Scientific Papers. Series A. Agronomy, Vol. LXVII, No. 1, 2024
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF THE MONOECIOUS HEMP *(CANNABIS SATIVA* L.) SEED OIL, VARIETY MARA 21

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Abstract

Cannabis sativa L. is one well-known medicinal plant that has attracted interest recently and throughout the years. Our research aimed to highlight the antimicrobial activity of hemp seed oil, the Mara variety cultivated under the conditions of the University of Life Sciences in Timisoara, on 12 microbial strains. The results demonstrated antimicrobial efficacy dependent from one species to another, as follows: an upward trend, positively correlated with the concentration increase tested in the case of: Streptococcus pyogenes, Staphylococcus aureus, Shigella flexneri, Pseudomonas aeruginosa, Salmonella typhimurium and Candida albicans with MIC between 0.2 mg/mL-8 mg/mL. As well as a downward trend, negatively correlated with concentration in the case of strains of Escherichia coli, Listeria monocytogenes, Haemophilus influenzae, Bacillus cereus and Candida parapsilopsis, where MIC was present starting with the concentration of 0.2 mg/mL. In the case of certain strains (Clostridium perfringens), Cannabis sativa L. oil showed a pronounced strain-boosting effect, with significant stimulation of bacterial growth. The results support further research into the effect of Cannabis sativa L. vegetable oil as a potential antimicrobial agent for microbial strains.

Key words*: Cannabis sativa L., hemp seeds, seed oil, bacterial strains, fungal strains.*

INTRODUCTION

The recent concern of researchers is to find active biological compounds to replace synthetic chemical compounds, considering that nature can represent a potential source of new therapeutic options, including antimicrobial therapy. Worldwide, in the last decades, the emergence of multidrug-resistant bacteria was observed in most antibiotics used (Mancuso et al., 2021; World Health Organization, 2023), so phytochemical products could represent an alternative method of treatment with a great advantage considering that they do not create resistance.

Cannabis sativa L., a species of the genus Cannabis, family Cannabaceae (Hillig, 2005.), is a herbaceous plant originally from Central and Northeast Asia and nowadays spread through the world due to its medicinal properties (Baldini et al., 2018, Balant et al., 2021; Barčauskaitė et al., 2022). The pharmacological properties of hemp include anti-anxiety, pain relief, anti-psychotic, anti-nausea, immunomodulatory, antivirals (Sea et al., 2023) anti-inflammatory, and antimicrobial activity (Andre et al., 2016; Aqawi et al., 2021; Chen et al., 2021). These properties are due to the chemical composition of cannabinoids, polyphenols, flavonoids and terpenes. The variability of the active biological compounds in hemp-based products is due to the geographical area, the weather and climate conditions in which the plant was grown, the portion of the plant used and the method of harvesting, storing, and extracting the compounds (Alonso-Esteban et al., 2022; Motiejauskaitė et al., 2023).

Of all the compounds, flavonoids, phenolic acids, and lignans of hemp are the most important for antimicrobial activity (Izzo et al., 2020; Albuquerque et al., 2021). The mechanism of action is not fully elucidated, but it is considered that the antimicrobial activity is due to the action of the molecules on the membrane permeability of the bacteria (Schofs et al., 2021). Regardless of the mechanism of action, studies show that hemp has both activity

against Gram-positive and Gram-negative bacteria, as well as antifungal effects. Ali et al. (2012), demonstrated that the seeds oil of *Cannabis sativa* L. had pronounced antibacterial activity against *B. subtilis* and *S. aureus*, *Pseudomonas* and moderate activity against *E. coli*. The author demonstrated no atifungal activity against *Aspergillus niger* and low activity against *Candida albicans* (Ali et al., 2012). Khan and Javaid, 2020, highlighted the antifungal effect of *Cannabis sativa* L. leaf extracts against *Aspergillus flavipes* (Khan and Javaid, 2020). Moreover, recently, Al Khoury et al., 2021, demonstrated anti-aflatoxigenic properties and fungal growth partial inhibition of cannabis extracts (Al Khoury et al., 2021). The minimum inhibitory concentrations vary according to the studies, on the one hand, due to the chemical composition of the hemp-based products, and on the other hand, depending on the bacterial or fungal strain studied (Schofs et al., 2021; Karas et al., 2020; Ali et al., 2012; Khan & Javaid, 2020).

The present study aimed to highlight the antimicrobial activity of *Cannabis sativa* L. oil against some ATTC strains, such as *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19114), *Clostridium perfringens* (ATCC 13124), *Bacillus cereus* (ATCC 10876), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Haemophilus influenzae* tip B (ATCC 10211), *Candida albicans* (ATCC 10231) și *Candida parapsilopsis* (ATCC 22019).

MATERIALS AND METHODS

1. Field experiences

The monoecious hemp variety Mara 21 was cultivated in the farm of the Young Naturalists' Resort, which belongs to the University of Life Sciences "King Mihai I" from Timişoara (Figure 1).

The experiment was arranged in the field with different distances: 12.5 cm, 100 cm and 150 cm between rows.

Hemp seeds were harvested when the seeds ripened in the BBCH 87-70% ripe fruits phenophase. The seed samples were labelled in paper bags and transported to the laboratory for specific tests.

The hemp seeds were subjected to Shoxlet extraction by continuous washing with ether, the oil obtained was subsequently used for microbiological assay.

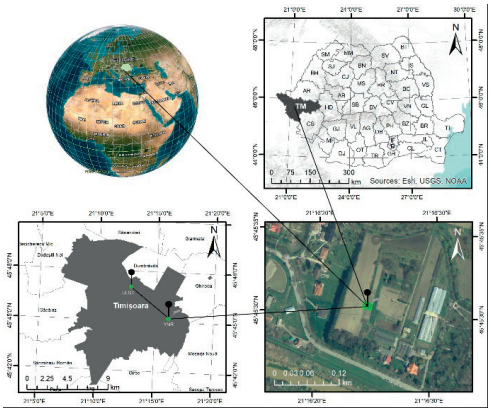

Figure 1. Research location (processing after Geospatial, 2023; ANCPI, 2023)

2. Laboratory tests

The microdilution assay was performed to demonstrate the antimicrobial activity of the *Cannabis sativa* L. oil (CSO). The method is similar to the one described in our previous study (Obiștioiu et al., 2023; Hulea et al., 2022).

The concentrations of the hemp seeds oil tested were 0.2 µl, 0.4 µl, 0.8, 1.6 µl, 2 µl, 4 µl, 8 µl, and 16 µl.

The ATCC strains used in the present study are a part of the culture collection of the Microbiology Laboratory of the Interdisciplinary Research Platform within the University of Life Sciences "King Mihai I" of Timisoara. The tested ATTC stains were *S. pyogenes* (ATCC 19615), *S. aureus* (ATCC 25923), L. *monocytogenes* (ATCC 19114), *Cl. perfringens* (ATCC 13124), *B. cereus* (ATCC 10876), *S. flexneri* (ATCC 12022), *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *S. typhimurium* (ATCC 14028), *H. influenzae* tip B (ATCC 10211), *C. albicans* (ATCC 10231) și *C. parapsilopsis* (ATCC 22019).

2.1. Bacterial culture

The ATTC strains were revived by overnight growth in Brain Heart Infusion (BHI) broth

(Oxoid, CM1135), subsequently passed to BHI Agar (Oxoid, CM1136). The samples were incubated in aerobic conditions, for 24 hours at 37°C.
The bacterial culture used for the microdilution assay consisted of a dilution of 10^{-3} of the fresh culture, equivalent to a 0.5 McFarland standard, which was spotted (100 µL) in each well of the 96 microdilution's well plate, using a Calibra digital 852 multichannel pipette. The tested hemp seed oil was added to the bacterial suspension, and then the plates were covered and incubated for 24 hours under aerobic conditions at 37°C. After 24 hours, the OD was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA).
Triplicate tests were performed.
The negative control consisted of suspensions of strain and BHI.
To interpret the results, BIR (bacterial inhibition rate) % was calculated by the following formulas (formulas 1 and 2):

$$BGR = \frac{OD_{sample}}{OD_{negative\ control}} \times 100 \quad (\%) \quad (1)$$

$$BIR\% = 100 - BGR\ (\%) \quad (2)$$

where:
BGR - bacterial growth rate;
OD sample - optical density at 540 nm as the mean value of triplicate readings for EOs in the presence of the selected bacteria;
OD negative control - optical density at 540 nm as the mean value of triplicate readings for the selected bacteria in BHI.
BIR - bacterial inhibition rate.

2.2. Fungal culture

The fungal ATTC strain was revived in Brain Heart Infusion (BHI) broth (Oxoid, CM1135) and subsequently passed on BHI Agar (Oxoid, CM1136).
The samples were incubated for 48 hours in aerobic conditions at 37°C. A 10^{-2} dilution of the fresh culture was performed to obtain an inoculum equivalent to 0.5 McFarland standard. 100 µL of fungal suspension was spotted in each well of the 96 microdilution wells plate, and the hemp seeds oil was added in different concentrations.
The plates were covered and incubated for 48 hours at 37°C, in aerobic conditions. After 48 hours, the OD was measured at 540 nm.
Triplicate tests were performed for all samples.
To interpret the results it was used MIR (mycelial inhibition rate)%. The values of this indicator were calculated by following formulas (formulas 3 and 4):

$$MGR = \frac{OD_{sample}}{OD_{negative\ control}} \times 100 \quad (\%) \quad (3)$$

$$MIR\% = 100 - MGR\ (\%) \quad (4)$$

where:
MGR - mycelial growth rate;
OD sample - optical density at 540 nm as the mean value of triplicate readings for EOs in the presence of the selected fungi;
OD negative control - optical density at 540 nm as the mean value of triplicate readings for the selected fungi in BHI;
MIR - mycelial inhibition rate.

BIR and MIR are the indicators that show the inhibition of microbial growth. If their values are positive, the tested oil has antimicrobial efficacy. The minimum concentration necessary for their value to be positive represents the minimum inhibitory concentration.

RESULTS AND DISCUSSIONS

In our study, we studied the antimicrobial activity of hemp oil against gram-positive bacteria: *S. pyogenes, S. aureus, L. monocytogenes, B. cereus* and *Cl. perfringens.*
As can be seen in Figure 2, the BIR values varied from one strain to another, depending on the concentration of CSO used.
For *S. pyogenes*, the BIR% value varied between -59.94% and 6.83%, becoming positive at a concentration of 4 µl, corresponding to MIC. In the case of *S. aureus,* the values of BIR% were positive, starting with the lowest concentration tested, respectively the concentration of 0.2 µl. Thus, the BIR% values for this bacterial strain were between 25.49% and 79.97%. Regarding the *L. monocytogenes*

and *B. cereus* ATTC strains, the growth of both strains was inhibited in the presence of 0.2 µl CSO. However, increasing the oil concentration caused a decrease in the BIR% value, demonstrating the potentiating effect at a higher concentration. Thus, the BIR% values for *L. monocytogenes* were 75.45-6.14%, while for *B. cereus,* between 53.83-7.37%. For *C. perfringens*, the BIR% values were negative for all tested concentrations, respectively, between -26.22% and -168.90%.

Briefly, the MIC (minimum inhibitory concentration) values for each strain were 4 µl for *S. pyogenes*, 0.2 for *S. aureus*, *L. monocytogenes*, and *B. cereus*. CSO has no antimicrobial activity against *C. perfringens*.

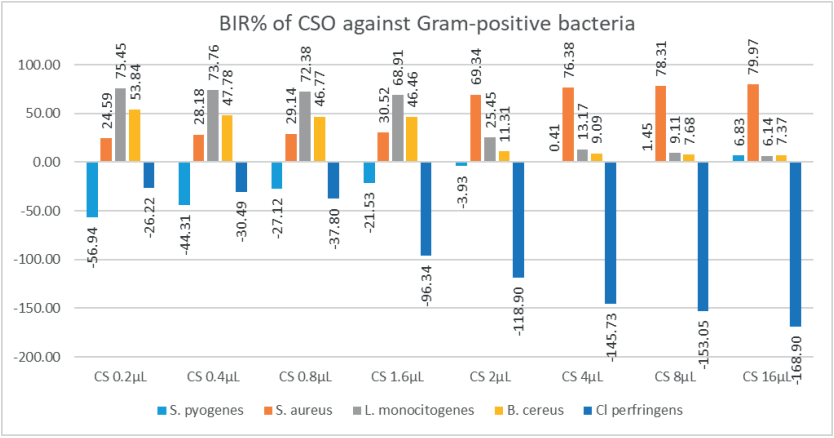

Figure 2. The BIR% of CSO against Gram-positive ATTC strains tested

Similar to Gram-positive strains, the BIR% values for Gram-negative bacteria varied from one strain to another (Figure 3), being between -257.39% and 63.17%. For *S. flexneri*, BIR% values varied between -1.34% and 63.17%, with a positive value starting with the concentration of 0.4 µl. In the case of *P. aeruginosa* strains, the BIR% value was negative until the last concentration tested, respectively of 16 µl. Thus, for this strain, this indicator varied between -257.38% and 20.22%. For *E. coli*, *S. typhimurium, and H. influenzae,* the values of BIR% were positive, starting with the first concentration of CSO tested. Although the BIR% for values *S. typhimurium* falls on an upward slope with maximum effectiveness at the concentration of 16 µl, in the case of strains of *E. coli* and *H. influenzae*, the values follow a downward slope. The values of BIR% were between 60.50 and 3.42% for *E. coli*, 48.45-12.41% for *H. influenzae*, and 18.74-60.80% for *S. typhimurium.*

In terms of MIC, this is 0.4 µl for *S. flexneri*, 16 µl for *P. aeruginosa*, and 0.2 µl for *E. coli*, *S. typhimurium,* and *H. influenzae.*

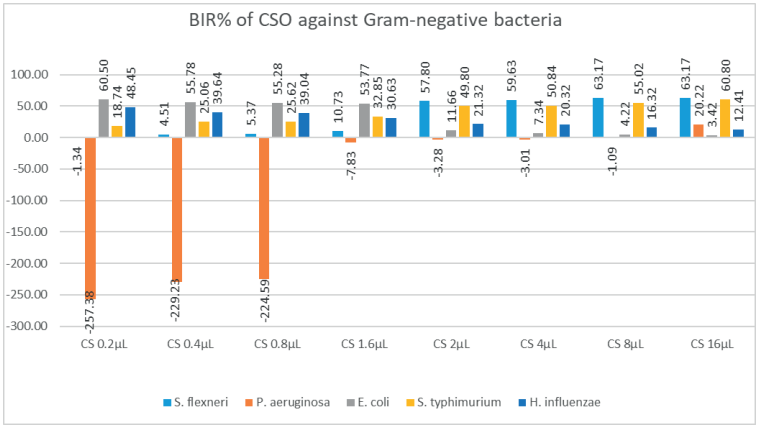

Figure 3. The BIR% of CSO against Gram-negative ATTC strains tested

The antifungal activity of CSO against *Candida spp*. is presented in Figure 4. Against *C. albicans,* the CSO determined values of BIR% between -22.15% and 69.03%, with positive values starting from the concentration of 0.8 µl. For *C. parapsilopsis*, the BIR% values fell on a downward slope, being 44.92% at a concentration of 0.2 µl and reaching the negative value of -27.38% at a concentration of 16 µl. This descending curve demonstrates the boosting effect of CSO as the concentration increases on the *C. parapsilosis* strain.

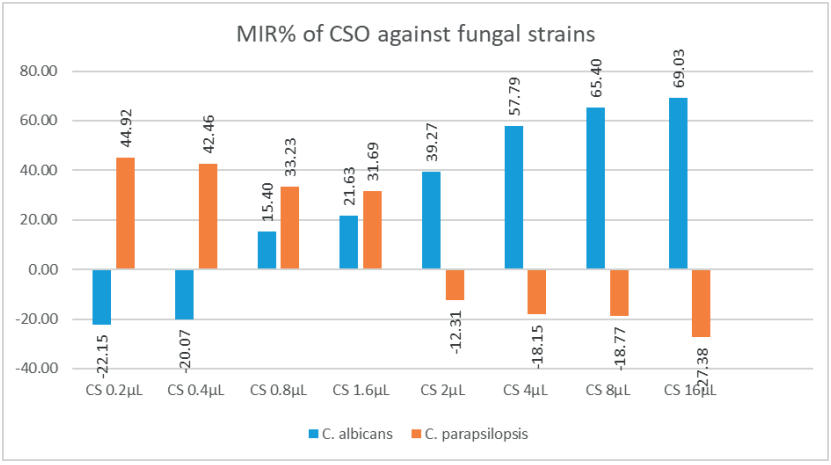

Figure 4. The MIR% of CSO against *C. albicans* and *C. parapsilosis*

Many studies have focused on the antibacterial properties of *Cannabis sativa* L. species and derived products, which were subsequently tested to discover new anti-infective agents.

Following the research of Blaskovich et al. (2021), they report that hemp cannabinoids exhibit a surprisingly consistent MIC of 1-4 µg mL^{-1} against a diverse range of over 20 types of Gram-positive bacteria. The MIC did not change appreciably against highly resistant strains such as *S. aureus.*

Lone T.A. and Lone R.A. (2012), following research on hemp leaf extract, concretises that the minimum inhibitory concentrations, between 5 pg/ml and 10 pg/ml, show an antibacterial and antifungal action.

CONCLUSIONS

The findings of the present study showed that antibacterial effectiveness varies depending on the species:

An upward trend that had a positive correlation with the concentration increase was observed in the cases of *S. typhimurium, C. albicans, P. aeruginosa, S. aureus, S. flexneri*, and *S. pyogenes*, with MICs ranging from 0.2 mg/mL to 8 mg/mL. In the case of strains of *E. coli, L. monocytogenes, H. influenzae, B. cereus*, and *C. parapsilopsis*, where MIC was present starting with the concentration of 0.2 mg/mL, there was also a downward trend that negatively correlated with concentration. *Cannabis sativa* L. oil has shown a strong strain-boosting effect in the case of specific strains (*Cl. perfringens*), with a notable enhancement of bacterial growth. The findings encourage additional investigation into the possible benefits of *Cannabis sativa* L. vegetable oil.

REFERENCES

Agenţia Naţională de Cadastru şi Publicitate Imobiliară – ANCPI. (2023). Baza de date geospaţiale - https://geoportal.ancpi.ro/portal/home/ (accessed on 15.02.2024).

Albuquerque, B.R., Heleno, S.A., Oliveira, M.B.P.P., Barros, L., & Ferreira, I.C.F.R. (2021). Phenolic compounds: Current industrial applications, limitations and future challenges. *Food Funct*., 12, 14–29, DOI: 10.1039/d0fo02324h.

Ali, E., Almagboul, A., Khogali, S., & Gergeir, U. (2012). Antimicrobial Activity of *Cannabis sativa* L. *Chinese Medicine*, 3(1), 61-64. doi: 10.4236/cm.2012.31010.

Al Khoury, A., Sleiman, A.R., Atoui, A., Hindieh, P., Maroun, R.G., Bailly, J.-D., & El Khoury, A. (2021). Antifungal and anti-aflatoxigenic properties of organs of *Cannabis sativa* L.: relation to phenolic content and antioxidant capacities. *Arch. Microbiol*., 203, 4485-4492, https://doi.org/10.1007/s00203-021-02444-x.

Alonso-Esteban, J.I., Pinela, J., Ćirić, A., Calhelha, R.C., Soković, M., Ferreira, I.C.F.R., Barros, L., Torija-Isasa, E., & Sánchez-Mata, M.C. (2022). Chemical composition and biological activities of whole and dehulled hemp (*Cannabis sativa* L.) seeds. *Food Chem.* 374:131754. doi: 10.1016/j.foodchem.2021.131754. Epub 2021 Dec 2. PMID: 34891087.

Andre, C.M., Hausman, J.F., & Guerriero, G. (2016). *Cannabis sativa*: The Plant of the Thousand and One Molecules. *Front. Plant Sci*., 7(19), doi: 10.3389/fpls.2016.00019.

Aqawi, M., Sionov, RV., Gallily, R., Friedman, M., & Steinberg, D. (2021). Anti-Bacterial Properties of Cannabigerol Toward *Streptococcus mutans*. *Front Microbiol.* Apr 22;12:656471. doi: 10.3389/fmicb.2021.656471.

Balant, M., Gras, A., Ruz, M., Valles, J., Vitales, D., & Garnatje, T. (2021). Traditional uses of Cannabis: An analysis of the CANNUSE database. *J. Ethnopharmacol*. 279, 114362. doi:10.1016/j.jep.2021.114362.

Baldini, M., Ferfuia, C., Piani, B., Sepulcri, A., Dorigo, G., Zuliani, F., Danuso, F., & Cattivello, C. (2018). The Performance and Potentiality of Monoecious Hemp (*Cannabis sativa* L.) Cultivars as a Multipurpose Crop. *Agronomy*, 8, 162, https://doi.org/10.3390/agronomy8090162.

Barčauskaitė, K., Žydelis, R., Ruzgas, R., Bakšinskaitė, A., & Tilvikienė, V. (2022). The Seeds of Industrial Hemp (*Cannabis Sativa* L.) a Source of Minerals and Biologically Active Compounds. *J. Nat. Fibers*, 19, 13025–13039, https://doi.org/10.1080/15440478.2022.2084486.

Blaskovich, M.A.T., Kavanagh ,A.M., Elliott, A.G., Zhang, B., Ramu, S., Amado, M., Lowe, G.J., Hinton, A.O., Pham, D.M.T., Zuegg, J., Beare, N., Quach, D., Sharp, M.D., Pogliano, J., Rogers, A.P., Lyras, D., Tan, L., West, N.P., Crawford, D.W., Peterson, M.L., Callahan, M., & Thurn, M. (2021). The antimicrobial potential of cannabidiol. *Commun Biol. Jan,* 19;4(1):7. doi: 10.1038/s42003-020-01530-y.

Chen, C., & Pan, Z. (2021). Cannabidiol and terpenes from hemp—Ingredients for future foods and processing technologies. *J. Future Foods*, 1, 113–127, https://doi.org/10.1016/j.jfutfo.2022.01.001.

Geospatial (2023). România: seturi de date vectoriale generale – http://geo-spatial.org/vechi/download/romania-seturi-vectoriale (accessed on 18.08.2022).

Hillig, K.W. (2005). Genetic evidence for speciation in *Cannabis* (Cannabaceae). *Genet Resour Crop Evol* ,52, 161–180, https://doi.org/10.1007/s10722-003-4452-y.

Hulea, A., Obiștioiu, D., Cocan, I,. Alexa, E., Negrea, M., Neacșu, A.G., Hulea, C., Pascu, C., Costinar, L., Iancu, I., Tîrziu, E., & Herman, V. (2022). Diversity of Monofloral Honey Based on the Antimicrobial and Antioxidant Potential. *Antibiotics (Basel)*, Apr 28;11(5):595, doi: 10.3390/antibiotics11050595.

Izzo, L., Castaldo, L., Narváez, A., Graziani, G., Gaspari, A., Rodríguez-Carrasco, Y., & Ritieni, A. (2020). Analysis of Phenolic Compounds in Commercial *Cannabis sativa* L. Inflorescences Using UHPLC-Q-Orbitrap HRMS. *Molecules*, 25, 631, https://doi.org/10.3390/molecules25030631.

Karas, J.A., Wong, L.J.M., Paulin, O.K.A., Mazeh, A.C., Hussein, M.H., Li, J., & Velkov, T. (2020). The Antimicrobial Activity of Cannabinoids. *Antibiotics (Basel),* Jul 13;9(7):406, doi: 10.3390/antibiotics9070406.

Khan, I.H., & Javaid, A. (2020). Antifungal activity of leaf extract of *Cannabis sativa* against *Aspergillus flavipes*. *PJWSR*, 27, 447-453, 10.28941/pjwsr.v26i4.883.

Lone, T.A., & Lone, R.A. (2012). Extraction of cannabinoids from *Cannabis sativa* L plant and its potential antimicrobial activity. *Univ J Med Dent*, 1(4):51-55, DOI:10.13140/RG.2.2.21906.94401.

Mancuso, G., Midiri, A., Gerace, E., & Biondo, C. (2021). Bacterial Antibiotic Resistance: The Most Critical Pathogens. *Pathogens*, Oct 12;10(10):1310, doi: 10.3390/pathogens10101310.

Motiejauskaitė, D., Ullah, S., Kundrotaitė, A., Žvirdauskienė, R., Bakšinskaitė, A., & Barčauskaitė, K. (2023). Isolation of Biologically Active Compounds from *Cannabis sativa* L. Inflorescences by Using Different Extraction Solvents and Evaluation of Antimicrobial Activity. *Antioxidants*, 12, 998, https://doi.org/10.3390/antiox12050998.

Obiștioiu, D,. Hulea, A., Cocan, I., Alexa, E., Negrea, M., Popescu, I., Herman, V., Imbrea, I.M., Heghedus-Mindru, G., Suleiman, M.A., Radulov, I., & Imbrea, F. (2023). Boswellia Essential Oil: Natural Antioxidant as an Effective Antimicrobial and Anti-Inflammatory Agent. *Antioxidants (Basel)*, 12(10):1807, https://doi.org/10.3390/ antiox12101807.

Schofs, L., Sparo, M.D., & Sánchez Bruni, S.F. (2021). The antimicrobial effect behind *Cannabis sativa*. *Pharmacol Res Perspect*, Apr;9(2):e00761, doi: 10.1002/prp2.761.

Sea, Y.L., Gee, Y.J., Lal, S.K., & Choo, W.S. (2023). Cannabis as antivirals. *Journal of Applied Microbiology,* 134(1), [lxac036]. https://doi.org/10.1093/jambio/lxac036.

World Health Organization, (2023). Antimicrobial resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance, (accessed on 18.10.2023)