Journal of Population Therapeutics & Clinical Pharmacology

Research Article
DOI: 10.53555/b774x573

ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL POTENTIAL OF *EUPHORBIA HELIOSCOPIA* L. COLLECTED FROM SIALKOT PAKISTAN

Hafsa Khalid[1], Zahid Ali Butt[1], Fatima Idrees[1*], Fiza Munir[1], Anfal Arshad[1], Maria Akhtar[1], Salma Khalid[1], Samra Riasat[1*] Asra Imtiaz[1] , Javed Kamar[2], Sajid Hussain[2*]

[1]Department of Botany, GC Women University, Sialkot.
[2]Department of Botany, PMAS Arid Agriculture University, Rawalpindi

***Corresponding Authors:** Fatima Idrees, Samra Riasat, Sajid Hussain
*Email: fatimaadrees4@gmail.com, samrariasat786@gmail.com, sajidhussaindgk121@gmail.com

Abstract
A study was conducted to evaluate the antimicrobial activity and phytochemical analysis of *Euphorbia helioscopia* L. Plants were collected and tested against several common species and human pathogenic microorganisms including *Escherichia coli, Klebsiella sp*. and the pathogenic fungus *Aspergillus niger*. Different solvents viz., ethanol, methanol, acetone and water were used for plant extract preparation. The extract then goes through a preliminary screening process to determine the presence of important phytochemicals. Phytochemical analysis has shown *that Euphorbia helioscopia* contains many active compounds, including flavonoids, alkaloids, tannins and saponins. Antifungal and antibacterial activity was evaluated by agar well diffusion method. Maximum zone of inhibition was observed by ethanolic and methanolic extract of *E. helioscopia* against *E. coli, Klebsiella sp* and *A. niger* as compared to other extracts. The extracts prepared in the water showed minimum zone of inhibition.

Introduction

Since ancient times, people have used plants to treat common infectious diseases, and some of these traditional medicines are still included in the daily treatment of various diseases. Long before humanity discovered the existence of microbes, certain plants were widely believed to have medicinal properties and contain what we now call antimicrobial principles (Saleem et al., 2015). In each discipline of pharmacology, agro-alimentary and agricultural production, the herbal remedies are becoming more and more well-liked as a natural supply of physiologically active compounds (Jaouadi et al., 2022). The secondary metabolic products that are produced by medicinal plants exhibit amazing structural alterations and are useful to humans in multiple ways. Phenolic compounds, glycosides, alkaloids, volatile oils, saponins, terpenoids, tannins, resins, steroids, and other substances are examples of secondary metabolites (Mehmood et al., 2021).

There are 5000 species and 300 genera in the family Euphorbiaceae, also known as the spurge family. The genus *Euphorbia* is the biggest in Euphorbiaceae and sixth largest genus among flowering plants, which has around 2000 species (Bakht et al., 2017). Toxic chemicals derived from the milky sap of *Euphorbia* species primarily serve as repellent to pests and livestock. Exposure with the eyes, nose, mouth, or even skin can cause severe pain and inflammation, possibly as a result of a protein- Kinase C enzymatic being activated (Singh et al., 2023).

Euphorbia helioscopia is herbaceous annual plant belongs to family Euphorbiaceae. It has significant medical value and is widespread in nature (Rafiq et al., 2014). It is extensively available across Northern Africa, Europe, and Asia (Dou et al., 2024). It appears in November - December and destroys winter vegetables and crops such potatoes, peas, lentils, and wheat. Flies fertilize the hermaphrodite blooms, which have both male and female parts (Singh., 2018). In traditional Chinese medicine, the aerial portions of this plant, known as "Ze Qi," are used to cure a variety of ailments, including ringworm, phlegm, coughing, ascites, edema, and TB (Zhu et al., 2020).

Native people in Hebei, Hunan, Sichuan, Guizhou, Jiangxi and other regions have long used *E. helioscopia* as a folk remedy due to its bitter and caustic flavor and frigid nature (Yang et al., 2021) Wolf's milk or sun spurge are two English names for the plant *Euphorbia helioscopia* L. (Beltagy,2019). Euphorbia is used to treat cancer and has been used in traditional medicine for hundreds of years to treat tumors and warts (Waheed et al., 2020).
It's believed that there are antifungal and antibacterial qualities in this plant. A variety of diterpenoids and triterpenoids are produced by species within the Euphorbia family, based on analysis (Deshpande et al., 2024). As a form of vermifuge and a fibrifuge, the leaves and stems of *Euphorbia helioscopia* are utilized. The seeds have been used to treat cholera when combined with roasted pepper, the roots are used as an anthelmintic and the oil from the seeds of the plant has purgative qualities. *E. helioscopia* is utilized by the locals in Pakistan as a cathoratic, antihelminthic and purgative. According to several ethnobotanical surveys for medicinal herbs the *Euphorbia spp*. used traditionally in different nations (Abou-El-Hamd H. Mohamed. et al., 2012).

1. Materials and Methods

2. Collection of plants

E. helioscopia was collected from the fields of Sialkot District. After two or three cycles of washing with tap water the plants were washed with distilled water and then shade dried.

3. Preparation of plants extracts

The collected plants were dried in shade and ground into fine powder. Then this powder was stored in airtight jars for further use. Plant extract was prepared by adding dried plant powder of 10 g into 100 ml of four different solvents viz., methanol, ethanol, acetone and water. Then these solvents were kept in water bath for 60 min at 60°C and then were kept at room temperature for 24h. After that the extracts were filtered with watt-man filter paper no.1. A rotatory evaporator was used for drying of sample to get the final concentration from the extract. Then dried extract was mixed with DMSO and stored in the refrigerator at 4°Cfor further use.
Different concentration of extracts 10%, 50% and100% were used.

4. Qualitative analysis of Phytochemicals

The primary phytochemical elements, including alkaloids, flavonoids, phenolics, saponins, steroids, tannins, carbohydrates, and volatile oils, were qualitatively confirmed by dissolving crude plant extract samples in the suitable solvents.

4.1. Dragendorff's Test for Alkaloids.

One milliliter of extract was placed in a test tube, and a couple of drops of Dragendorff's solution were added., and color development was observed. The presence of alkaloids is indicated by an orange appearance (Kebede et al., 2021).

4.2. Alkaline Reagent Test for Flavonoids.

To a test tube 2ml plants extract was added with few drops of 2% NaoH solution. Presence of flavonoids was observed by seeking the appearance of a yellow color that fades off after a diluted solution of hydrogen chloride is added (Mehmood et al., 2021).

4.3. Ferric Chloride Test for Phenolics.

For an indication of the presence of phenolics, add 2 milliliters of plant extract to a test tube, drops of one percent ferric chloride solution should be added. Emergence of a blue, green, or black coloration can be observed (Kebede et al., 2021).

4.4. Frothing Test for Saponins.

In a test tube with 2 ml of the plant extract, add 5 ml of distilled water to it. For two to three minutes, give the mixture an intensive shake. let the mixture settle for over ten minutes. When persistent foam forms, it means that saponins are present. This foam should remain in place for at least ten minutes (Kebede et al., 2021).

4.5. Liebermann-Burchard Test for Steroids.

Take a test tube with 2 milliliters of plant extract. Two milliliters of chloroform were added and then add two milliliters of acetic anhydride. After these One or two drops of strong sulfuric acid should be carefully added to the test tube's side. Check for the appearance of a blue or green hue, which demonstrates the existence of steroids (Falana and Nurudeen., 2022).

4.6. Ferric Chloride Test for Tannins.

Take a test tube with 2 ml of the plant extract. Two or three drops of one percent solution of ferric chloride need to be poured. When a blue-black or green-black hue forms, tannins are present (Mehmood et al., 2021).

4.7. Fehling's Test for Carbohydrates.

Merge an equal amount of Fehling's A and B solutions. Pour 2 milliliters of this blend into a test tube holding 2 milliliters of plant extract. For a period of two to three minutes, heat the mixture in a bath of water that is boiling. When a red precipitate forms, it means that reducing sugars are present (Mehmood et al., 2021).

4.8. Copper acetate Test for Volatile oils.

A little amount of diluted hydrochloric acid and 0.1 ml of diluted sodium hydroxide were added to 2 ml of extract. The emergence of a white precipitate is indicative of volatile oils are present (Kuppusamy et al., 2015).

4.9. Alkaline reagent Test for coumarins

Prepare a test tube with two milliliters of plant extract. Add two milliliters of a 10% solution of sodium hydroxide (NaOH). Thoroughly shake the blend and note any variations in hue. The emergence of a yellow hue signifies the existence of coumarins (Ahmad et al.,2019).

4.10. Borntrager's Test for anthraquinones:

Dissolve two to three milliliters of diluted HCl with two to three milliliters of extract, bring to a boil, and then strain. Add chloroform in equal parts and give it an extensive shake. After removing the chloroform layer, apply the same amount of diluted ammonia. Anthraquinones are present in the ammoniacal layer when it is pink, red, or violet in hue (Kebede et al., 2021).

5. Microbial strains

Pre-identified bacterial strains *(E. coli* and *Klebsiella sp.)* and fungal strain (*A. niger) were* obtained from sustainable development study center (SDSC), from GC University Lahore. These identified bacterial strains were inoculated on slants and isolated by regular sub culturing method.

6. Preparation of bacterial growth media

Nutrient agar media was prepared by adding 8.0 g of nutrient Broth in some quantity of distilled water. By constantly shaking the solution add 15.0 g of agar to it and the final volume will be raised to 1000ml. Gently warm the solution for a clear solution formation and after its formation autoclaved it at 121℃ temp, 15 lbs/inch² pressure for 15 min.

7. Bacterial inoculum preparation

For inoculum preparation, nutrient broth (0.8 g) was dissolved in water and the volume was raised up to 100ml in a conical flask. Then, the media was autoclaved at 121°c temperature, 15 lbs/inch ² pressure for 15 minutes. Autoclaved media was cooled at room temperature. In laminar air flow, a sterilized inoculating needle was used for inoculating a loopful of bacteria in the nutrient broth solution. Then, the flask was kept in the incubator shaker for 24h.

8. Antibacterial activity

Well diffusion method was used for determining the antibacterial activity of plant extracts. After 24h of being kept at 37℃ in an incubator, the inhibition zones on the plates were measured and noted in millimeter from the bottom side of the Petri plate.

9. Preparation of fungal growth media

For preparation of potato dextrose agar (PDA) media, 40 g of PDA was added in some distilled water and then raised the final volume up to 1000 ml. The media was warmed gently until a clear solution was formed. Then the solution was autoclaved at 121°C, 15Ibs/inch^2 pressure for 15 min.

10. Fungal inoculum preparation

For the preparation of fungal inoculum, (0.9%) saline water was added in a slant full of fungal spores. Spore suspension was formed by shaking the slant gently. This spore suspension was used as inoculum for further studies.

11. Antifungal activity

Well the diffusion method was used for the determination of the antifungal activity of plant extracts. The plates were inoculated with the identified fungi and plates was then incubated for 72h at 28 °C. The inhibition zone was measured and recorded from the back side of the petri plate in millimeters.

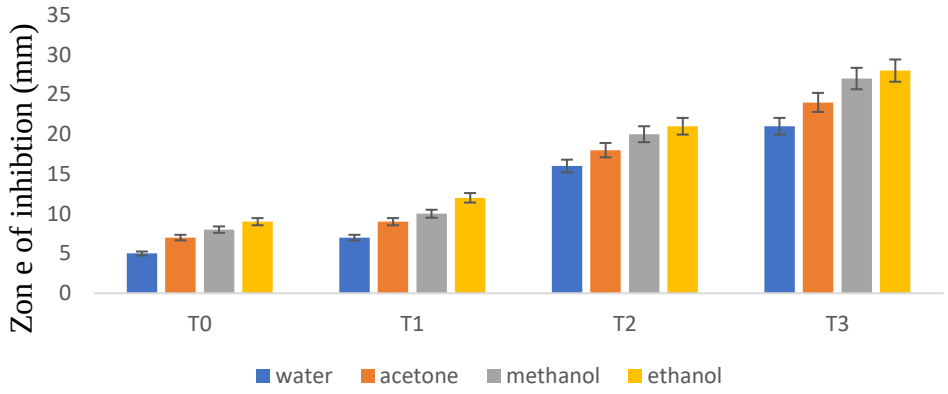
12. Statistical analysis

All measurements were performed in triplicate. GraphPad Prism-10 software was utilized to analyze the data and determine the mean ± standard deviation. All the results were significant at $p < 0.05$.

Results

The effect of different concentrations of ethanolic extract of *E. helioscopia* on the growth of the bacteria *E. coli*

Figure 3.1 illustrates the impact of different concentrations of ethanolic, methanolic, acetone, and water extracts of *E. helioscopia* on the growth of *E. coli*. For ethanolic extracts, T3 (100%) exhibited the biggest inhibition zone (28 mm), while T1 (10%) had the smallest (12 mm), and T2 (50%) showed 21 mm. Methanolic extracts showed the biggest inhibition with T3 (100%) at 21 mm, the smallest with T1 (10%) at 10 mm, and T2 (50%) at 20 mm. Acetone extracts showed 24 mm for T3 (100%), 9 mm for T1 (10%), and 18 mm for T2 (50%). Water extracts resulted in 21 mm for T3 (100%), 7 mm for T1 (10%), and 16 mm for T2 (50%). The control (T0) is also run parallel. All results were significant at $p \leq 0.05$



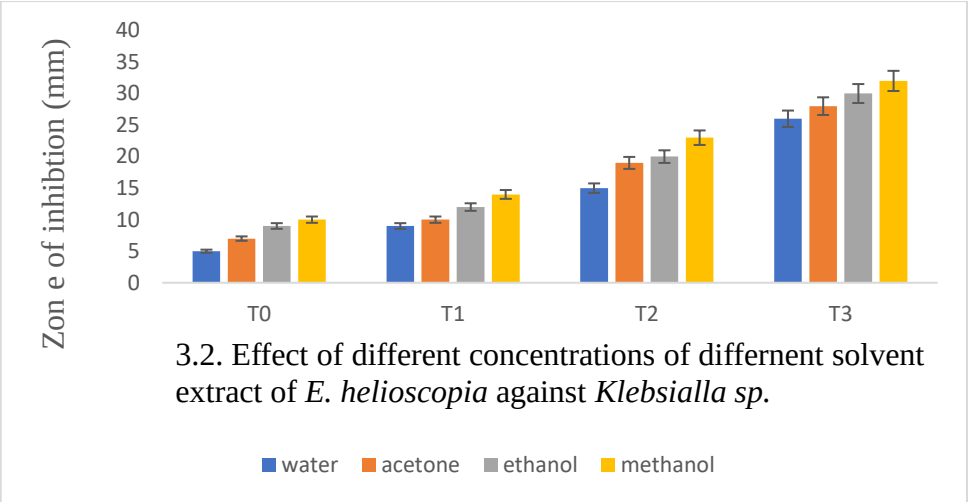
3.1. Effect of different concentrations of differnent solvent extract of *E. helioscopia* against *E. coli*

Plate: 1. Antibacterial activity of different solvent extracts of *Euphorbia helioscopia* on the growth of *E. coli*

The effect of different concentrations of ethanolic extract of *E. helioscopia* on the growth of the bacteria *Klebsiella sp.*

Figure 3.2 shows the impact of various concentrations of *E. helioscopia* extracts on *Klebsiella sp.* growth. For ethanolic extracts, T3 (100%) exhibited the biggest inhibition zone (32 mm), T1 (10%) the smallest (14 mm), and T2 (50%) had 23 mm. Methanolic extracts showed T3 (100%) with 30 mm, T1 (10%) with 12 mm, and T2 (50%) with 20 mm. Acetone extracts resulted in 28 mm for T3 (100%),

10 mm for T1 (10%), and 19 mm for T2 (50%). Water extracts showed 26 mm for T3 (100%), 9 mm for T1 (10%), and 15 mm for T2 (50%). The control (T0) is also run parallel. All results were significant at $p \leq 0.05$.

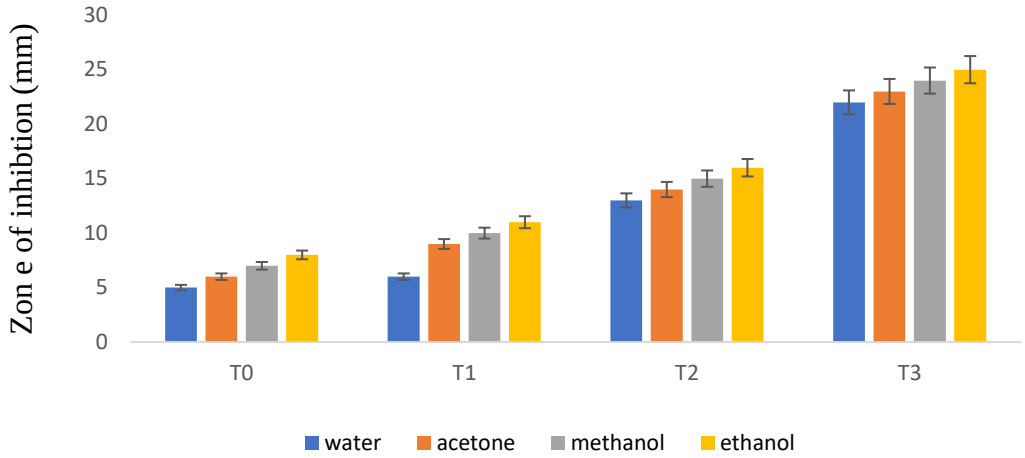


3.2. Effect of different concentrations of differnent solvent extract of *E. helioscopia* against *Klebsialla sp.*

Plate: 2. Antibacterial activity of different solvents extracts of *Euphorbia helioscopia* on the growth of *Klebsiella sp.*

The effect of different concentrations of ethanolic extract of *E. helioscopia* on the growth of the bacteria *A. niger*.

Figure 3.3 shows the effects of various concentrations of *E. helioscopia* extracts on *A. niger* growth. Ethanolic extracts exhibit the highest inhibition zone at 25 mm for T3 (100%), the lowest at 11 mm for T1 (10%), and 16 mm for T2 (50%). Methanolic extracts show 24 mm for T3 (100%), 10 mm for T1 (10%), and 15 mm for T2 (50%). Acetone extracts result in 23 mm for T3 (100%), 9 mm for T1 (10%), and 14 mm for T2 (50%). Water extracts showed 22 mm for T3 (100%), 6 mm for T1 (10%), and 13 mm for T2 (50%). The control (T0) is also run parallel. All results were significant at $p \leq 0.05$.



3.3. Effect of different concentrations of differnent sovent extract of *E. helioscopia* against *A .niger*

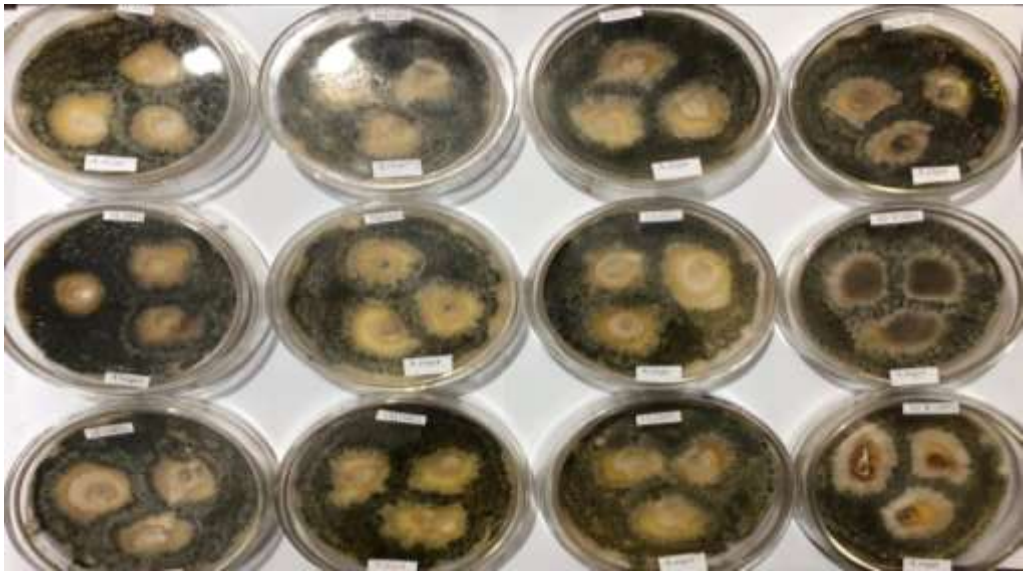


Plate: 3. Antifungal activity of different solvents extracts of *Euphorbia helioscopia* on the growth of *A. niger.*

Results of phytochemical analysis of different solvents of *Euphorbia helioscopia*

Table .1.	Qualitative analysis of phytochemicals of plants extracts in different solvents of *Euphorbia helioscopia*			
Secondary metabolites	Ethanol	Methanol	Acetone	Water
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Coumarins	+	+	+	+
Terpenoids	+	+	+	+
Phenolics	+	+	+	+
carbohydrates	+	+	+	+
Anthraquinones	+	+	+	+
Volatile oil	-	-	+	-

Present = +, Absent = -

Discussion

Plants contain many active compounds including various phenolic compounds that contribute to antimicrobial activity. These metabolites act as natural defense mechanisms and have been cited for their potential in medicine and health. The properties of these phenolic compounds depend upon the choice of extraction method and on factors such as the type of compounds one want to extract, the plant material used and the desired extract purity. The well diffusion method, sometimes referred to as the agar well diffusion method, is a popular method for determining how effective antibiotics, plant extracts, and other chemicals are as antimicrobial agents. It has been reported by Valgas et al., 2007 that well diffusion method is best for antimicrobial activity of plants extract in despite of other methods like disc diffusion methods.

Good antimicrobial activity is demonstrated by ethanolic, methanolic, acetonic, and water e *E. helioscopia* showed good antimicrobial activity against the microbes and fungi viz., *A. niger*, *Klebsiella sp. and E. coli* (Amtaghri et al., 2022). The reason behind it could be that *E. helioscopia* abundantly extracts phenolic compounds which are responsible for their batter antimicrobial activity. (Mahomood et al., 2020). Because these phenolic compounds have ability by interfering with the metabolism and cellular properties of microbial cells, which can inhibit their growth or kill them. Adil et al. (2024) reported same kind of results in his research. In comparison to water extracts, ethanol and methanol extracts can frequently produce more concentrated solutions of bioactive chemicals which may account for some of their increased antibacterial action (Waheed et al., 2020). The acetone extract has good antibacterial properties because it contains a variety of bioactive substances, including tannins, terpenoids, alkaloids, flavonoids and phenolic compounds. (Mehmood et al., 2021). These compounds have been known to exhibit antimicrobial properties by interfering with the growth and proliferation of microorganisms, including bacteria and fungi (Yang et al., 2021). While water extracts may not be as efficient as methanol, acetone and ethanol in extracting certain antimicrobial compounds such as alkaloids or terpenoids, but water extracts can still exhibit antimicrobial activity. The organic solvents can exhibit good antimicrobial activity, this might be due to the reason that compound extracted by these solvents may alter the base pair sequence in the microbes DNA that may create problem in the DNA synthesis or it may rupture the cell membrane in the result the death of microorganisms may occur (Doughari., 2012).

Conclusion

From the results it is concluded that the *Euphorbia helioscopia* L. has such type of phytochemicals which are responsible for the antimicrobial activity. The *Euphorbia helioscopia* may also be used to synthesize new antimicrobial drugs or may be used as an antimicrobial agent in its crude form.

References

1. Abou-El-Hamd, E. M., Mohamed-Elamir, F. H., and Moustafa, M. F. (2012). *Euphorbia helioscopia*: chemical constituents and biological activities. *International Journal of Phytopharmacology*, 3(1), 78-90.
2. Adil, M., Filimban, F. Z., Ambrin, Quddoos, A., Sher, A. A., and Naseer, M. (2024). Phytochemical screening, HPLC analysis, antimicrobial and antioxidant effect of *Euphorbia parviflora* L. (Euphorbiaceae Juss.). *Scientific Reports*, 14(1), 5627.
3. Ahmed, S. R., Roy, R., Romi, I. J., Hasan, M., Bhuiyan, M. K. H., and Khan, M. M. H. (2019). Phytochemical screening, antioxidant and antibacterial activity of some medicinal plants grown in Sylhet region. *IOSR J. Pharm. Biol. Sci*, 14, 26-37.
4. Amtaghri, S., Akdad, M., Slaoui, M., and Eddouks, M. (2022). Traditional uses, pharmacological, and phytochemical studies of *Euphorbia*: A review. *Current Topics in Medicinal Chemistry*, 22(19), 1553-1570.
5. Bakht, J., Shafi, M., and Iqbal, A. (2017). In vitro antibacterial and antifungal activities of different solvent extracted samples from the stem of *Euphorbia heliscopia*. *Pakistan Journal of Pharmaceutical Sciences*, 30(4), 1371-1377.

6. Beltagy, A. M. (2019). Chemical composition and antibacterial activity of medicinally useful essential oil from the inflorescence of *Euphorbia helioscopia* L. grown in Egypt. *International Journal of Pharmaceutical Sciences and Research*, 10(8), 3660-7.
7. Deshpande, R. K., Reddy, V. J. S., Jallepalli, V. R., and Dwivedee, M. (2024). Evaluation of acute oral toxicity of aqueous extract of *Euphorbia helioscopia*. 6(si2):552. DOI:10.48047/AFJBS.6.Si2.2024.5527-5539
8. Dou, M., Zhu, D., Cui, G., Li, H., Di, L., & Wang, L. (2024). *Euphorbia helioscopia* L. exhibits promising therapeutic effects on hemangioendothelioma and melanoma through angiogenesis inhibition. *Phytomedicine*, 129, 1556-66.
9. Doughari, J. H. (2012). *Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents* (pp. 1-33). Rijeka, Croatia: INTECH Open Access Publisher.
10. Falana, M. B and Nurudeen, Q. O. (2022). Phytochemical screening and in vitro antimicrobial activities of *Euphorbia Lateriflora* on selected pathogens. *Iraqi Journal of Science*, 1402-1412.
11. Jaouadi, R., Elimem, M., Sayari, N., Kalboussi, M., Lahfef, C., Hammadi, J. and Rouz, S. (2022). Antioxidant and insecticidal properties of *Euphorbia helioscopia* L. Aqueous extracts. *International Journal of Research in Agronomy*. 5(2): 91-94.
12. Kebede, T., Gadisa, E. and Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PloS one*, 16(3). e0249253.
13. Kuppusamy, P., Yusoff, M. M., Parine, N. R. and Govindan, N. (2015). Evaluation of in-vitro antioxidant and antibacterial properties of *Commelina nudiflora* L. extracts prepared by different polar solvents. *Saudi journal of biological sciences*, 22(3), 293-301.
14. Mahomoodally, M. F., Dall'Acqua, S., Sinan, K. I., Sut, S., Ferrarese, I., Etienne, O. K. and Zengin, G. (2020). Phenolic compounds analysis of three *Euphorbia* species by LC-DAD-MSn and their biological properties. *Journal of pharmaceutical and biomedical analysis*, 189, 113477.
15. Mehmood, S., Syed, F., Khan, M. I., & Aadil, R. M. (2021). Bioactivity evaluation and phytochemical screening of *Euphorbia helioscopia* and *Rumex dentatus*. *Food Science and Technology*, 42, e66721.
16. Rafiq, S., Qadir, S., Wani, I. H., Ganie, S. A., Masood, A., & Hamid, R. (2014). Purification and partial characterization of a fructose-binding lectin from the leaves of Euphorbia helioscopia. *Pak. J. Pharm. Sci*, 27(6), 1805-1810.
17. Saleem, U., Saleem, M., Ahmad, B., Hussain, K., Ahmad, M., Bukhari, N. I., & Anjum, A. A. (2015). In-vitro antimicrobial susceptibility testing of leaves methanol extract and latex of *Euphorbia helioscopia* using agar well diffusion and broth dilution methods. *The Journal of Animal and Plant Sciences*. 25(1):261-267.
18. Singh, M., Rani, A., & Gupta, D. K. (2018). *Euphorbia helioscopia* L: past, present and future prospects. *Bulletin of Pure & Applied Sciences-Botany*. 37(1), 20-26.
19. Singh, M., Rani, A., & Gupta, D. K. (2023). *Euphorbia helioscopia*: Past, Present and Future Prospects. *Advances and Challenges in Science and Technology*. 84.
20. Valgas, C., Souza, S. M. D., Smânia, E. F., & Smânia Jr, A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian journal of microbiology*, 38, 369-380.
21. Waheed, K., Muhammad, S. K., Shomaila, A., Muhammad, Z., Izhar, U., & Ullah, S. (2020). Antimicrobial activity and phytochemical screening of *Euphorbia helioscopia*. *Planta daninha*, 38, e020213727.
22. Wang, W. P., Jiang, K., Zhang, P., Shen, K. K., Qu, S. J., Yu, X. P. and Tan, C. H. (2018). Highly oxygenated and structurally diverse diterpenoids from *Euphorbia helioscopia*. Phytochemistry, 145, 93-102.
23. Yang, Y., Chen, X., Luan, F., Wang, M., Wang, Z., Wang, J., & He, X. (2021). *Euphorbia helioscopia* L.: A phytochemical and pharmacological overview. *Phytochemistry*. 184, 112649.

24. Zhu, Q., Jiang, M. L., Shao, F., Ma, G. Q., Shi, Q., & Liu, R. H. (2020). Chemical composition and antimicrobial activity of the essential oil from *Euphorbia helioscopia* L. *Natural Product Communications*. 15(9), 1934578X20953249.