Journal of science faculty at Sirte University
JSFSU
2021
Peer-review Periodical Journal

جامعة سرت
Sirte University
1989

ISSN INTERNATIONAL STANDARD SERIAL NUMBER
eISSN: 2789-858X

SCIENTIFIC JOURNAL FOR THE FACULTY OF SCIENCE – SIRTE UNIVERSITY

DOI: 10.37375/issn.2789-858X – Indexed by Crossref, USA

VOLUME 3 ISSUE 1 APRIL 2023

Bi-Annual, Peer- Reviewed, Indexed, and Open Accessed e-Journal

SJFSSU

معامل التأثير العربي = 1.02

Legal Deposit Number@NationaL Library (Benghazi): 990/2021

Sjsfsu@su.edu.ly
journal.su.edu.ly/index.php/JSFSU

Scientific Journal for the Faculty of Science-Sirte University Vol. 3, No. 1 (2023) 115-121

Scientific Journal for the Faculty of Science-Sirte University

Journal home page: http://journal.su.edu.ly/index.php/JSFSU/index

DOI: 10.37375/issn.2789-858X

Assessment of the Antimicrobial Activity of Three *Silene* Species (Caryophyllaceae) Against Some Microorganisms

Miloud M. Miloud[1] and Najma A. Senussi[2]

[1]*Botany Department, Science Faculty, Benghazi University, Al-abyar branch, Libya.*

[2]*Botany Department, Science Faculty, Ajdabiya University, Libya.*

DOI: https://doi.org/10.37375/sjfssu.v3i1.1089

ARTICLE INFO:

Received:.26 February 2023

Accepted: 5 April 2023

Published: 17 April 2023

Keywords:

Silene species, Antimicrobial activity, well diffusion assay, MIC, MBC/MFC.

A B S T R A C T

Three *Silene* species (*Silene gallical L., Silene succulent Forsk., and Silene apetala* Willd) were tested for potential anti-microbial activity against some microorganisms (*Staphylococcus aureus*, *Serratia marcescens*, *Acinetobacter boumannii*, *Klebsella sp.*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides*, and *Alternaria alternata* using the well diffusion assay. Solvents used in the extraction process are ethanol, methanol and acetone. The obtained results from all plant extracts showed clear antimicrobial activity against all tested microbial species, except *S. succulent* extracts, which had no inhibitory activity against *Klebsiella sp.*, *A. niger* and *A. flavus*. Moreover, the acetone extract of *S. gallical* and *S. apetala* was the most effective plant extract and showed bacteriostatic, bactericidal, fungistatic and fungicidal activities against the highly susceptible species of microbes (*S. aureus*, *S. marcescens*, *C. cladosporioides*, and *A. alternata*) with MIC ranged from 3.12 to 6.25 mg/ml, MBC and MFC of 6.25 and 12.5 mg/ml. The experiments confirmed the efficacy of selected plant extracts as natural antimicrobials and suggested that they could be used in drugs to treat infectious diseases caused by the tested microbes.

1 Introduction

Despite the progress that has been made in medical science, infectious pathogens remain an important cause of morbidity and mortality (Moellering et al., 2007). Those circumstances have propelled scientists to explore new antimicrobial effective substances from different sources such as medicinal plants (Cordell, 2000). Medicinal plants are a source of efficient substances that act as antibacterial and antifungal agents (Chandra, 2013). Medicinal plant extracts are used for the treatment or prevention of diseases and promotion of good health (El Astal et al., 2005). In this study, we targeted three plants growing southeast of Benghazi, Libya. The selected plants are *Silene gallical* L., *Silene succulent* Forsk. and *Silene apetala* Willd. (The used part is the leaves), but known for their uses in traditional medicine for various diseases. Many species belongs to *Silene* have been used to treat inflammations, bronchitis, colds, and infections (Ali et al., 1999; Hirst, 2005). The *Silene* belongs to Caryophyllaceae family which comprises annuals, biennials and perennials (Greuter 1995). Most of its species are hermaphrodites and very few numbers of its species are dioecious or gynodioecious (Greuter, 1995). Some *Silene* species contain effective chemical compounds such as ecdysteroids, Phyto-ecdysteroids, flavonoids, saponins and triterpenes (Mamadalieva, 2012). The main objective of current study is to evaluate the antimicrobial activity of leaf extracts of *Silene*

Corresponding author: E-mail: *Miloud.elagaily@uob.edu.ly*

gallical, Silene succulent and *Silene apetala* against some microorganisms.

2 Materials and Methods

2.1 Plants extraction preparation

Fresh samples were collected from the leaves of the selected plants in the middle of the spring month of 2022 from the southeast of Benghazi, Libya. The collected plants were watery washed and dried in the shade for 2 weeks. The dried plant leaves of each plant species were crushed into a fine powder using an electric blender. According to the method of Mohammadi *et al.,* (2015), with minor modifications, 50 g of the powder of *Silene gallical, Silene succulent, and Silene apetala* were filled in the thimble and extracted successively with 200 ml each of ethanol, methanol, and acetone using a Soxhlet apparatus for 24 hours. All the extracts were evaporated using a rotary evaporator. All the crude extracts were dissolved in the same used solvents. One concentration of extracts was prepared, which is 50 mg/ml, and stored at 4 °C in airtight bottles until further use.

2.2 Collection of microorganisms

Bacterial species were collected from the microbiology laboratory of Benghazi Medical Centre (BMC). Fungal species were collected from the Botany Department, Ajdabiya University. In total, eight microorganisms, four bacterial species (*S. aureus*, *S. marcescens*, *A. boumannii*, *Klebsiella sp.*) and four fungal species (*A. niger*, *A. flavus*, *C. cladosporioides*, and *A. alternata*). The bacterial species were maintained on nutrient agar slants and the fungal species were maintained on potato dextrose agar slants at 4 °C.

2.3 Inoculums preparation

Bacteria stock cultures were sub-cultured onto Nutrient Agar (NA) plates and incubated overnight at 37°C (bacterial cultures are 24 hours old). Three to four bacterial colonies were inoculated into 10 ml of Mueller Hinton broth (MHB) and incubated at 37 °C. The overnight bacterial suspensions were adjusted to 0.5 McFarland Standard with sterile MHB broth, approximately1.5×10^6 cell/ml. To aid comparison, the adjustment of bacterial suspensions to the density of the 0.5 McFarland Standard was done against a white background with contrasting black lines (Teh et al., 2017). The fungal inoculum (spores) was prepared according to the method of Surapuram et al., (2014) with minor modification, by suspending five representative colonies, obtained from fresh, mature (3 to 7 days-old) cultures grown at 27°C on PDA medium, in potato dextrose broth (PDB). Then the inoculum was adjusted to 0.5 McFarland standard, approximately $1\text{-}5\times10^6$ spores/ml, by measuring the absorbance in a spectrophotometer at a wavelength of 625 nm.

2.4 Antimicrobial activity of plant extracts

The obtained crude extracts were tested against four bacterial species and four fungal species by MHA medium and PDA medium. A well diffusion assay was used for evaluating the antimicrobial activity (Pawaskar and Kale, 2006; Athanassiadis et al., 2009). Some antibiotics were used as the standard antimicrobial agents. The media was poured into the sterile Petri plates and allowed to solidify to make a base layer. The microbial inoculum was evenly spread over the media. A sterile cork borer was used to punch wells (five wells) in the media. Subsequently, wells were filled with 100 µl of each extract at a concentration of 50 mg/ml and allowed to diffuse at room temperature for 1 hour, then the plates were placed in an incubator at 37 °C for 24 hours in the case of bacteria and at 27 °C for 72 hours in the case of fungi. The resulting diameters of inhibition zones were measured using a ruler in millimeters. The experiments were conducted three times, and the mean zone of inhibition was calculated for each crude extract and standard antibiotic.

2.5 Determination of Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the effective plant extracts

The MIC test was prepared according to the method of Mostafa et al., (2018), with minor modifications. MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of microbes after overnight incubation. The crude plant extracts which exhibited strong antimicrobial activity at 50 mg/ml were tested to determine their MIC using a well diffusion assay and to evaluate their efficiency in controlling microbial species causing diseases. different concentrations of the tested plant extracts (1.56, 3.12, 6.25, 12.5, 25, and 50 mg/ml). Mueller-Hinton agar and Potato dextrose agar were poured into sterile Petri dishes and seeded with microbial suspensions of the tested microbes. Wells were filled with 100µl of various plant extracts concentrations and allowed to diffuse at room temperature for 1 hour before being incubated in the incubator at 37 °C for 24 hours (for bacteria) and at 27 °C for 48-72 hours (for fungi). The zones of inhibition were measured by a ruler in millimeters. While, the MBC and MFC are the

concentrations that cause growth inhibition by % 99.9, and this was confirmed by taking a swab from the zones of inhibition and cultivating it on MHA medium and PDA medium again to make sure the bacteria and fungi are killed. The concentration of the plant extract that did not show any microbial growth on the freshly inoculated used media was determined as the MBC and MFC.

3 Results

The results from the experiments were recorded in Table 1 and in Figures 1-3. *S. gallical*, *S. succulent* and *S. apetala* were investigated to evaluate their antimicrobial activity against eight microorganisms, including *S. aureus*, *S. marcescens*, *A. boumannii*, *Klebsiella sp*., *A. niger*, *A. flavus*, *C. cladosporioides* and *A. alternata*, using a well diffusion assay with a concentration of 50 mg/ml. Ciprofloxacin, Imipenem, Colistin, and Fluconazole were used as a positive controls for the antibacterial and antifungal assays, respectively. The ethanol, methanol, and acetone solvents were used as a negative controls. The obtained results from all plant extracts showed clear antimicrobial activity against all tested microbial species, except *S. succulent* extracts, had no inhibitory activity against *Klebsiella sp.*, *A. niger* and *A. flavus*. Moreover, the acetone extract of *S. gallical* and *S. apetala* was the most effective plant extract against four microbial species (*S. aureus*, *S. marcescens*, *C. cladosporioides*, and *A. alternate*) compared to the other extracts (ethanolic and methanolic extracts). In the present study, the acetone extract of *S. gallical* and *S. apetala* was the most efficient plant extract in inhibiting microbial species *S. aureus*, *S. marcescens*, *C. cladosporioides*, and *A. alternata*, so the MIC, MBC and MFC values of this extract were tested. The MIC results were recorded in Table 2 and illustrated in Figures 4 and 5. The inhibitory effect of *S. gallical* acetone extract started at 3.12 mg/ml with inhibition zones of 9,8,9 and 8 mm against *S. aureus*, *S. marcescens*, *C. cladosporioides*, and *A. alternata*, while acetone extract of *S. apetala* suppressed microbial growth of these species at a concentration of 6.25 mg/ml with inhibition zones of 9,8,9 and 9 mm respectively. *S. gallical* acetone extract showed potentially bactericidal and fungicidal activities against four microbial species (*S. aureus*, *S. marcescens*, *C. cladosporioides*, and *A. alternata*) with MBC and MFC of 6.25 mg/ml, while the MBC and MFC of *S. apetala* acetone extract reached 12.5 mg/ml against these species.

Table (1). Average zones of inhibition of *Silene* leaf extracts against tested microbial species.

No.	Plant species	The zone of inhibition is measured in millimeter													
		Concentration 50 mg/ml													
		S. gallical			*S. succulent*			*S. apetala*			Antibiotics				Controls
	Microbial species	A	B	C	A	B	C	A	B	C	Ci	Im	Co	Fl	ABC
1	***S. aureus***	13	12	16	12	13	13	11	11	16	23	R	R	R	R
2	***S. marcescens***	11	10	16	11	10	12	10	12	15	R	21	R	R	R
3	***A. boumannii***	13	13	13	10	9	11	10	10	12	R	R	13	R	R
4	***Klebsiella sp.***	11	10	13	R	R	R	12	11	12	18	R	R	R	R
5	***A. niger***	12	10	12	R	R	R	9	9	10	R	R	R	13	R
6	***A. flavus***	11	11	12	R	R	R	9	9	10	R	R	R	13	R
7	***C. cladosporioides***	10	11	17	10	10	13	11	13	15	R	R	R	19	R
8	***A. alternata***	11	13	16	11	11	12	13	13	16	R	R	R	16	R

A:Ethanol B:Methanol C:Acetone R:Resistant / Ci:Ciprofloxacin, Im:Imipenem, Co:Colistin, Fl:Fluconazole

Table (2). MIC values of acetone extract of *S. gallical* and *S. apetala* against four microbial species.

No.	Plant extract	The zone of inhibition is measured in millimeter				
		Microbial species				
		Concentrations in mg/ml	*S. aureus*	*S. marcescens*	*C. cladosporioides*	*A. alternata*
1	***S. gallical***	1.56	R	R	R	R
		3.12	9	8	9	8
		6.25	9	8	9	10
		12.5	12	10	13	11
		25	14	13	15	14
		50	16	16	17	16
2	***S. apetala***	1.56	R	R	R	R
		3.12	R	R	R	R
		6.25	9	8	9	9
		12.5	12	11	10	10
		25	13	14	12	13
		50	16	15	15	16

R:Resistant

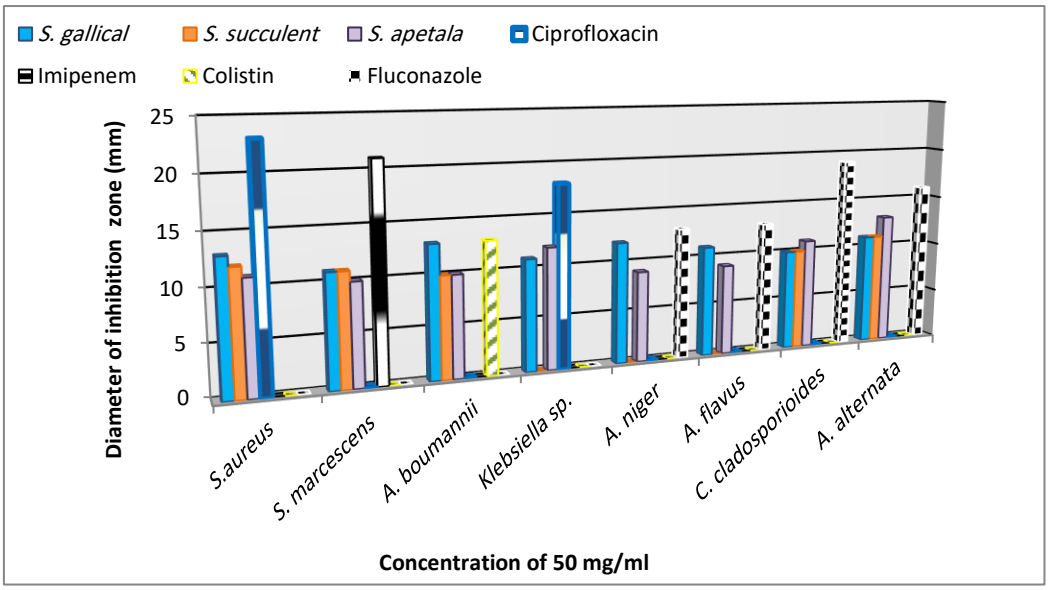

Figure (1): Effect of ethanol extract of *Silene* species against tested microbial species

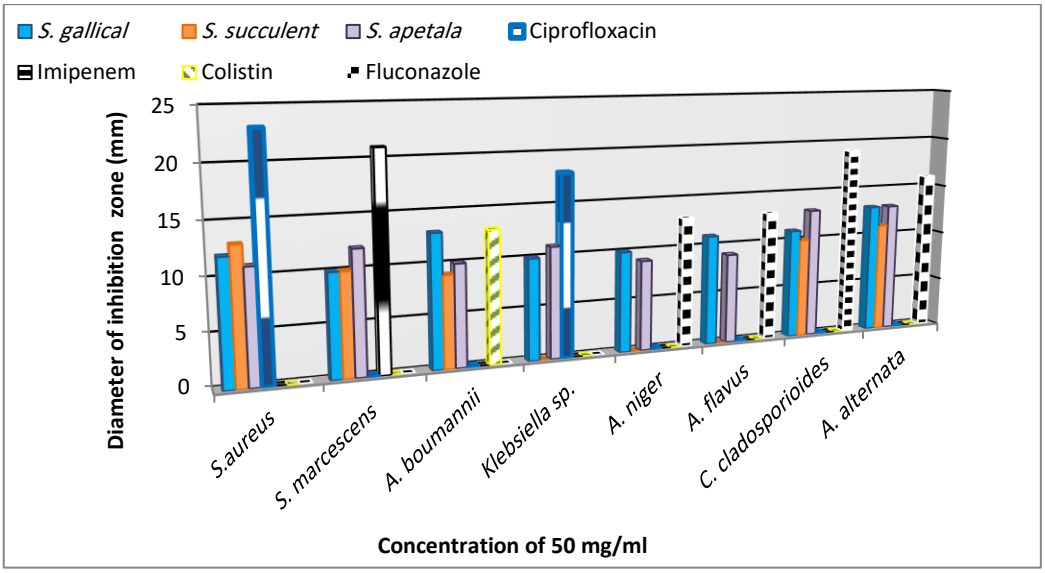

Figure (2): Effect of methanol extract of *Silene* species against tested microbial species

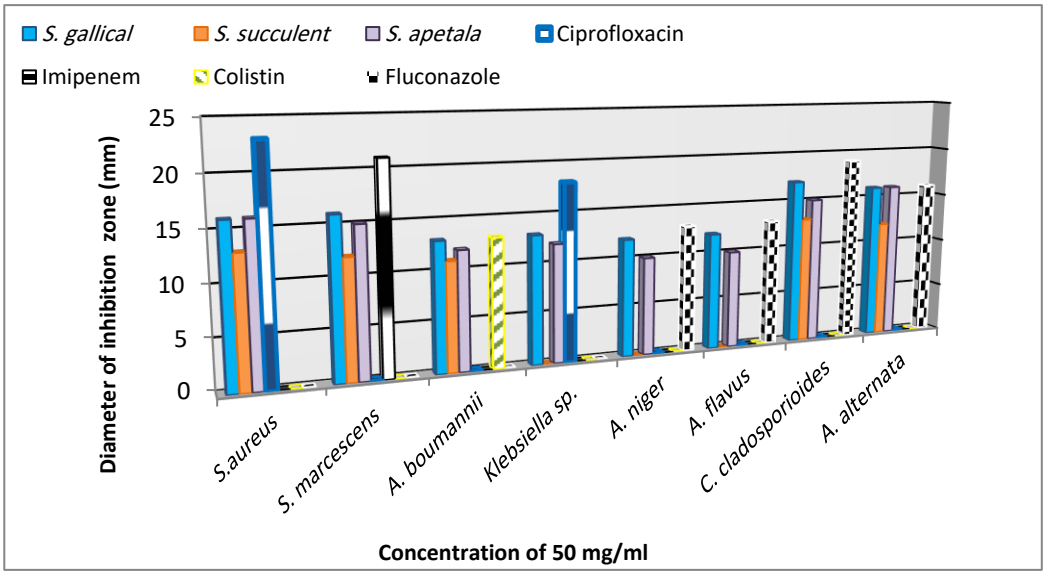

Figure (3). Effect of acetone extract of *Silene* species against tested microbial species.

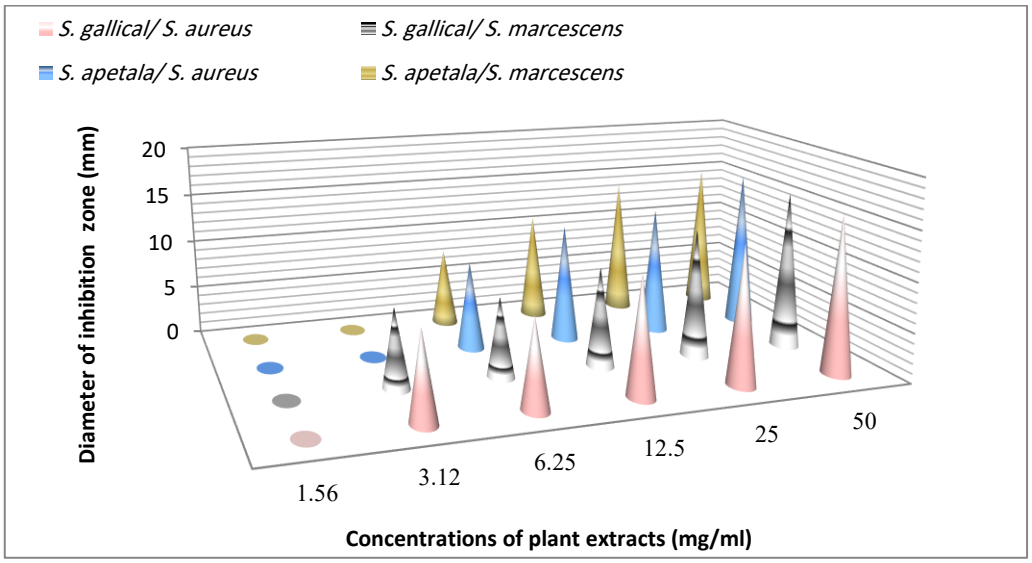

Figure (4). MIC of acetone extract against *S. aureus* and *S. marcescens*

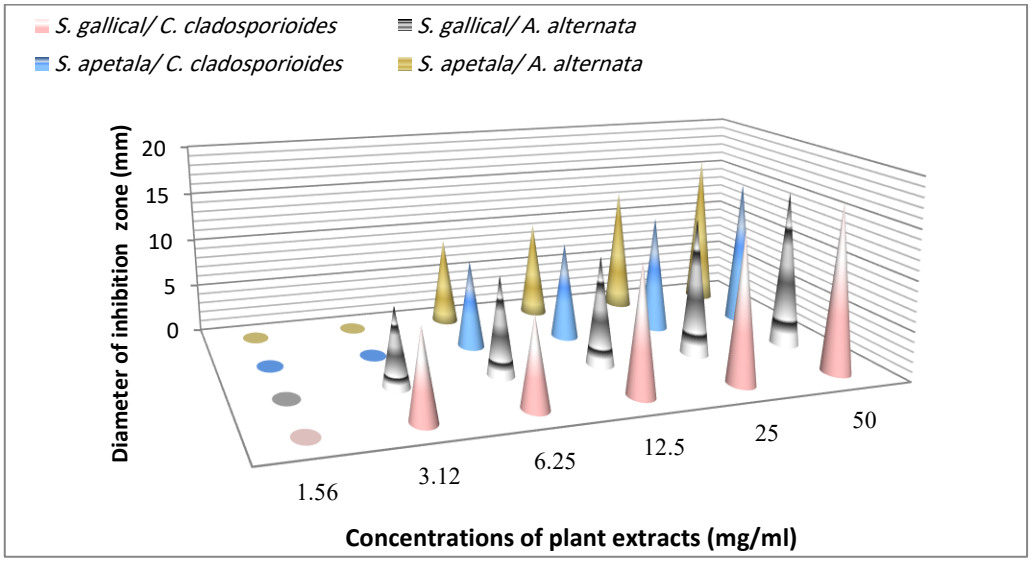

Figure (5). MIC of acetone extract against *C. cladosporioides*, and *A. alternata*

4 Discussion

The agar well diffusion method has been used in this research because it is more sensitive than the agar disc diffusion method (Valgas et al., 2007). Many studies have shown that obtained crude extracts from the *Silene* species inhibit the growth of different microorganisms at various concentrations (Miloud and Senussi, 2021; Keskin et al., 2016; Toroglu et al., 2013; Ertürk et al., 2006) .Several studies were conducted to determine the efficacy of plant extracts and their active compounds as antimicrobial agents for microbial growth control. High phenolic compounds, flavonoids, aldehydes, ketones, saponins, and alcohols cause antimicrobial activity (Akgul, 1989; Sindhu and Manorama, 2012). These compounds are known to be abundant in the Caryophyllaceae family. The flavonoids from plant extracts have been found to possess antimicrobial and antioxidant properties in various studies (Amarlal et al., 2009; Lin et al., 2008). Gram positive bacteria were to be more susceptible than Gram negative bacteria. This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lack the natural sieve effect against large molecules due to the small pores in their cell envelope (Hawkey, 1998). Some researchers have suggested that antimicrobial components of plant extracts (terpenoid, alkaloid, and phenolic compounds) interact with enzymes and proteins of the microbial cell membrane, causing its disruption to disperse a flux of protons towards the cell exterior, which induces cell death or may inhibit enzymes necessary for aminoacid biosynthesis (Burt, 2004; Gill and Holley, 2006).

5 Conclusions

In conclusion, the used crude extracts of *Silene* species exhibited a good antibacterial and antifungal activities against all tested microbial species, except *S. succulent* extracts had no activity against (*Klebsiella sp., A. niger* and *A. flavus*). The acetone extract of *S. gallical* and *S. apetala* was highly significant against the highly susceptible species of microbes (*S. aureus*, *S. marcescens*, *C. cladosporioides*, and *A. alternate*). Finally, the results of this study clearly elucidate the antimicrobial activity of these plants and provide an evidence to support their use in folk medicine.

Acknowledgements

We would like to thank the Department of Botany, Faculty of Science, Ajdabiya University for providing all the facilities to conduct this study.

Conflict of Interest: The authors declare that there are no conflicts of interest.

References

Akgul, A. (1989). Antimicrobial activity of black cumin (Nigella sativa L.) essential oil. *Gazi Universitesi Eczacilik Fakultesi Dergisi*, *6*(1), 63-68.

Ali, Z., Ahmad, V. U., Ali, M. S., Iqbal, F., Zahid, M., & Alam, N. (1999). Two new C-glycosylflavones from *Silene conoidea*. *Natural Product Letters*, *13*(2), 121-129. https://doi.org/10.1080/10575639908048832

Amaral, S., Mira, L., Nogueira, J. M. F., da Silva, A. P., & Florêncio, M. H. (2009). Plant extracts with anti-inflammatory properties—A new approach for characterization of their bioactive compounds and establishment of structure–antioxidant activity relationships. *Bioorganic & medicinal chemistry*, *17*(5), 1876-1883. https://doi.org/10.1016/j.bmc.2009.01.045

Athanassiadis, B., Abbott, P. V., George, N., & Walsh, L. J. (2009). An *in vitro* study of the antimicrobial activity of some endodontic medicaments and their bases using an agar well diffusion assay. *Australian Dental Journal*, *54*(2), 141-146. https://doi.org/10.1111/j.1834-7819.2009.01107.x

Burt, S. (2004). Essential oils: their antibacterial properties and potential application in foods: A review. *International Journal of Food Microbiology*, *94*(3), 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Chandra, M. (2013). Antimicrobial activity of medicinal plants against human pathogenic bacteria. *International journal of biotechnology and bioengineering research*, *4*(7), 653-658. http://www.ripublication.com/ijbbr.htm

Cordell, G. A. (2000). Biodiversity and drug discovery—a symbiotic relationship. *Phytochemistry*, *55*(6), 463-480. https://doi.org/10.1016/S0031-9422(00)00230-2

El Astal, Z. Y., Ashour, A. E. R. A., & Kerrit, A. A. M. (2005). Antimicrobial activity of some medicinal plant extracts in Palestine. *Pakistan Journal of Medical Sciences*, *21*(2), 187-193.

Ertürk, Ö., Kati, H., Yayli, N., & Demirbağ, Z. (2006). Antimicrobial properties of *Silene multifida* (Adams) Rohrb. plant extracts. *Turkish Journal of Biology*, *30*(1), 17-21. https://journals.tubitak.gov.tr/biology

Gill, A. O., & Holley, R. A. (2006). Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *International Journal of Food Microbiology*, *108*, 1–9. https://doi.org/10.1016/j.ijfoodmicro.2005.10.009

Greuter, W. (1995). *Silene* (Caryophyllaceae) in Greece: a subgeneric and sectional classification. *Taxon*, *44*(4), 543-581. https://doi.org/10.2307/1223499

Greuter, W. (1995). Studies in Greek Caryophylloideae: Agrostemma, Silene, and Vaccaria. *Willdenowia*, 105-142. https://www.jstor.org/stable/3996977

Gould D, Booker C. Applied microbiology for nurses. Aardvark Editorial, Mcndham, Suffolk; 2000:75-94

Hawkey, P. M. (1998). The origins and molecular basis of antibiotic resistance. *British Medical Journal*, *317*(7159), 657-660. https://doi.org/10.1136/bmj.317.7159.657

Hirst, M. (2005). Dreams and medicines: The perspective of Xhosa diviners and novices in the Eastern Cape, South Africa. *Indo-Pacific Journal of Phenomenology*, *5*(2), 1-22. https://hdl.handle.net/10520/EJC46926

Keskin, D., Guvensen, N. C., & Yildiz, K. (2016). Antimicrobial activity of *Silene cariensis* subsp cariensis and *Silene pungens* from Turkey. *Advances in Environmental Biology*, *10*(7), 167-173. http://www.aensi.org/aeb.html

Lin, S. Y., Wang, C. C., Lu, Y. L., Wu, W. C., & Hou, W. C. (2008). Antioxidant, anti-semicarbazide-sensitive amine oxidase, and anti-hypertensive activities of geraniin isolated from *Phyllanthus urinaria*. *Food and Chemical Toxicology*, *46*(7), 2485-2492. https://doi.org/10.1016/j.fct.2008.04.007

Mamadalieva, N. Z. (2012). Phytoecdysteroids from *Silene* plants: distribution, diversity and biological (antitumour, antibacterial and antioxidant) activities. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, *11*(6), 474-497. https://www.redalyc.org/articulo.oa?id=85624607001

Miloud, M.M., & Senussi, N.A. (2021). Antibacterial activity of leaf extracts of *Silene succulent* Forsk. (Caryophyllaceae) against clinically important bacteria. *Academia Journal of Microbiology Research*, 9(1): 013-020. http://www.academiapublishing.org/ajmr

Moellering Jr, R. C., Graybill, J. R., McGowan Jr, J. E., & Corey, L. (2007). Antimicrobial resistance prevention initiative—an update: proceedings of an expert panel on resistance. *American journal of infection control*, *35*(9), S1-S23. https://doi.org/10.1016/j.ajic.2007.08.001

Mohammadi, S., Asgary, V., Shandiz, S. A. S., Heidari, E., Jozaghkar, H., Cohan, R. A., & Mirzaie, A. (2015). Antimicrobial activity of methanolic root extracts of *Euphorbia condylocarpa* against pathogenic bacteria. *Advanced Studies in Biology*, *7*(2), 55-64. https://doi.org/10.12988/asb.2015.41049

Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, *25*(2), 361–366. https://doi.org/10.1016/j.sjbs.2017.02.004

Pawaskar, S. M., & Kale, K. U. (2006). Antibacterial activity of successive extracts of Mimosa pudica. *INDIAN DRUGS-BOMBAY-*, *43*(6), 476.

Sindhu, S., & Manorama, S. (2012). Screening of Polycarpaea corymbosa lam.(Caryophylaceae) for its in vitro antioxidant activity. *Asian Journal of Pharmaceutical and Clinical Research*, *5*(4), 175-8.

Surapuram, V., Setzer, W. N., McFeeters, R. L., & McFeeters, H. (2014). Antifungal activity of plant extracts against *Aspergillus niger* and *Rhizopus stolonifer*. *Natural Product Communications*, *9*(11), 1603 – 1605. https://doi.org/10.1177/1934578X1400901118

Teh, C. H., Nazni, W. A., Nurulhusna, A. B., Norazah, A., & Lee, H. L. (2017). Determination of antibacterial activity and minimum inhibitory concentration of larval extract of flyvia resazurin-based turbidometric assay. *BMC Microbiology*, *17*(36), 1-8. https://doi.org/10.1186/s12866-017-0936-3

Toroglu, S., Keskin, D., Dadandi, M. Y., & Yildiz, K. (2013). Comparision of antimicrobial activity of *Silene laxa* Boiss. & Kotschy and *Silene caramanica* Boiss. & Heldr different extracts from Turkey. *JOURNAL OF PURE AND APPLIED MICROBIOLOGY*, *7*, 1763-1768.

Scientific Journal For the Faculty of Science – Sirte University

Sjsfsu@su.edu.ly

journal.su.edu.ly/index.php/JSFSU