

Investigation antibacterial effect of euphorbia plant against different types of bacteria

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Abstract

In the present study, antibacterial activity assays were conducted using the extract of *Euphorbia lucida* Waldst. & Kit. against a range of bacterial strains. The efficacy of different dosage of the plant extract against different bacterial species was evaluated employing the disk diffusion method. The tested microorganisms included *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, *Salmonella kentucky*, *Listeria innocua*, *Serratia marcescens*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. The results demonstrated that *Euphorbia lucida* Waldst. & Kit extract exhibits notable antibacterial activity, with the most pronounced inhibitory effects observed against *Bacillus subtilis* (29.35 mm) and *Klebsiella pneumoniae* (24.95 mm). These findings suggest the potential utility of Euphorbia-derived compounds in both medical and environmental disinfection applications.

Keywords: Antibacterial test; Bacterium; Disinfection; Euphorbia plant

1. Introduction

Disinfection refers to the systematic process of eliminating or neutralizing pathogenic microorganisms to inhibit the transmission of infectious diseases and sustain hygienic standards in both personal and communal environments [1]. Particularly in the post-pandemic period, the critical role of effective disinfection practices has intensified significantly within individual and societal contexts. The rapid proliferation of pathogenic microorganisms on various surfaces, equipment, and water systems necessitates the deployment of robust and safe disinfection methods [2]. Historically, heavy metals such as silver and zinc have been extensively studied and utilized for their antibacterial properties; however, the utilization of these metals has raised concerns regarding environmental pollution, toxicity, and potential health risks from direct human contact [3]. Furthermore, the inadvertent release of such metallic compounds into ecosystems has been linked to substantial ecological harm, affecting both aquatic and terrestrial organisms adversely [4]. Recent studies show that some of the heavy metals pose a threat to human health even at very low concentrations [5-13], and even those necessary for living things can be harmful at high concentrations [14-19]. Therefore, it is recommended to reduce the release of heavy metals into nature and even to carry out studies to reduce their concentrations in nature [20-27]. Consequently, there is an escalating demand for exploring novel, naturally sourced disinfectants to complement or replace conventional chemical methods, leading to increased scientific attention towards plant-based antimicrobial agents

Plants fulfill many ecological, economic and social functions in nature [28-41]. In addition, they are widely used in medicine. Medicinal plants, deeply embedded in human culture, tradition, and history, continue to grow in prominence for their therapeutic and antibacterial potentials [42]. The appeal of plant-derived disinfectants is primarily due to their natural bioactive constituents, including alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins, known for their ability to disrupt microbial cell walls or interfere with vital microbial

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metabolic pathways, thus offering potent biological defense mechanisms [43,44]. Among these medicinal plants, *Euphorbia* species have a notable history in traditional medicine practices, and contemporary research consistently highlights their substantial antimicrobial activities [45,46]. These studies indicate promising opportunities for employing certain *Euphorbia* species in natural disinfection practices.

Methanolic extracts of various *Euphorbia* species including *Euphorbia aleppica* L, *E. macroclada*, and *E. virgate* have demonstrated significant inhibitory effects against a range of pathogenic microorganisms. Kirbag et al. [45] reported the antimicrobial activity of methanolic extracts and latex from several *Euphorbia* species (*Euphorbia aleppica*, *E. szovitsii* var. *harputensis*, *E. falcata* etc.) used medicinally in Türkiye. Extracts from eight species were tested against various bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans* etc.) and the results showed varying levels of microbial inhibition, with MIC values ranging from 31.2 to 1000 µg. Similarly, Ribinskas et al. [47] evaluated methanolic extracts of *Euphorbia helioscopia* L. against methicillin-resistant *Staphylococcus aureus* (MRSA) strains using the disc diffusion method demonstrating notable antibacterial effects. *Euphorbia hirta* has also been extensively studied for antimicrobial potential. Perumal et al. [48] assessed the antimicrobial and cytotoxic properties of different solvent extracts derived from *Euphorbia hirta*. Among the tested extracts, the ethanolic fraction displayed the most potent antimicrobial effect, particularly against *Salmonella typhi*. The study suggest that *E. hirta* contains bioactive compounds with significant antimicrobial potential and low cytotoxicity. These findings indicate that *Euphorbia* species contain bioactive compounds specifically phenolic compounds and flavonoids that contribute to their antimicrobial effectiveness. Consequently, *Euphorbia* species represent promising candidates for the development of plant-based disinfectants and novel antimicrobial agents.

Euphorbia lucida Waldst. & Kit. is a perennial herbaceous species belonging to the Euphorbiaceae family, commonly found in moist habitats such as river valleys, wet meadows, and forest edges. Native to regions stretching from Central Europe to Central Asia, it is also part of the natural flora of Türkiye. The plant is characterized by its glossy dark green lanceolate leaves and yellowish-green flowers. While *E. lucida* is considered rare or locally distributed in countries such as Germany and Austria, it is more widespread in parts of Eastern Europe and Türkiye. Moreover, several *Euphorbia* species have long been used in traditional medicine due to their bioactive compounds with reported antimicrobial, cytotoxic, and anti-inflammatory properties [49]. In this context, *Euphorbia lucida* holds potential for further phytochemical and pharmacological exploration.

In this study, the antibacterial activity of various concentrations of *Euphorbia lucida* extract was evaluated against different bacterial strains. Antibacterial assays were conducted using the standard disk diffusion method, and the tested organisms included *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, *Salmonella kentucky*, *Listeria innocua*, *Serratia marcescens*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. The aim of the study was to assess the potential of *Euphorbia lucida* as a natural antimicrobial agent and to investigate whether its efficacy varies by dose and bacterial species..

2. Material and methods

2.1. Pereparation of Euphorbia Plant Extract

Euphorbia lucida Waldst. & Kit. was collected from Bartın, Türkiye and its extract was separated. Then the extract of this plant was preserved at 4 °C.

2.2. Types of Bacteria and Paper Disc Preparation

Klebsiella pneumoniae, *Pseudomonas aeruginosa* (DSMZ 50071), *Salomonella kentucky*, *Listeria innocua*, *Saratia marrescens* (ATCC 13048), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (DSMZ 1971) and *Escherichia coli* (ATCC 25922) were used in the study. The filter paper was perforated with a punch and placed in a glass petri dish. Then it was sterilized under 1 atm pressure at 200°C for 2 hours.

2.3. Experimental Study

The solution to be used was obtained by adding the desired concentration of bacteria to sterile distilled water. The desired initial bacterial concentration (105 CFU/mL) was obtained by making the necessary dilution from the bacterial culture incubated overnight at 37°C the day before. Disk diffusion method was used in the study and studies were carried out at ambient temperature. Nutrient broth and nutrient agar were used for bacterium as a medium. Studies were carried out with *Euphorbia lucida* Waldst. & Kit.extract undiluted and 3 different concentrations (2%, 10%, 100%). Samples taken during the studies were incubated for 1 day at 37 °C after being planted in suitable media.

3. Results and Discussion

In the study, studies were conducted with different *Euphorbia lucida* Waldst. & Kit. extract dosages and the results are shown in Table 1. Antibacterial effect of *Euphorbia lucida* Waldst. & Kit. on some bacteria is shown in Fig.1. In the study, the best bacterial effect was seen in *Bacillus subtilis* and *Klebsiella pneumoniae* bacteria. This suggests that the phytochemical components present in *Euphorbia* may exert variable effects on both Gram-positive and Gram-negative bacteria. The strong inhibitory effect observed against *Bacillus subtilis* is likely due to the cell wall structure of this Gram-positive bacterium, which may be more susceptible to the active compounds in the extract. *Klebsiella pneumoniae* also showed considerable sensitivity to the extract. This indicates that the *Euphorbia* extract may contain bioactive compounds capable of penetrating even more resilient bacterial defenses, pointing to a promising potential for broad-spectrum application. On the other hand, relatively lower antibacterial activity was observed against *Pseudomonas fluorescens*, *Salmonella kentucky*, *Listeria innocua*, *Serratia marcescens*, *Staphylococcus aureus*, and *Escherichia coli*. These results suggest that the efficacy of the extract is strain-specific, and certain bacteria may possess intrinsic or acquired resistance to its active constituents. In particular, the lower effect observed on *Pseudomonas fluorescens*, a bacterium known for its environmental resilience, supports this interpretation. These findings highlight the potential of *Euphorbia* extract as a natural antimicrobial agent.

Table 1 Antibacterial Effect Results of Different Dilutions of *Euphorbia* Plant Extract

Types of Bacteria	Undiluted (mm)	%2 (mm)	%10 (mm)	%100 (mm)
<i>Klebsiella pneumoniae</i> (4)	24.95	17.61	6	6
<i>Pseudomonas aeruginosa</i> (5)	20.11	12.50	8.14	-
<i>Salomonella kentucky</i> (7)	11.43	-	-	-
<i>Listeria innocua</i> (9)	10.31	9.89	-	-
<i>Saratia marrescens</i> (19)	19.59	11.63	-	-
<i>Staphylococcus aureus</i> (15)	17.05	16.67	10.01	7.10
<i>Bacillus subtilis</i>	29.35	15.87	8.51	-
<i>Escherichia coli</i>	22.76	-	-	-

- : no zone formation

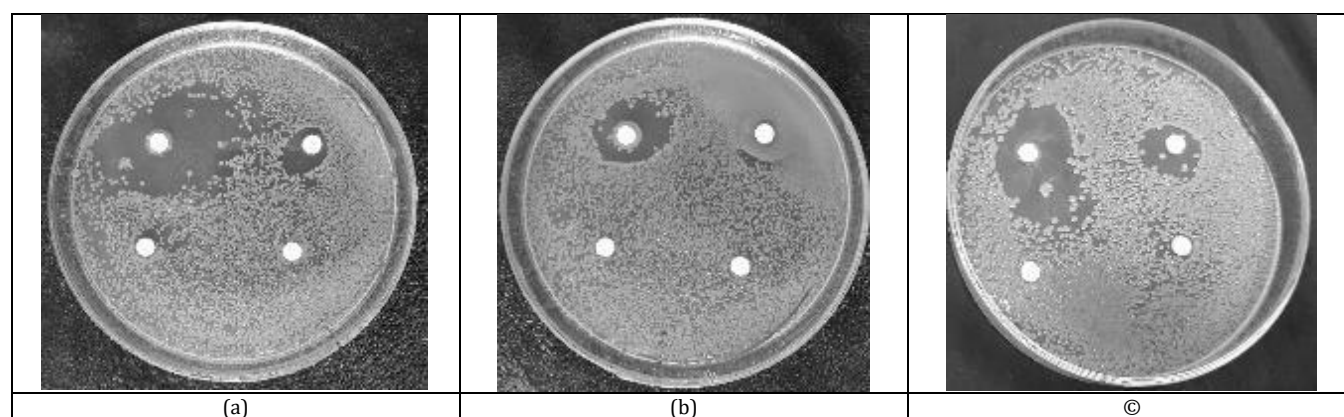


Figure 1 Antibacterial effect of euphorbia extract on some bacteria (a) *Bacillus subtilis* (b) *Escherichia coli* (c) *Klebsiella pneumoniae*

4. Conclusion

In this study, the antibacterial effects of *Euphorbia* plant extract were evaluated against eight different bacterial strains. The findings revealed that the extract exhibited notably high antibacterial activity against *Bacillus subtilis* and *Klebsiella pneumoniae*. These findings suggest that *Euphorbia* extract exhibits strong disinfectant properties, particularly against these two bacterial species indicating the extract's potential effectiveness against both Gram-positive and some resistant Gram-negative bacteria. Limited effects were recorded against *Pseudomonas fluorescens*, *Salmonella kentucky*,

Listeria innocua, *Serratia marcescens*, *Staphylococcus aureus*, and *Escherichia coli*, suggesting that the extract's efficacy varies depending on the bacterial species. Overall, this study supports the potential use of plant-derived extracts as alternative antimicrobial agents. However, further research is needed to isolate and identify the specific bioactive compounds responsible for the observed effects.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they no conflict of interest. The none of the authors have any competing interests in the manuscript.

References

- [1] Rutala, W. A., Boyce J.M., & Weber, D. J. (2019). Disinfection, sterilization and antisepsis: An overview. *American Journal of Infection Control*, 51, A3-A12.
- [2] Kampf, G., Todt, D., Pfaender, S., & Steinmann, E. (2020). Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. *Journal of Hospital Infection*, 104(3), 246-251.
- [3] Evans, A., & Kavanagh K.A. (2021). Evaluation of metal-based antimicrobial compounds for the treatment of bacterial pathogens. *Journal of Medical Microbiology*, 70(5). <https://doi.org/10.1099/jmm.0.001363>
- [4] Singh, R., Gautam, N., Mishra, A., & Gupta, R. (2011). Heavy metals and living systems: An overview. *Indian Journal of Pharmacology*, 43(3), 246-253
- [5] Koç İ, Canturk U, Cobanoğlu H, Kulac S, Key K, & Sevik H. (2025). Assessment of 40-year Al Deposition in some Exotic Conifer Species in the Urban Air of Düzce, Türkiye. *Water, Air, & Soil Pollution*, 236(2), 1-14.
- [6] Özel HB, Şevik H, Yıldız Y, & Çobanoğlu H. (2024). Effects of Silver Nanoparticles on Germination and Seedling Characteristics of Oriental Beech (*Fagus orientalis*) Seeds. *BioResources*, 19(2). 2135-2148
- [7] Isinkaralar K, Isinkaralar O, Özel HB, & Şevik H. (2024). A Comparative Study About Physical Properties of Copper Oxide and Zinc Oxide Nanoparticles on *Fagus orientalis* L. as Bioindicator. *Water, Air, & Soil Pollution*, 235(11), 738.
- [8] Sevik H, Ozel HU, Yildiz Y, & Ozel HB. (2025). Effects of Adding Fe2O3 and Fe3O4 Nanoparticles to Soil on Germination and Seedling Characteristics of Oriental Beech. *BioResources*, 20(1), 70-82.
- [9] Isinkaralar O, Isinkaralar K, & Sevik H. (2025). Health for the future: spatiotemporal CA-MC modeling and spatial pattern prediction via dendrochronological approach for nickel and lead deposition. *Air Quality, Atmosphere & Health*, 1-13.
- [10] Ghoma WEO, Sevik H, & Isinkaralar K. (2023). Comparison of the rate of certain trace metals accumulation in indoor plants for smoking and non-smoking areas. *Environmental Science and Pollution Research*, 30(30): 75768-75776.
- [11] Key K, Kulaç Ş, Koç İ, & Sevik H. (2023). Proof of concept to characterize historical heavy-metal concentrations in atmosphere in North Turkey: determining the variations of Ni, Co, and Mn concentrations in 180-year-old *Corylus colurna* L. (Turkish hazelnut) annual rings. *Acta Physiologiae Plantarum*, 45(10); 1-13.
- [12] Sevik H, Yildiz Y, Ozel HB. (2024). Phytoremediation and Long-term Metal Uptake Monitoring of Silver, Selenium, Antimony, and Thallium by Black Pine (*Pinus nigra* Arnold), *BioResources*, 19(3). 4824-4837.
- [13] Erdem R, Koç İ, Çobanoğlu H, & Şevik H. (2024). Variation of Magnesium, One of the Macronutrients, in Some Trees Based on Organs and Species. *Forestist*, 74(1). 84-93
- [14] Sevik H, Cetin M, Ozel HB, & Pinar B. (2019). Determining toxic metal concentration changes in landscaping plants based on some factors. *Air Quality, Atmosphere & Health*, 12, 983-991.

- [15] Sevik H, Cetin M, Ozturk A, Ozel HB, & Pinar B. (2019). Changes in Pb, Cr and Cu concentrations in some bioindicators depending on traffic density on the basis of species and organs. *Applied Ecology And Environmental Research* 17(6):12843-12857
- [16] Koc I, Cobanoglu H, Canturk U, Key K, Kulac S, & Sevik H. (2024). Change of Cr concentration from past to present in areas with elevated air pollution. *International Journal of Environmental Science and Technology*, 21(2), 2059-2070.
- [17] Ozturk Pulatoglu A, Koç İ, Özel HB, Şevik H, & Yıldız Y. (2025). Using Trees to Monitor Airborne Cr Pollution: Effects of Compass Direction and Woody Species on Cr Uptake during Phytoremediation. *BioResources*, 20(1), 121-139.
- [18] Cobanoglu H, Sevik H, & Koç İ. (2023). Do annual rings really reveal Cd, Ni, and Zn pollution in the air related to traffic density? An Example of the Cedar Tree. *Water, Air, & Soil Pollution*, 234(2); 65.
- [19] Koç İ, Canturk U, Isinkaralar K, Ozel HB, & Sevik H. (2024). Assessment of metals (Ni, Ba) deposition in plant types and their organs at Mersin City, Türkiye. *Environmental Monitoring and Assessment*, 196(3), 282.
- [20] Sevik H, Koç İ, & Cobanoglu H. (2024). Determination of Some Exotic Landscape Species As Biomonitors That Can Be Used for Monitoring and Reducing Pd Pollution in the Air. *Water, Air, & Soil Pollution*, 235(10), 615.
- [21] Canturk U, Koç, İ, Ozel HB, & Sevik H. (2024). Identification of proper species that can be used to monitor and decrease airborne Sb pollution. *Environmental Science and Pollution Research*, 1-11.
- [22] Yaşar İsmail, T.S., İsmail, M.D, Çobanoğlu, H., Koç, İ., & Sevik, H. (2024). Monitoring arsenic concentrations in airborne particulates of selected landscape plants and their potential for pollution mitigation, *Forestist*. <https://doi.org/10.5152/forestist.2024.24071>
- [23] Koç İ, Cobanoglu H, Canturk U, Key K, Sevik H, & Kulac S. (2025). Variation of 40-year Pb deposition in some conifers grown in the air-polluted-urban area of Düzce, Türkiye. *Environmental Earth Sciences*, 84(7), 186.
- [24] Gültekin Y, Bayraktar MK, Sevik H, Cetin M, & Bayraktar T. (2025). Optimal vegetable selection in urban and rural areas using artificial bee colony algorithm: Heavy metal assessment and health risk. *Journal of Food Composition and Analysis*, 139, 107169.
- [25] Isinkaralar K, Isinkaralar O, Koç İ, Özel HB, & Şevik H. (2024). Assessing the possibility of airborne bismuth accumulation and spatial distribution in an urban area by tree bark: A case study in Düzce, Türkiye. *Biomass conversion and biorefinery*, 14(18), 22561-22572.
- [26] Isinkaralar K, Isinkaralar O, Koc I, Sevik H, & Ozel HB. (2025). Atmospheric Trace Metal Exposure in a 60-Year-Old Wood: A Sustainable Methodological Approach to Measurement of Dry Deposition. *Int J Environ Res* 19, 112 (2025). <https://doi.org/10.1007/s41742-025-00783-x>
- [27] Kuzmina N, Menshchikov S, Mohnachev P, Zavvalov K, Petrova I, Ozel HB, Aricak B, Onat SM, and Sevik H. (2023). Change of aluminum concentrations in specific plants by species, organ, washing, and traffic density, *BioResources*, 18(1); 792-803.
- [28] Hrivnák M, Krajmerová D, Paule L, Zhelev P, Sevik H, Ivanković M, Goginashvili N, Paule J, Gömöry D. (2024). Are there hybrid zones in *Fagus sylvatica* L. sensu lato?. *European Journal of Forest Research*, 143, 451–464. <https://doi.org/10.1007/s10342-023-01634-0>
- [29] Sevik H, Yahyaoglu Z, & Turna I. (2012). Determination of genetic variation between populations of *Abies nordmanniana* subsp. *bornmulleriana* Mattf according to some seed characteristics, genetic diversity in plants. *Chapter*, 12; 231-248.
- [30] Kurz M, Koelz A, Gorges J, Carmona BP, Brang P, Vitasse Y, ... & Csillery K. (2023). Tracing the origin of Oriental beech stands across Western Europe and reporting hybridization with European beech–Implications for assisted gene flow. *Forest Ecology and Management*, 531; 120801.
- [31] Tandoğan M, Özel HB, Gözet FT, & Şevik H. (2023). Determining the taxol contents of yew tree populations in western Black Sea and Marmara regions and analyzing some forest stand characteristics. *BioResources*, 18(2), 3496-3508.
- [32] Kravkaz-Kuscu IS, Sariyildiz T, Cetin M, Yigit N, Sevik H, & Savaci G. (2018). Evaluation of the soil properties and primary forest tree species in Taskopru (Kastamonu) district. *Fresenius Environmental Bulletin*, 27(3), 1613-1617.

- [33] Sevik H, Cetin M, Ozturk A, Yigit N, & Karakus O. (2019). Changes in micromorphological characters of *Platanus orientalis* L. leaves in Turkey. *Applied Ecology and Environmental Research*, 17(3);5909-5921.
- [34] Yigit N, Öztürk A, Sevik H, Özel HB, Ramadan Kshkush FE, & Işık B. (2023). Clonal Variation Based on Some Morphological and Micromorphological Characteristics in the Boyabat (Sinop/Turkey) Black Pine (*Pinus nigra subsp. pallasiana* (Lamb.) Holmboe) Seed Orchard. *BioResources*, 18(3): 4850-4865
- [35] Cantürk U, Koç İ, Özel HB, & Şevik H. (2024). Possible changes of *Pinus nigra* distribution regions in Türkiye with the impacts of global climate change. *BioResources*, 19(3), 6190-6214
- [36] Özdikmenli G, Yiğit N, Özel HB, & Şevik H. (2024). Altitude-dependent Variations in Some Morphological and Anatomical Features of Anatolian Chestnut. *BioResources*, 19(3). 4635-4651
- [37] Sevik H, Çetin M, & Kapucu O. (2016). Effect of light on young structures of Turkish fir (*Abies nordmanniana subsp. bornmulleriana*). *Oxidation Communications*, 39(1), 485-492.
- [38] Sevik H, & Topacoglu O. (2015). Variation and inheritance pattern in cone and seed characteristics of Scots pine (*Pinus sylvestris* L.) for evaluation of genetic diversity. *Journal of Environmental Biology*, 36(5), 1125.
- [39] Özel HB, Sevik H, Cetin M, Varol T, & Isik B. (2024). Impact of employment policies on disabled individuals in silvicultural activities. *Environment, Development and Sustainability*, 1-14.
- [40] Sevik H, Cetin M, Kapucu O, Aricak B, & Canturk U. (2017). Effects of light on morphologic and stomatal characteristics of Turkish Fir needles (*Abies nordmanniana subsp. Bornmulleriana* Mattf.). *Fresenius Environmental Bulletin*, 26 (11), 6579-6587.
- [41] Özel HB, Şevik H, Onat SM, & Yigit N. (2022). The effect of geographic location and seed storage time on the content of fatty acids in stone pine (*Pinus pinea* L.) seeds. *BioResources*, 17(3), 5038.
- [42] Jamshidi Kia, F., Lorigooini, Z., & Amini Khoci, H. (2018). Medicinal plants: Past history and future perspective, *Journal of Herbmmed Pharmacology*, 7(1), 1-7.
- [43] Elisha, I. L., Botha, F. S., McGaw, L. J., & Eloff, J. N. (2017). The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. *BMC Complementary and Alternative Medicine*, 17(1). <https://doi.org/10.1186/s12906-017-1645-z>
- [44] Das, K., Tiwari, R.K.S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agents: current methods and future trends. *J Med Plants Res*. 4:104–11.
- [45] Kirbag, S., Erecevit, P., Zengin, F., & Guvenc, A. (2013). Antimicrobial activities of some Euphorbia species. *African Journal of Traditional, Complementary and Alternative Medicines*, 10(5). 305-309
- [46] Kamba A., & S Hassan L. G. (2010) Phytochemical screening and antimicrobial activities of Euphorbia balsamifera leaves, stems and root against some pathogenic microorganisms. *African Journal of Pharmacy and Pharmacology*, 4(9):645-652.
- [47] Ribinskas, T., Vitkauskienė, A., Kareiviene, V., & Zevzikoviene, A. (2024). Antimicrobial Activity of Euphorbia helioscopia Against Methicillin-Resistant Staphylococcus aureus (MRSA) In Vitro. *Cureus*. 16(9): e69840.
- [48] Perumal, S., Mahmud, R., Pillai, S., Lee, W. C., & Ramanathan, S. (2012). Antimicrobial activity and cytotoxicity evaluation of Euphorbia hirta (L.) extracts from Malaysia. *APCBEE Procedia*, 2, 80-85.
- [49] Đurović, S., Ranimirović, M., Tomović, G., Petkovski, G., & Niketić, M. (2022). Genus Euphorbia L. (Euphorbiaceae juss.) in Serbia based on herbarium data from the collections BEO and BEOU. *Bulletin of the Natural History Museum*, 15, 97-120.