) evaluated

the antifungal activity of ethanolic and aqueous leaves extracts on *C. albicans* strains. However, the effect of leaves extracts of VA-C against human *Candida* sp. have not been extensively studied.

Therefore, this study aims to investigate the anticandidal activity and efficiency of VA-C leaves extracts (water, methanol and ethanol) against three most frequently isolated *Candida* sp. (*Candida albicans*, *Candida tropicalis* and *Candida ciferrii*). And screening the phytochemical of these extracts using the Gas Chromatography-Mass Spectrometry (GC-MS).

Materials & Methods

Plant material

Vitex agnus-castus VA-C leaves was collected from a garden in Dammam city, Saudi Arabia then was identified using Brickell and Zuk (1997).

Preparation of plant extracts

VA-C leaves were washed with tap water and left to dry for two days at room temperature then grinded to fine powder. Maceration method described by Pandey and Tripathi (2014) was used with little modification, in which 60g of the leaves powder was transferred to three Erlenmeyer flasks containing 300 mL of three different solvents; distilled water, methanol (80%), and ethanol (80%) to a final concentration of 20% g/mL. Leaves mixtures were shaken for 72 hours at speed 300 rpm/min/ 20°C, to allow better extraction of active compounds. The leaves mixtures were filtered twice; first using Whatman No. 1 filter paper and the second using bacterial filters then filtrates were concentrated using an oven at 80°C. The residues were re-suspend using Dimethyl Sulfoxide (DMSO) to a final concentration of 20% all flasks were kept at 4°C for further use³³

Agar well diffusion method

Three different prepared VA-C leaves extracts were screened for their anticandidal activity at the concentration of 20% (mg/ml) using the agar well diffusion method (NCCLS, 1993), against three unicellular fungi. *Candida tropicalis* and *Candida albicans* were kindly provided by King Fahd Hospital, Al Khobar – kingdom of Saudi Arabia, and the third *Candida ciferrii* was obtained from the Biology Department – College of Science – Imam Abdulrahman Bin Faisal University. Inoculums of the yeasts were prepared from new cultures in Potato Dextrose broth (PDB). Biomerieux DensiCHEK plus meter device was used to adjust the cell suspensions turbidity at 5×10^6 CFU/ml which represents 0.5 McFarland standards, then each Petri dish was inoculated in a well with 0.5ml of the previous suspensions. Melted Potato Dextrose Agar (PDA) was poured over the inoculums and to ensure even distribution of the inoculums the plates were rotated then left to harden at room temperature for 5min. Five wells were made on the inoculated PDA using 6 mm sterile cork-borer then each well was filled by 100 μ L of the plant extracts. Positive and negative controls were included; nystatin (10 mcg) was used as a positive control and DMSO was used as a negative control. Then plates were incubated at 37°C for 24 hours. Anticandidal activity of the plant extracts was estimated in millimeters (mm) using ruler by measuring the free growth zones around the wells. To ensure the reliability of the results the experiments were performed in three replicates.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of VA-C leaves extracts was estimated using the

Two- fold dilution method (Omura et al., 1993). The plant extracts were diluted with PDB media using 96-well microtiter plates in wells 1 to 10. Standard candida's inoculums at the concentration of $1-2 \times 10^6$ CFU/mL were transferred to the wells to make a final concentration of 50%. Growth media with candida's inoculum in well 11 and growth media with plant extract in well 12, were used as positive and negative controls, respectively. The turbidity was examined after overnight incubation at 37°C with the naked eye and the lowest concentrations of plant extract showing no Candida growth was recorded as MICs. All experiments were performed in three replicates.

Determination of Minimum Fungicidal Concentration (MFC)

Classic pour plate technique was used to determine the MFC (NCCLS, 1997). Concentrations that showed no candida growth from previous MIC experiment were transferred to Petri dishes, then 15 mL of melted PDA was poured over it and gently rotated then left to solidify. Inoculated Petri dishes were incubated at 37°C for 48 hours. The lowest concentration that showed no visible candida colonies were recorded as MFC. All experiments were performed in three replicates.

Determination of anticandidal efficiency

The anticandidal efficiency of VA-C leaves extracts (ethanol, methanol and water) was determine by calculating the ratio of MFC/MIC₃₇.

Gas Chromatography-Mass Spectrometry (GC-MS)

Gas Chromatography-Mass Spectrometer (Shimadzu-Japan) Model QP2010 SE, with 5 Sil MS 5% diphenyl/ 95% dimethyl polysiloxane capillary column (0.25- μ m df, 30 meter, 0.25 mmID) was used to analysis the VC-A leaves' bioactive compounds. One μ L of diluted plant extract (100/1400, V/V in DMSO) was injected in the split mode and the split ratio was 1:10. For GC-MS exposure or detection, electron impact ionization system at 70eV ionization energy was used. Pure helium (99.999%) was used as a carrier gas, at a constant column flow 0.7ml/ min and total flow was 10.4 ml/min. Flow control mode was linear velocity of 29.6cm/sec. Injector temperature was set at 250°C and ion-source temperature 250°C. The column temperature was programmed from 50°C to 300°C, hold time was 3 min, and total run time was 29 min. The chemical compounds were identified using National Institute of Standards and Technology (NIST 08) library match and the quantitative data were generated automatically as percentage (Adams, 2007).

Statistical analysis

The anticandidal activity of VA-C leaves extract between the solvents and the Candida sp. had been conducted using one-way Anova test. *P-value* of <0.01 were considered statistically significant. Statistical data were analyzed using Statistical Packages for Software Sciences (SPSS, 2013) version 21 Armonk, New York, IBM Corporation.

Results

Anticandidal activity of VA-C leaves extracts

Based on the results, VA-C extracts were able to inhibit the growth of all tested *Candida* sp. This inhibition activity depends on the solvent type and *Candida* species. The results showed that *C. tropicalis* was the most inhibited by all the extracts follow by *C. albicans* and *C. ciferrii* was the last effected (all $P=0.01$). The extract of ethanol was significantly higher compared to both extracts of water and methanol against *C. tropicalis*, *C. albicans* and *C. ciferrii* at $P=0.01$, $P=0.037$ and $P=0.047$, respectively (Table 1).

In general MICs results were between 12.5 µg/ml to 25 µg/ml, all extracts showed similar activity against all *Candida* sp. at MIC 25 µg/ml except *C. tropicalis* which was the most sensitive to the ethanol extract at MIC 12.5 µg/ml. The results of MFCs were between 25 µg/ml to 100 µg/ml. Most extracts showed similar MFCs values against all *Candida* sp. at MFC 50 µg/ml except *C. tropicalis* the MFC was at 25 µg/ml by the ethanol extract this considered as the highest anticandidal activity and *C. albicans* the MFC was at 100 µg/ml by the methanol extract and this considered as the lowest anticandidal activity. The results revealed that are both MICs and MFCs values for all three solvents were narrow where the differences between values were one to two concentration only. The ratios MFC/MIC in all the three extracts were only one to two-fold and this means that VA-C leaves extracts have a candidacidal potential.

Gas Chromatography -Mass Spectrometry (GC-MS) analysis

The results revealed that VA-C leaves extracts are rich in Phytochemical components with difference in the concentrations. In general, 101 chemical compounds were extracted depending on the solvent type, of these 12 compounds were extracted by all three solvents and the total number of extracted compounds was 50, 48 and 52 by ethanol, methanol, water, respectively (Table 3).

Discussion

Due to the phenomenon of antibiotic resistance, which has spread to include a large number of microorganisms such as *Candida* sp., regarding the interest in searching for alternatives has increased. Since the fact that plants contain secondary metabolites, they have been used for a long time in many fields, such as treating a variety of diseases, as flavors, preserving food, pesticides, perfumery and cosmetics. Recently its ability to inhibit the microbial growth. VA-C leaves extracts have been reported with mild and reversible side effects such as headache, acne, nausea, gastrointestinal disturbances, erythematous rash, pruritus and menstrual disorders; moreover, no drug interactions have been associated (Daniele et al., 2005). Therefore, VA-C leaves extracts (ethanol, methanol and water) were investigated for their ability to inhibit the growth of three *Candida* species that resistance to the antibiotic which are *C. ciferrii*, *C. albicans* and *C. tropicalis* that resistance to Azoles (Romald et al., 2019; Bhakshu, et al., 2016).

The results of the current study showed that the alcoholic extract (methanol and ethanol) and the aqueous extract have the ability to inhibit the growth of all test *Candida* species. These results are in agreement with the previous study of Kalhora et al. (2014) where they found that the ethanol extract of VA-C leaves has the potential to inhibit the growth of *C. albicans* and also our study is consistent with Maltaş et al., 2010 who observed that the methanol extract of VA-C leaves inhibits the growth of *C. albicans*. Moreover, our result showed that inhibitory capacity of

the solvents varies significantly in descending order of ethanol then water then methanol, this result is compatible with recent study carried by Keikha et al. (2018) who found that ethanol leave extract of VC-A has the highest inhibiting effect than water extract against *C. albicans* isolates.

The result of current study of the MIC showed that the ethanol extracts of VC-A was relatively higher than water and methanol. Where MIC values were between concentration 12.5 µg/ml and 25 µg/ml for ethanol and they represent dilution 4 and 3 respectively, and for water and methanol the MIC values are specified in concentration 25 µg/ml which represents the dilution 3. These results are close to a study of Keikha et al. (2018) who also found that the ethanol extract of VC-A was more effective than the aqueous extract where the MIC values of ethanol against isolates of *Candida* species were between the concentration of 0.78 µg/ml and 1.56 µg/ml and they represent dilution 7 and 8, respectively, and the values of the aqueous extract were between 6.25 µg/ml and 1.562 µg/ml which represent the dilutions are 5 and 7, respectively.

Also, the present study showed convergence of MFC values which represents only the three dilutions from 1 to 3 (100 µg/ml and 25 µg/ml) respectively. Where the VC-A extract of ethanol was the most influential on *C. tropicalis* with the value of MFC 25 µg/ml and the aqueous extract less effective on *C. albicans* with a value of 100 µg/ml.

The nature of the antibiotic's work is a very important in determining the appropriate type for treatment the infection, as it works in two different ways, either killing the microbe (microbicidal) or inhibiting its growth of the microbe (microbistatic) (Etebu1 and Ariekpar, 2016). Antibiotics with inhibitory effect are usually prescribed to patients who do not have problems with their immune system, while antibiotics with a fatal effect are prescribed for patients with low immunity or severe infections (Davies and Davies, 2010). Since *Candida* species are generally opportunistic and affect the group of people with low immunity, thus antibiotics that use for treatment candida infection are inevitably more effective if they are of the fatal type. Therefore, the inhibitory efficiency of the VC-A extract was estimate using the ratio between MFC and MIC. The result of calculation showed that the ratio of MFC/MIC between one to two-fold according to this result the VC-A extracts possess candidacidal effect (Levison and Levison, 2009). To our best of knowledge, there is no previous study investigate this finding.

In the current study, the extracts of VA-C differed in their inhibitory effect according to the type of solvent and this is maybe due to the difference in the degree of polarity between the solvent, where the water is the highest polarity of 1,000 followed by methanol 0.762 the least is ethanol 0.654. Thus, the compounds that extracted by these highly polar solvents differ in quantity and quality (Abubakar and Haque, 2020). Many studies have demonstrated the effect of the solvent type on the inhibitory potential of plant extracts (Aljuraifani 2017; Ababutain 2015).

The GC-MC analysis result revealed that all three VA-C extracts were rich in chemical compounds that have important activity as an anti-inflammatory, anticancer, anti-alzheimer, anti-diarrhea, anti-diabetic, anti-viral, antioxidant, anti-allergic, nematicide, antibacterial, antifungal, as well as other uses as food preservatives, flavorings and (Table 3). Moreover, several of these secondary metabolism compound belongs to an important chemical group such as polyphenols, fatty acids, terpenes, terpenoid, steroids, aldehydes, alcohol and esters. These results are in agreement with a previous study of (Keikha et al., 2018) stated that the VC-A extract is rich in chemical compounds, where they found that the alcoholic extract contains 36 chemical compounds that belong to different chemical groups. The difference in the number of the

phytochemical compounds may be attributed to the variations among VA-C genotypes. (Karaguzel and Girmen, 2009)

The inhibitory activity of VA-C extracts maybe attributed to the presence of important bioactive compounds (Abdal Sahib et al., 2019), which maybe targeting different structures of the *Candida* species such as, cell wall, cell membrane, mitochondria enzymes. Also, some of these compounds can reduces or prevent the virulence factors includes; adhesins, enzymes production, germ tubes (Pseudohyphal), biofilm formation, Quorum sensing (de Oliveira Santos et al., 2018; Liu et al., 2017, Sardi et al., 2013). The results of this study showed the diversity of the compounds extracted from VC-A plant leaves that belonging to several effective biochemical compound with different anticandidal activity including, polyphenols that can destroys the *Candida* cell membrane leading to permeability of the cell contents (Peralta et al., 2015; Hwang et al., 2011; Hwang et al., 2010), inhibit the mitochondria enzymes activity in the *Candida* cell (Yang et al., 2014) and inhibit the germ tubes formation (Seleem et al., 2016). Fatty acids with moderate carbon chain between 10-12 carbons has good inhibitory activity against *Candida* species (Ababutain, 2019; Bergsson et al., 2001). Terpenes has reported to have inhibitory activity against *C. albicans* and also prevent the biofilm formation (Pemmaraju et al., 2013). Terpenoid, inhibit *C. albicans* cell growth by affecting the efficacy of the membrane and prevents adhesins, biofilm formation and germ tubes formation (Touil et al., 2020; Raut et al., 2013; Zore et al., 2011).

Conclusions

The results of the current study showed that the extract of the VA-C leaves is rich in bioactive compounds with broad spectrum activity that inhibited all the tested *Candida* sp. despite different species levels. Accordingly, the VA-C leaves extracts were superior to antibiotics in general, in which antibiotics affect a specific species or even a strain of species. This necessitates an accurate diagnosis of the *Candida* isolation in order to choose the appropriate antibiotic. The inhibitory activity of the ethanol solvent was better than methanol and water, which may indicate the importance of choosing the appropriate solvent to extract phytochemicals with high inhibiting effectiveness and in higher quantities. Moreover, the results showed that the extract had candidacidal effect on test *Candida* sp. at low concentrations, which is an advantage to reduce the side effect of the extract, if any. These results add an advantage to VA-C leaves extracts in its use as a promising source to develop an alternative anticandidal agent.

Acknowledgements

The authors thank the Director of Basic and Applied Scientific Research Centre (BASR) at Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia for her continuous support and encouragement. The authors like to thank Dr. Ahmed Alsayyah, Dr. Reem AlJindan, and Mrs. Nouf Alromaihi at King Fahd Hospital, Al Khobar – kingdom of Saudi Arabia for providing us with the tested microorganisms.

References

- Ababutain, I., 2015. Impact of solvent type on antibacterial activities of Lawsonia inermis leaves. J. Food Agric. Environ. 13 (1),51-53.
- Ababutain, I.M., 2019. Antimicrobial activity and gas chromatography-mass spectrometry (GC-MS) analysis of Saudi Arabian Ocimum basilicum Leaves Extracts, J. Pure Appl. Microbiol. 13(2), 823-833.
- Ababutain, I.M., Alghamdi, A.I., 2018. Phytochemical analysis and antibacterial activity of Vitex agnus-castus L. leaf extracts against clinical isolates. Asia Life Sci. 27(1), 11-20.
- Abdal Sahib, A.H., Al-Shareefi, E., Hameed, I.H., 2019. Detection of Bioactive Compounds of Vitex agnus-castus and Citrus sinensis Using Fourier-transform infrared spectroscopic profile and Evaluation of Its Anti-microbial Activity. Indian J. Public. Health Res. Dev.10(1), 954-959.
- Abdulrasheed, M., Ibrahim I.H, Luka, A, Maryam, A. A, Hafsat, L, Ibrahim, S, Maigari, F.U., Gidado, M.B., 2019. Antibacterial effect of Cinnamon (Cinnamomum zeylanicum) bark extract on different bacterial isolates. JEMAT. 7(1),16-20.
- Abubakar, A.R., Haque M., 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J. Pharm. Bioall. Sci.12,1-10.
- Adams, R.P., 2007. Identification of essential oil component by gas chromatography/ mass spectrometry. Allured Publishing Corporation, Carol Stream, Illinois.
- Aljuraifani, A., 2017. Impact of solvent types on antimicrobial activities of pumpkin (Cucurbita pepo L.) pulp extracts. Asia Life Sci. 26 (2), 229-235.
- Al-Marzoqi, A.H., Hadi, M.Y., Hameed, I.H., 2016. Determination of metabolites products by Cassia angustifolia and evaluate antimicrobial activity. J. Pharmacognosy Phytother. 8(2), 25-48.
- Al-Salih, D.A., Aziz, F.M., Mshimesh, B.A., Jehad, M.T., 2013. Antibacterial Effects of Vitamin E: in Vitro Study. J. Biotechnol. Res. Center 7(2), 17-23.
- Arokiyaraj, S., Perinbam, K., Agastian, P., Kumar M.R., 2009. Phytochemical analysis and antibacterial activity of Vitex agnus-castus. IJGP. 3(2),162-164.
- Asdadi, A., Idrissi Hassani L.M., Chebli, B., Moutaj, R., Gharby, S., Harhar, H., Salghi, R., EL Hadek, M. 2014. Chemical composition and antifungal activity of vitex agnus-castus l. seeds oil growing in Morocco. J. Mater Environ. Sci. 5(3), 823–830.
- Bergsson, G., Arnfinnsson, J.H., Steingrímsson, O., Thormar, H., 2001. In Vitro killing of Candida albicans by fatty acids and monoglycerides. Antimicrob. Agents Chemother. 45(11), 3209–3212.
- Bhakshu, L.Md., Ratnam, K.V., Raju, R.R.V., 2016. Anticandidal activity and phytochemical analysis of certain medicinal plants from Eastern Ghats, India. Indian J. Nat. Prod. Resour. 7(1), 25-31.
- Bidossi, A., Bortolin, M., Toscano, M., De Vecchi, E., Romanò, C.L., Mattina, R., Drago, L., 2017. In vitro comparison between α -tocopheryl acetate and α -tocopheryl phosphate against bacteria responsible of prosthetic and joint infections. PLoS One 12(7), e0182323. doi:10.1371/journal. Pone.0182323

346 Birkbeck, T.H., Reid, H.I., Darde, B., Grant, A.N., 2006. Activity of bronopol (Pyceze®) against
347 bacteria cultured from eggs of halibut, *Hippoglossus hippoglossus* and cod, *Gadus morhua*.
348 *Aquaculture* 254, 125–128.

349 Brandt, M.E., Lockhart, S.R., 2012. Recent Taxonomic Developments with *Candida* and Other
350 Opportunistic Yeasts. *Curr. Fungal Infect. Rep.* 6(3), 170–177.

351 Brickell, C., Zuk, J.D., 1997. A-Z Encyclopedia of garden plants. New York, United States, The
352 American Horticultural Society, DK Publishing Inc. 1095 p.

353 Carlomagno, G., Unfer, V., 2011. Inositol safety: clinical evidences. *Eur. Rev. Med. Pharmacol.*
354 *Sci.* 15, 931-936.

355 Chatelon, J., Cortegiani, A., Hammad, E., Cassir, N., Leone, M., 2019. Choosing the Right
356 Antifungal Agent in ICU Patients. *Adv. Ther.* 36(12), 3308-3320.

357 Cheng, S-S., Chung, M-J., Lin, C-Y., Wang, Y-N., Chang, S-T., 2012. Phytochemicals from
358 *Cunninghamia konishii* Hayata act as antifungal agents. *J. Agric. Food Chem.* 60(1), 124–128.

359 da Rocha, A. B., Lopes, R. M., Schwartzmann, G. 2001. Natural products in anticancer therapy.
360 *Curr. Opin. Pharmacol.* 1, 364–369. doi: 10.1016/s1471-4892(01)00063-7.

361 Daniele, C., Coon, J.T., Pittler, M.H., Ernst, E., 2005. *Vitex agnus castus*: A systematic review
362 of adverse events. *Drug Saf.* 28(4), 319-332.

363 Davies, J., Davies, D., 2010. American Society for Microbiology. All Rights Reserved. Origins and
364 Evolution of Antibiotic Resistance. *Microbiol. Mol. Biol. R.* 74(3), 417–433.

365 de Oliveira Santos, G.C., Vasconcelos, C.C., Lopes, A.J.O., de Sousa Cartágenes, M.dS., Filho,
366 A.K.D.B., do Nascimento, F.R.F., Ramos, R.M., Pires, E.R.R.B., de Andrade, M.S., Rocha,
367 F.M.G., de Andrade, M.C., 2018. *Candida* Infections and Therapeutic Strategies: Mechanisms of
368 Action for Traditional and Alternative Agents. *Front. Microbiol.* 9,1351. doi:
369 10.3389/fmicb.2018.01351.

370 Dervishi-Shengjergji, D., Vilma, P., Xhulieta, H., Aurel, N., Blerta, K., 2014. Antibacterial activity
371 and chemical composition of *Vitex agnus castus* fruits essential oils from Mbishkodra, Albania.
372 *JIEAS.* 9(4), 521-424.

373 Drobnica, L., Sturdík, E., 1980. Antimicrobial Activity of 2-vinylfuran Derivatives. *Folia Microbiol*
374 (Praha). 25(6), 467-75.

375 Eryigit, T., Çig, A., Okut, N., Yildirim, B., Ekici, K., 2015. Evaluation of chemical composition and
376 antimicrobial activity of *Vitex agnus castus* L. fruits' essential oils from West Anatolia, Turkey.
377 *J. Essent. Oil Bear Plants* 18, 208-214.

378 Eseyin, O.A., Sattar M.A., Rathore, H.A., Aigbe, F., Afzal S., Ahmad, A., Lazhari, M., Akthar, S.,
379 2018. GC-MS and HPLC profiles of phenolic fractions of the leaf of *Telfairia occidentalis*. *Pak. J.*
380 *Pharm. Sci.* 31(1), 45-50.

381 Etebu, E., Ariekpar, I., 2016. Antibiotics: Classification and mechanisms of action with emphasis
382 on molecular perspectives. *Int. J. Appl. Microbiol. Biotechnol. Res.* 4, 90-101.

383 Ganapaty, S., Vidyadhar. K.N., 2005. Phytoconstituents and biological activities of *Vitex*: A
384 review. *JNR.* 5, 75-95.

385 Ghannadi, N., Bagherinejad, M.R., Abedi, D., Jalali, M., Absalan, B., Sadeghi, N., 2012.
386 Antibacterial activity and composition of essential oils from *Pelargonium graveolens* L' Herit. and
387 *Vitex agnus-castus* L. *Iran. J. Microbiol.* 4(4), 171-176.

- 388 Ghimire, G.P., Thuan, N.H., Koirala, N., Sohng, J.K., 2016. Advances in biochemistry and
389 microbial production of squalene and its derivatives. J. Microbiol. Biotechnol. 26(3), 441–451.
- 390 Huntley, N. F., Patience, J. F., 2018. Xylose: absorption, fermentation, and postabsorptive
391 metabolism in the pig. J. Anim. Sci. Biotechnol. 9(4), DOI 10.1186/s40104-017-0226-9.
- 392 Hwang, B., Cho, J., Hwang, I.-S., Jin, H.-G., Woo, E.-R., Lee, D. G., 2011. Antifungal activity of
393 larciresinol derived from *Sambucus williamsii* and their membrane-active mechanisms in *Candida*
394 *albicans*. Biochem. Biophys. Res. Commun. 410(3), 489–493.
- 395 Hwang, B., Lee, J., Liu, Q.-H., Woo, E.-R., Lee, D. G., 2010. Antifungal effect of (+)-pinoresinol
396 isolated from *Sambucus williamsii*. Molecules 15(5), 3507–3516, 2010.
- 397 Ibrahim, I.S., Ali, M., Zage, A.U., 2016. Phytochemistry of methanolic and aqueous extracts of
398 *Eucalyptus camaldulensis* leaves, seeds and stem bark. Int J Advan.Academic Res. Sci. 2, 75-
399 80.
- 401 Jegadeeswari, P., Nishanthini, A., Muthukumarasamy. S., Mohan. V.R. 2012. GC-MS analysis of
402 bioactive components of *Aristolochia krysagathra* (Aristolochiaceae). J. Curr. Chem. Pharm. Sci.
403 2, 226-232.
- 404 Johnny, A.K., Darre, M.J., Donoghue, A.M., Donoghue, D.J., Venkitanarayanan, K., 2010.
405 Antibacterial effect of *trans*-cinnamaldehyde, eugenol, carvacrol, and thymol on *Salmonella*
406 *enteritidis* and *Campylobacter jejuni* in chicken cecal contents in vitro. J. Appl. Poultry Res. 19
407 (3), 237–244.
- 408 Joller, C., De Vrieze, M., Moradi, A., Fournier, C., Chinchilla, D., L'Haridon, F., Buisson, S.,
409 Weisskopf, L., 2020. S-methyl Methanethiosulfonate: Promising Late Blight Inhibitor or Broad
410 Range Toxin? Pathogens 9(6), 496 doi.org/10.3390/pathogens9060496.
- 412 Kalhor, M.A., Farheen, S., Aqsa, N.U., 2014. The antimicrobial activity of ethanol extract of *Vitex*
413 *agnus-castus*. Am. Int. J. Contemp. Res. 1(1), 47-50.
- 414 Karthikeyan, S. C., Velmurugan, S., Donio, M. B., Michaelbabu, M., Citarasu, T., 2014. Studies
415 on the antimicrobial potential and structural characterization of fatty acids extracted from Sydney
416 rock oyster *Saccostrea glomerata*. Ann. Clin Microb. Anti. 13, 332. doi.org/10.1186/s12941-014-
417 0057-x
- 418 Karaguzel, O., Girmen, B., 2009. Morphological variations of chaste tree (*Vitex agnus-castus*)
419 genotypes from southern Anatolia, Turkey, New Zeal. J. Crop. Hort. 37(3), 253-261.
- 420 Katirae, F., Mahmoudi, R., Tahapour, K., Hamidian, G., Emami, S.J., 2015. Biological properties
421 of *Vitex agnus-castus* essential oil (phytochemical component, antioxidant and antifungal activity).
422 Biotechnol. Health. Sci. 2(2), 267–97.
- 423 Keikha, N., Shafaghat, M., Mousavia, S.M., Moudi, M., Keshavarzi, F., 2018. Antifungal effects of
424 ethanolic and aqueous extracts of *Vitex agnus-castus* against vaginal isolates of *Candida*
425 *albicans*. Curr. Med. Mycol. 4(1), 1–5.
- 426 Kim, G-Y., Jeon J-S., Jae Kyung Kim, J.K., 2016. Isolation Frequency Characteristics of *Candida*
427 Species from Clinical Specimens. Mycobiology 44(2), 99-104.
- 428 Kirti, K., Amita, S., Priti, S., Mukesh Kumar, A., Jyoti. S., 2014. Colorful World of Microbes:
429 Carotenoids and Their Applications. Adv. Biol. 2014, 1–13.

- 430 Koehler, P., Stecher, M., Cornely O.A., Koehler, D., Vehreschild, M.J.G.T., Bohlius, J.,
431 Wisplinghoff, H., Vehreschild, J.J., 2019. Morbidity and mortality of candidaemia in Europe: an
432 epidemiologic meta-analysis. Clin. Microbiol. Infect. 25(10), 1200-1212.
- 433 Kumar, R.N., Vasantha, K., Mohan, V.R., 2014. GC-MS analysis of bioactive components of
434 tubers of *Ruellia tuberosa* L. (Acanthaceae). Am. J. Phytomed. Clin. Ther. 2(2), 209-216.
- 435 Kumaravel, S., Muthukumaran, P., Shanmugapriya, K., 2017. Chemical composition of *Trigonella*
436 *foenum-graecum* through gas chromatography mass spectrometry analysis. J. Med. Plants Stud.
437 5, 1-3.
- 438 Lamba, A., 2007. Antimicrobial activities of aldehydes and ketones produced during rapid
439 volatilization of biogenic oils. Masters Theses.
- 440 Levison M.E., Levison J.H., 2009. Pharmacokinetics and pharmacodynamics of antibacterial
441 agents. Infect. Dis. Clin. North Am. 23(4), 791-9.
- 442 Liu, X., Ma, Z., Zhang, J., Yang, L., 2017. Antifungal compounds against *Candida* infections from
443 traditional Chinese medicine. BioMed Res. Int. 2017, 1-12. doi.org/10.1155/2017/4614183
- 444 Ma, K., Thomason, L.A., McLaurin, J., 2012. Scyllo-Inositol, preclinical, and clinical data for
445 Alzheimer's disease. Adv. Pharmacol. 64,177-212.
- 446 Madan R.K., Levitt J., 2014. A review of toxicity from topical salicylic acid preparations. J. Am.
447 Acad. Dermatol. 70(4), 788-92.
- 448 Maltaş, E., Uysal, A., Yıldız, S., Durak, Y., 2010. Evaluation of antioxidant and antimicrobial
449 activity of *Vitex agnus castus* L. Fresenius Environ. Bull. 19, 3094-3099.
- 450 McDonnell, G., 2009. Sterilization and Disinfection. In Encyclopedia of Microbiology, 3rd Edition,
451 Moselio Schaechter (Editor), Academic press, Elsevier Ltd, 529-548.
- 452 Miceli, M.H., Diaz, J.A., Lee, S.A., 2011. Emerging opportunistic yeast infections. Lancet. Infect.
453 Dis. 11, 142–151.
- 454 Miguel, V., Lestard, M.E.D., Tuttolomondo, M.E., Díaz, S.B., BenAltabef, A., Puiatti, M., Pierini,
455 A.B., 2016. Molecular view of the interaction of S-methyl methane-thiosulfonate with DPPC
456 bilayer. Biochim. Biophys. Acta. Biomembr. 1858(1), 38-46.
- 457 Mincea, M.M., Lupşa, I.R., Cinghiţă, D.F., Radovan, C.V., Talpos, I., Ostafe, V., 2009.
458 Determination of methylparaben from cosmetic products by ultraperformance liquid
459 chromatography. J. Serb. Chem. Soc. 74 (6), 669–676.
- 460 Naragani, K., Mangamuri, U., Muvva, V., Poda, S., Munaganti, R.K., 2016. antimicrobial potential
461 of *Streptomyces cheonanensis* vuk-a from mangrove origin. Int. J. Pharm. Pharm. Sci. 8(3), 53-
462 57.
- 463 National Committee for Clinical Laboratory Standards (NCCLS), 1993. Performance Standards
464 for Antimicrobial Disk Susceptibility Tests. Approved Standard, NCCLS Document M2-A5,
465 National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania, USA, 13(24), 35.
- 466 National Committee for Clinical Laboratory Standards (NCCLS), 1997. Performance Standards
467 for Antimicrobial Disk Susceptibility Tests. Approved Standard M2-A6. National Committee for
468 Clinical Laboratory Standards, Wayne, Pennsylvania, USA, 17(1).
- 469 Neeraj, Vasudeva, N., Sharma, S., 2019. Chemical composition of *Fagopyrum*
470 *esculentum* Moench seed through GC-MS. Int. J. Pharm. Sci. Res. 10(5), 2392-2396.

- 471 Neves, R.C.S., da Camara, C.A.G., 2016. Chemical composition and acaricidal activity of the
472 essential oils from *Vitex agnus-castus* L. (Verbenaceae) and selected monoterpenes. *Annals of*
473 *the Brazilian Academy of Sciences. An. Acad. Bras. Cienc.* 88(3), 1221-1233.
- 474 Niroumand, M.C., Heydarpour, F., Farzaei, M.H., 2018. Pharmacological and therapeutic effects
475 of *Vitex agnus-castus* L.: A review. *Phcog. Rev.* 12,103-14.
- 476 Okoye, N.N., Ajaghaku, D.L., Okeke, H.N., Ilodigwe, E.E., Nworu, C.S., Okoye, F.B. 2014. Beta-
477 Amyrin and alpha-amyrin acetate isolated from the stem bark of *Alstonia boonei* display profound
478 anti-inflammatory activity. *Pharm. Biol.* 52(11), 1478–14786.
- 479 Olajuyigbe, O.O., Onibudo, T.E., Cooposamy, R.M., Ashafa, A.O.T., Afolayan, A.J., 2018.
480 Bioactive compounds and in vitro antimicrobial activities of ethanol stem bark extract of
481 *Trilepisium madagascariense* DC. *Int. J. Pharmacol.* 14(7), 901-912
- 482 Omura, S., Pyl, D.V.D., Inokoshi, J., Takahashi, Y., Takeshima, Y., 1993. Peptidicinnamylsnew
483 farnesyl-protein transferase inhibitors produced by an actinomycete I. Producingstrain,
484 fermentation, isolation and biological activity. *J. Antibiot.* 46, 222-228.
- 485 Ovesná, Z., Vachálková, A., Horváthová, K., 2004. Taraxasterol and beta-sitosterol: new naturally
486 compounds with chemoprotective/chemopreventive effects. *Neoplasma* 51(6), 407-414.
- 487 Özçelik, B., Kartal, M., Orhan, I., 2011. Cytotoxicity, antiviral and antimicrobial activities of
488 alkaloids, flavonoids, and phenolic acids. *Pharm. biol.* 49(4), 396-402.
- 489 Pandey, A., Tripathi, S., 2014. Concept of standardization, extraction and pre phytochemical
490 screening strategies for herbal drug. *J. Pharmacogn. Phytochem.* 2 (5), 115-119.
- 491 Paramythiotou, E., Frantzeskaki, F., Flevari, A., Armaganidis, A., Dimopoulos, G., 2014. Invasive
492 Fungal Infections in the ICU: How to Approach, How to Treat. *Molecules* 19(1),1085-119.
- 493 Pemmaraju, S.C., Pruthi, P.A., Prasad, R., Pruthi, V., 2013. *Candida albicans* biofilm inhibition
494 by synergistic action of terpenes and fluconazole. *Indian J. Exp. Biol.* 51, 1032-1037.
- 495 Peralta, M. A., Da Silva, M. A., Ortega, M. G., Cabrera, J. L., Paraje, M. G., 2015. Antifungal
496 activity of a prenylated flavonoid from *Dalea elegans* against *Candida albicans* biofilms.
497 *Phytomedicine*, 22(11), 975–980.
- 498 Rani, A., Sharma, A., 2013. The genus *Vitex*: A review. *Pharmacogn Rev.* 7(14), 1-5.
- 499 Raut, J.S., Shinde, R.B., Chauhan, N.M., Karuppayil, S.M., 2013. Terpenoids of plant origin
500 inhibit morphogenesis, adhesion, and biofilm formation by *Candida albicans*. *Biofouling*
501 29(1), 87-96.
- 502 Rodrigues, C.F., Rodrigues, M.E., Henriques, M., 2019. *Candida* sp. Infections in Patients with
503 Diabetes Mellitus. *J. Clin. Med.* 8(1): 76. doi: 10.3390/jcm8010076
- 504 Romald, P.N., Sridharan, K.S., Mohanty, S., Anupma Jyoti Kindo, A.J., 2019. Rare isolate of
505 *Stephanoascus ciferrii* from the aural discharge of post-mastoidectomy patient-A case report. *J.*
506 *Clin. Diagn. Res.* 13(3), 1-3.

507 Sardi, J.C.O., Scorzoni, L., Bernardi, T., Fusco-Almeida A.M., Mendes Giannini M. J. S., 2013.
 508 *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal
 509 products and new therapeutic options. *J. Med. Microbiol.* 62, 10-24.

510 Saud, R., Pokhrel, S., Yadav, P.N., 2019. Synthesis, characterization and antimicrobial activity of
 511 maltol functionalized chitosan derivatives, *Journal of Macromolecular Science, Part A*, DOI:
 512 10.1080/10601325.2019.1578616.

513 Seleem, D., Benso, B., Noguti, J., Pardi, V., Murata, R.M., 2016. In vitro and in vivo antifungal
 514 activity of lichochalcone-A against *Candida albicans* biofilms. *PLoS ONE*, 11(6), e0157188.
 515 doi:10.1371/journal.pone.0157188.

516 Sermakkani, M., Thangapandian, V., 2012. GC-MS analysis of *Cassia italica* leaf methanol
 517 extract. *Asian J. Pharm. Clin. Res.* 5(2), 90-4.

518 Shahina, P., Shahzad, A., Upadhyaya, P., Yadav, V. 2016. Gas chromatography-mass
 519 spectrometry analysis of methanolic leaf extract of *Cassia angustifolia* Vahl. *Asian J. Pharm. Clin.*
 520 *Res.* 9, 111-6.

521 Sharma, V., Singh, G., Kaur, H., Saxena, A.K., Ishar, M.P., 2012. Synthesis of β -ionone derived
 522 chalcones as potent antimicrobial agents. *Bioorg. Med. Chem. Lett.* 22(20), 6343-6.

523 Shibula, K., Velavan, S., 2015. Determination of phytocomponents in methanolic extract of
 524 *Annona muricata* leaf using GC-MS technique. *Int. J. Pharmacog. Phytochem. Res.* 7, 1251-5.

525 Solanki, S., Singh, A., Sood, H., 2018. GC analysis of invitro developed shoots of *Stevia*
 526 *rebaudiana* through rapid tissue culture. *International Conference on New Horizons in Green*
 527 *Chemistry & Technology (ICGCT) 2018.*
 528 Available at SSRN: <http://dx.doi.org/10.2139/ssrn.3298672>

529 Statistical Packages for Software Sciences (SPSS). 2013. version 21.0 Armonk, New York, IBM
 530 Corporation. Released.

531 Tan, K.H., Nishida, R., 2012. Methyl eugenol: Its occurrence, distribution, and role in nature,
 532 especially in relation to insect behavior and pollination. *J. Insect. Sci.* 12(1), 1-60.

533 Tolstikov, G.A., Flekhter, O.B., Shul'ts, E.E., Baltin, L.A., Tolsikov, A.G., 2005. Betulin and its
 534 derivatives. chemistry and biological activity. *Khim. Interes. Ust. Razv.* 13, 1-30.

535 Touil, H.F.Z., Boucherit, K., Boucherit-Otmani, Z., Kohder, G., Madkour, M., Soliman, S.S.M.
 536 2020. Optimum inhibition of amphotericin-B-Resistant *Candida albicans* strain in single- and
 537 mixed-species biofilms by *Candida* and non-*Candida* terpenoids. *Biomolecules* 10(2), 342.
 538 doi: 10.3390/biom10020342

539 Treasurer, W. Cochrane, E., Grant, A., 2005. Surface disinfection of cod *Gadus morhua* and
 540 haddock *Melanogrammus aeglefinus* eggs with bronopol, *aquaculture* 250(1-2), 27–35.

541 Tripathi, N., Kumar, S., Singh, R., Singh, C.J., Singh, P., 2013. Varshney V.K. Isolation and
 542 Identification of γ -sitosterol by GC-MS from Roots of *Girardinia heterophylla*. *OJC.* 29(2), 705-7.

543 Tyagi, T., Agarwal, M., 2017. Phytochemical screening and GCMS analysis of bioactive
 544 constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms.
 545 *J. Pharmacogn. Phytochem.* 6(1), 195-206.

- 546 Ulukanli, Z., Çenet, M., Öztürk, B., Bozok, F., Karabörklü, S., Demirci, S.C., 2015. Chemical
547 characterization, phytotoxic, antimicrobial and insecticidal activities of *Vitex agnus-castus*:
548 Essential oil from East Mediterranean Region. J. Essent. Oil-Bear. Plants. 18(6), 1500-1507.
- 549 Wei, J.H., Yin, X., Welandar, P.V., 2016. Sterol Synthesis in Diverse Bacteria. Front Microbiol.
550 7,990. doi:10.3389/fmicb.2016.00990.
- 551 World Health Organization. (WHO). 2002. Monographs on Selected Medicinal Plants. 2, 55–65.
- 552 Yang, S., Fu, Y., Wu X., Zhou, Z., Xu, J., Zeng, X., Kuang, N., Zeng, Y., 2014. Baicalin prevents
553 *Candida albicans* infections via increasing its apoptosis rate. Biochem. Biophys. Res. Commun.
554 451(1), 36–41.
- 555 Yilar, M., Bayan, Y., Onaran, A., 2016. Chemical Composition and Antifungal Effects of *Vitex*
556 *agnus-castus* L. and *Myrtus communis* L. Plants. Not. Bot. Horti. Agrobi. 44(2), 466-471.
- 557 Zore, G.B., Thakre, A.D., Jadhav, S., Karuppayil, S.M., 2011. Terpenoids inhibit *Candida*
558 *albicans* growth by affecting membrane integrity and arrest of cell cycle. Phytomedicine
559 18(13), 1181-90.

Table 1(on next page)

Anticandidal activity of VA-C leaves extract at concentration of 20% by using well diffusion assay.

* P-value has been calculated using one-way Anova. ** Significant at $p < 0.01$ level. ND, not identified

<i>Candid</i> sp.	Zone of inhibition (mm) Mean \pm SD					P-value *
	Nystatin (10 mcg)	negative control	Ethanol	Water	Methanol	
<i>C. tropicalis</i>	11.0 \pm 1.00	0	7.50 \pm 0.50	5.67 \pm 0.29	5.33 \pm 0.29	0.01 **
<i>C. albicans</i>	5.83 \pm 0.29	0	5.83 \pm 0.29	5.00 \pm 0.50	5.00 \pm 0.50	0.047 **
<i>C. ciferrii</i>	ND	0	4.33 \pm 0.58	3.33 \pm 0.29	3.33 \pm 0.29	0.037 **
P-value	0.01 **	-	0.01 **	0.01 **	0.01 **	--

Table 2(on next page)

Minimal Inhibitory Concentration (MIC) $\mu\text{g/ml}$ and Minimal Fungal Concentration (MFC) $\mu\text{g/ml}$ and their ratio of VA-C leaves extracts.

*Ratio MFC/MIC

<i>Candid</i> sp.	Ethanol			Water			Methanol		
	MIC	MFC	Ratio*	MIC	MFC	Ratio	MIC	MFC	Ratio
<i>C. tropicalis</i>	12.5	25	1	25	50	1	25	50	1
<i>C. albicans</i>	25	50	1	25	50	1	25	100	2
<i>C. ciferrii</i>	25	50	1	25	50	1	25	50	1

Table 3(on next page)

GC-MS analysis of VA-C leaves extracts, their molecular formula , nature and biological activities.

No	Compound name	Peak Area%			Molecular Formula	Compound nature and biological activities
		EL	ML	WL		
1	4,5-Dichloro-1,3-dioxolan-2-one	7.43	7.45	1.39	C ₃ H ₂ Cl ₂ O ₃	No report was found.
2	Benzoic acid, 4-hydroxy-	2.13	3.95	5.99	C ₇ H ₆ O ₃	Phenolic compounds (Eseyin et al., 2018).
3	5-Hydroxymethylfurfural	1.18	1.61	0.82	C ₆ H ₆ O ₃	Organic compound Antioxidant and Antiproliferative (Ibrahim et al., 2016).
4	Phenol	1.12	1.73	1.41	C ₆ H ₅ OH	Phenolic compound, antiviral, antibacterial and antifungal activities (Özçelik et al., 2011).
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	0.75	0.82	1.41	C ₆ H ₈ O ₄	Flavonoids, Anti-inflammatory, analgesic, antimicrobial activity (Neeraj et al., 2019).
6	Catechol	0.50	0.57	1.14	C ₆ H ₄ (OH) ₂	Polyhydric phenol, antiviral, antimicrobial activities (Özçelik et al., 2011).
7	Benzeneacetaldehyd, alpha-methyl-	0.35	0.65	1.26	C ₉ H ₁₀ O	Hydrotropic aldehyde.
8	Benzeneacetic acid, 4-hydroxy3-methoxy,	0.39	0.38	0.58	C ₁₀ H ₁₂ O ₄	No report was found.
9	Pentanal	0.10	0.16	0.88	C ₅ H ₁₀ O	alkyl aldehyde, Inhibition bacteria (Lamba, 2007).
10	Squalene	0.13	0.37	0.40	C ₃₀ H ₅₀	Terpenoid, Anticandidal activity, antioxidant, anti-inflammatory, and anticancer agent (Ghimire et al., 2016; Zore et al., 2011).
11	Maltol	0.06	0.14	0.77	C ₆ H ₆ O ₃	Antimicrobial activity (Saud et al., 2019).
12	1H-Benzocyclohepten-7-ol,2,3,4,4a,5,6,7,8-	0.36	0.12	0.11	C ₁₅ H ₂₆ O	Sesquiterpenids (Solaki et al., 2018).
13	Triacetin	0.19	0.27	-	C ₉ H ₁₄ O ₆	Triester of glycerin and acetic acid.
14	3,5-Octadienoic acid, 7-hydroxy-2-methyl	0.85	0.08	-	C ₉ H ₁₄ O ₃	No report was found.
15	Eugenol	0.39	0.36	-	C ₁₀ H ₁₂ O ₂	Phenolic compounds, antimicrobial activity, insecticide nematocide and food additive (Tan and Nishida, 2012; Johny et al., 2010).
16	1,2,3-Benzenetriol	0.56	0.67	-	C ₆ H ₆ O ₃	No report was found.
17	Propylphosphonic acid, di(2-ethylhexyl) ester	2.57	1.18	-	C ₂₁ H ₄₀ O ₄	Ester.
18	Methylparaben	0.39	0.28	-	C ₈ H ₈ O ₃	Antimicrobial activity, food preservative, added to

						cosmetic products, and pharmaceutical products (Mincea et al., 2009).
19	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene	1.21	1.04	-	C ₁₅ H ₂₄ O	No report was found.
20	n-Hexadecanoic acid	0.70	0.56	-	C ₁₆ H ₃₂ O ₂	Palmitic saturated Fatty acid ester, antimicrobial and antitumor activities (Karthikeyan et al., 2014).
21	5-(1-Isopropenyl-4,5-dimethylbicyclo [4.3.0])	1.13	0.87	-	C ₂₂ H ₃₆ O ₂	No report was found.
22	2,4-Cholestadien-1-one	1.72	0.96	-	C ₂₇ H ₄₂ O	No report was found.
23	Phytol	3.31	1.81	-	C ₂₀ H ₄₀ O	Diterpene, antiviral and antimicrobial activities (Özçelik et al., 2011).
24	9,12,15-Octadecatrienoic acid, (Z, Z,Z)-	1.18	0.90	-	C ₁₈ H ₃₀ O ₂	Linolenic Omega-3 polyunsaturated fatty acid, anti-inflammatory (Sermakkani and Thangapandian, 2012).
25	Cedran-diol, (8S,14)-	0.13	0.12	-	C ₁₅ H ₂₆ O ₂	No report was found.
26	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.11	1.34	-	C ₂₀ H ₄₀ O	Terpene Alcohol, antimicrobial, antioxidant, anti-inflammatory and flavoring agent (Shibula and Velavan, 2015; Jegadeeswari et al., 2012; Sermakkani and Thangapandian, 2012).
27	Spiro [4.5] dec-9-en-1-ol,1,6,6,10-tetramethyl	0.54	0.39	-	C ₁₄ H ₂₄ O	No report was found.
28	4,5-Dichloro-1,3-dioxolan-2-one	7.43	7.45	-	C ₃ H ₂ Cl ₂ O ₃	No report was found.
29	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-	0.75	0.82	-	C ₅ H ₆ O ₄	No report was found.
30	Dodeca-1,6-dien-12-ol, 6,10-dimethyl	1.57	1.57	-	C ₁₄ H ₂₆ O	No report was found.
31	Octadecanoic acid	0.51	-	0.22	C ₁₇ H ₃₅ CO ₂ H	Stearic saturated fatty acid.
32	n-Hexadecanoic acid	0.70	-	0.96	C ₁₆ H ₃₂ O ₂	Palmitic saturated Fatty acid ester, antioxidant, pesticide, nematocidal, antiandrogenic and hypochloesterolemi (Sermakkani and Thangapandian, 2012; Tyagi and Agarwal., 2017).
33	Benzenediazonium, 2-hydroxy-, hydroxide, i	0.86	-	1.17	C ₆ H ₅ N ₂ O	No report was found.

34	Vitamin E	-	0.39	0.36	$C_{29}H_{50}O_2$	Lipid, antibacterial, anti-alzheimer, antiaging and antioxidant (Kumaravel et al., 2017; Al-Marzoqi et al., 2016; Shahina et al., 2016; Al-Salih et al., 2013).
35	Cedrol	-	0.22	0.12	$C_{15}H_{26}O$	sesquiterpene alcohol.
36	gamma-Sitosterol	-	0.89	0.56	$C_{29}H_{50}O$	Steroid, antidiabetic drug (Tripathi et al., 2013).
37	Paromomycin	-	0.12	0.22	$C_{23}H_{45}N_5O_1$ 4	Treatment of diarrhea and protozoa infections (Olajuyigbe et al., 2018).
38	Heptanal	0.20	-	-	$C_7H_{14}O$	aldehyde antibacterial activity (Lamba, 2007).
39	Ionone	0.33	-	-	$C_{13}H_{20}O$	Sesquiterpenoids, antimicrobial agents (Sharma et al., 2012).
40	Chloroxylenol	0.16	-	-	C_8H_9OCl	phenols with <u>antiseptic</u> activity, It is used in the manufacture of disinfectants and sterilizers (McDonnell, 2009).
41	1-Heptadecene	0.22	-	-	$C_{17}H_{34}$	unsaturated aliphatic hydrocarbons.
42	Undecanal	0.25	-	-	$C_{10}H_{21}CHO$	fatty aldehyde lipid molecule.
43	1H-Indene, 2,3-dihydro-1,1,2,3,3-pentamethyl	9.63	-	-	$C_{14}H_{20}$	No report was found.
44	Epiglobulol	0.97	-	-	$C_{15}H_{26}O$	Alcohol.
45	tau-Cadinol	1.64	-	-	$C_{15}H_{26}O$	No report was found.
46	alpha-Cadinol	0.38	-	-	$C_{15}H_{26}O$	Antifungal activity (Cheng et al., 2012).
47	Phytol, acetate	0.37	-	-	$C_{22}H_{42}O_2$	Food additive, antimicrobial, anti-inflammatory, anticancer and antidiuretic properties (Sermakkani and Thangapandian, 2012).
48	1S,2S,5R-1,4,4-Trimethyltricyclo [6.3.1.0(2,5)]	1.26	-	-	$C_{15}H_{24}$	No report was found.
49	beta-iso-Methyl ionone	0.39	-	-	$C_{14}H_{22}O$	No report was found.
50	Longipinane, (E)-	0.41	-	-	$C_{15}H_{24}$	No report was found.
51	(-)-Isolongifolol, methyl ether	0.70	-	-	$C_{16}H_{28}O$	Ether.
52	Taraxasterol	0.07	-	-	$C_{30}H_{50}O$	Anti-tumor and chemopreventive activity (Ovesná and Horvathova, 2004).
53	S-Methyl methanethiosulphonate	0.07	-	-	$CH_3SO_2SCH_3$	Ester, Antimutagenic agent and antimicrobial activity (Joller et al., 2020; Miguel et al., 2016).

54	1-Heptatriacotanol	0.23	-	-	C ₃₇ H ₇₆ O	Fatty alcohol.
55	2-Vinylfuran		0.83		C ₆ H ₆ O	Antimicrobial activity (Drobnica and Sturdik, 1980).
56	3,5-Octadienoic acid, 7-hydroxy-2-methyl-,	-	1.04	-	C ₉ H ₁₄ O ₃	No report was found.
57	Salicyl hydrazide	-	0.39	-	C ₇ H ₈ N ₂ O ₂	Phenolic compounds, antimicrobial activity, Anti-inflammatory (Madan et al., 2014).
58	Cedran-diol, 8S,13-	-	0.40	-	C ₁₅ H ₂₆ O ₂	No report was found.
59	Isobutyl 4-hydroxybenzoate	-	8.91	-	C ₁₁ H ₁₄ O ₃	No report was found.
60	Propylphosphonic acid, di(2-ethylhexyl) noneste	-	1.18	-	C ₂₁ H ₄₀ O ₄	No report was found.
61	Methyl(ethenyl)bis(but-3-en-1-ynyl) silane	-	1.62	-	C ₇ H ₁₆ Si	No report was found.
62	beta Carotene	-	0.16	-	C ₄₀ H ₅₆	Carotenoids used as food, nutrition, antioxidant, disease control, and antimicrobial agents (Kirti et al., 2014).
63	17-Norkaur-15-ene, 13-methyl-, (8.beta.,13.b	-	1.05	-	C ₂₀ H ₃₂	No report was found.
64	3-Hydroxy-2-(2-methylcyclohex-1-enyl) propan-	-	1.48	-	C ₁₀ H ₁₆ O ₂	No report was found.
65	Cyclopropanebutanoic acid, 2-[[2-[[2-(2-pen	-	1.20	-	C ₁₁ H ₂₂ N ₂ O ₄	No report was found.
66	Cholan-24-oic acid, methyl ester, (5.beta.)-	-	1.56	-	C ₂₅ H ₄₀ O ₃	No report was found.
67	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	-	1.12	-	C ₃₂ H ₅₂ O ₂	No report was found.
68	geranyl-.alpha.-terpinene	-	0.80	-	C ₂₀ H ₃₂	Terpinene.
69	Tungsten, tricarbonyl-(2,5-norbornadiene)	-	-	1.32	C ₁₄ H ₁₆	No report was found.
70	1,2-Cyclopentanedione	-	-	1.21	C ₇ H ₁₀ O ₂	Prevents gastrointestinal tumor growth (Neeraj et al., 2019).
71	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	-	-	0.34	C ₆ H ₈ O ₂	No report was found.
72	1,2,3-Propanetriol, 1-acetate	-	-	1.23	C ₅ H ₁₀ O ₄	No report was found.
73	Acetoacetic acid, 3-thio-, benzyl ester	-	-	0.16	C ₁₁ H ₁₂ O ₂	No report was found.
74	trans-Z-.alpha.-Bisabolene epoxide	-	-	1.11	C ₁₅ H ₂₄ O	No report was found.
75	2-Hydroxyoctanoic acid	-	-	0.62	C ₈ H ₁₆ O ₃	No report was found.

76	1-Tetradecene	-	-	0.67	C ₁₄ H ₂₈	Antimicrobial activity (Naragani et al., 2016).
77	Benzoic acid, 4-methoxy-	-	-	0.69	C ₈ H ₈ O ₃	No report was found.
78	Chlorozotocin	-	-	0.20	C ₉ H ₁₆ ClN ₃ O ₇	No report was found.
79	2-Isopropyl-5-methyl-6-oxabicyclo [3.1.0] hex	-	-	1.51	C ₁₀ H ₁₆ O ₂	No report was found.
80	Quinic acid	-	-	2.96	C ₇ H ₁₂ O ₆	Anti-viral activity (Özçelik et al., 2011).
81	3-Methylindene-2-carboxylic acid	-	-	1.11	C ₁₁ H ₁₀ O ₂	No report was found.
82	O, O-Dibutyl S-(2-acetamidoethylmercapto) p	-	-	1.32	C ₁₂ H ₂₂ O ₄	No report was found.
83	3-Deoxy-d-mannonic acid	-	-	1.21	C ₆ H ₁₂ O ₆	No report was found.
84	Cyclooctane-1,4-diol, cis	-	-	0.44	C ₈ H ₁₆ O ₂	No report was found.
85	cis, cis, cis-7,10,13-Hexadecatrienal	-	-	0.58	C ₁₆ H ₂₆ O	Unsaturated fatty aldehyde.
86	10-Iodo-7-oxa-2-thia-tricyclo [4.3.1.0(3,8)]de	-	-	1.08	C ₈ H ₁₁ IOS	No report was found.
87	Bicyclo [6.1.0] nonane, 9-(1-methylethylidene	-	-	3.66	C ₁₂ H ₂₀	No report was found.
88	Inositol	-	-	0.15	C ₆ H ₁₂ O ₆	Essential nutrient, Cancer chemoprevention agent, treatment for Polycystic Ovary Syndrome and insulin sensitizing agent (Carlomagno and Unfer, 2011).
89	Xylose	-	-	0.15	C ₅ H ₁₀ O ₅	Pentose sugar (Huntley and Patience, 2018).
90	Scyllo-Inositol	-	-	1.18	C ₆ H ₁₂ O ₆	treatment of Alzheimer's disease (Ma et al., 2012).
91	2,4-Pentadien-1-ol, 3-pentyl-, (2Z)-	-	-	0.94	C ₁₀ H ₁₈ O	No report was found.
92	Widdrol hydroxyether	-	-	0.23	C ₁₅ H ₂₆ O ₂	No report was found.
93	Stigmasterol	-	-	0.21	C ₂₉ H ₄₈ O	Steroid, antioxidant, antimicrobial, anticancer, antiarthritic, antiasthma, anti-inflammatory, diuretic (Tyagi and Agarwal, 2017; Kumar et al., 2014).
94	beta.-Amyrin	-	-	0.43	C ₃₀ H ₅₀ O	Triterpenes, anti-inflammatory (Okoye et al., 2014).
95	5,5'-Dihydroxy-3,3'-dimethyl-2,2'-binaphthal	-	-	1.31	C ₁₇ H ₁₄ O ₆	No report was found.

96	Lanosterol	-	-	0.22	C ₃₀ H ₅₀ O	Sterol, essential components of eukaryotic cells (Wei et al., 2016).
97	Betulin	-	-	0.58	C ₃₀ H ₅₀ O ₂	Anti-Viral and anti- tumour (Tolstikov et al., 2005).
98	alpha-Tocopheryl acetate	-	-	0.23	C ₃₁ H ₅₂ O ₃	Antimicrobial activity (Bidossi et al., 2017).
99	Geldanamycin	-	-	0.33	C ₂₉ H ₄₀ N ₂ O ₉	Chemotherapeutic agents (da Rocha et al., 2001).
100	Dihydrosteviobiside	-	-	0.26	C ₃₂ H ₅₂ O ₁₃	No report was found.
101	Bronopol	-	-	0.10	C ₃ H ₆ BrNO ₄	Antimicrobial activity (Birkbeck et al., 2006; Treasurer et al., 2005).
Total compounds		50	48	52		