# Antibacterial activity of *Plantago lanceolata* and *Helichrysum stoechas* extracts on *Escherichia coli* and *Pseudomonas aeruginosa*

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**Abstract:** The use of herbal medicinal products has recently increased because of bacterial resistance to conventional antibiotics. *Plantago lanceolata* and *Helichrysum stoechas* used in traditional medicine due to their medicinal properties. This current study aimed to perform perform the *in vitro* antibacterial activity of the extracts obtained from the fresh leaves of *P. lanceolata* and dry *H. stoechas* against Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* isolated from patients by studying the inhibition of the growth of the pathogen *E. coli* and *P. aeruginosa*. The antibacterial activity of the ethanolic and aqueous decoction extracts of the plants was examined. The results indicated that there was no antibacterial activity of *P. lanceolata* and *H. stoechas* observed against Gram-negative bacteria used in this study. Therefore, further investigations and future experiments need to be carried out to determine the effect of these herbal extracts on other types of bacteria.

***Keywords****: Plantago lanceolata, Helichrysum stoechas, Plant extraction, Soxhlet, antimicrobial activity.*

## 1. Introduction:

Medicinal plants contain several compounds that have a role in protecting the plant. These compounds are effective against microorganisms that cause diseases. Pathogenic microorganisms, whether bacteria or pathogenic microscopic fungi are eliminated using antibiotics. However, the production of microbes has increased over the last decades and bacteria resist antibiotics after periods of time, thus their effectiveness decreases. To solve this problem, antibiotics must be developed over periods of time by adding new active substances that are effective in eliminating those microbes. Medicinal plants are one of the sources of antibiotics, it has been tested in Latin America 122 medicinal plants, and it was documented that among the compounds extracted from these plants, twelve had an inhibitory effect on the bacteria *Staphylococcus aureus* and ten species had an inhibitory effect against *Escherichia coli* [1] [2].

The *Plantago lanceolata* and *Helichrysum stoechas* are among the herbs and plants that have been used and are still being used widely in folk medicine. *P. lanceolata* is a narrow-leaved plantain [3], and one of more than 250 species of the genus *Plantago* L. (Plantaginaceae) with a cosmopolitan distribution and the most important one [4], common in roadside grassland [5], with a mass of surface fibrous roots and frequently a few deep roots. Furthermore, *P. lanceolata* leaves have very wide medicinal use in a mixture with other herbs, for example, *Thymi herba and Salviae folium* and other [6].

Studies have confirmed that some *Plantago* species have substantial anti-inflammatory, antioxidant activities and antiviral [7, 8]. Moreover, Phytochemical studies have revealed that the genus *Plantago* contains a higher amount of phenolic compounds (flavonoids and tannins). Specifically, phenolic compounds appear to play a possible role in the control growth of bacteria and diminishing growth, and virulence of pathogenic oral flora [9]. In addition, it has been showed that methanol and water extracts of *P. lanceolata* are abundant in phenolic acids, with the benzoic acid derivatives hydroxybenzoate and 3,4,5-trihydroxybenzoate (Gallic acid) being the most represented [7]. The antimicrobial activity of *P. lanceolata* has been studied against *Streptococcus* species and *Lactobacillus* which demonstrated good antimicrobial activity *in vitro* [10]. Different species of *Plantago* were studied for example, a study by Monawer and Mammani 2023 [11], used different concentrations of the ethanolic extract of *P. major* leaves and showed different zones of inhibition against *P. aeruginosa*, this bacterium displayed a high level of resistance to the antibiotics, and the greatest inhibitory effect was using the 100% ethanolic extract *P. major*.

The genus *Helichrysum* consists of up to 600 species of flowering plants belonging to the Asteraceae family. *Helichrysum* genus are typically aromatic, perennial shrubs, with thick leaves and hardy flower heads that are dispersed all over. It has been shown that *Helichrysum* plants are rich in phenolic compounds, flavonoids, phloroglucinols, and pyrones, also some species contain terpenes [12; 13]. *Helichrysum stoechas* (L.) belonging to the family

Asteraceae, is common in the Mediterranean area, north-western Africa, eastern Turkey, southern India, Sri Lanka, and Australia, and the plants grow best in a neutral soil pH [14; 15; 16; 17].

*H. stoechas* has demonstrated anti-α-glucosidase, and antioxidative properties [18], and has antimicrobial activity against *E. coli* [17]. The microorganisms gram-negative bacteria *E.coli* and *P. aeruginosa* are the most abundant facultative bacterial species in the normal microbiota of the large intestine of humans [19; 20], and are among the most antibiotic-resistant types of bacteria. These bacteria cause urinary tract infections which is one of the most common infections. Increasing antibiotic resistance leading to increased interest in the development of new types of effective antimicrobial compounds. Although the great interest in the genus *Helichrysum* and *Plantago*, little is known about the effective antimicrobial compounds of *H. stoechas* and *P. lanceolata* in Libya.

The present study aims to determine the antimicrobial activity of the ethanol and aqueous decoction extract of *P. lanceolata* and *H. stoechas* plant species used in Libyan traditional medicine from the Sirte City region of Libya against two types of Gram-negative bacteria that cause diseases, especially for people suffering from urinary tract infections.

## 2. Materials and Methods:

**2.1 Plant collection:**

The fresh aerial parts of *Plantago lanceolata* (Figure 1 A) were harvested in February (2021) from the garden of the Faculty of Science, University of Sirte, Sirte, Libya. The *Helichrysum stoechas* plant (Figure 1 B) was purchased as a dried powder at the local market in Sirte city, Libya.

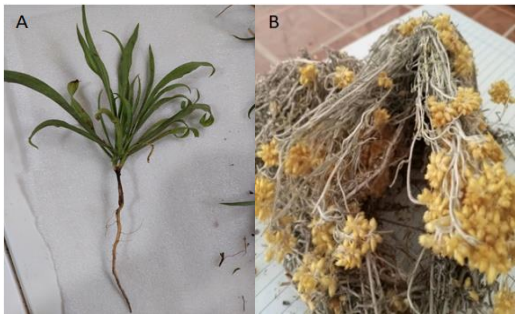


**Figure 1***:* plants used in this study (A) *P. lanceolata* and (B) *H. stoechas*

**2.2 Drying and grinding plants:** The fresh aerial parts of *P. lanceolata* were washed and dried in a shady place at room temperature after spreading on paper, taking into account constant stirring to ensure good drying for 9 days. It is then ground with a blender after removing the hard wood parts to obtain the plant in powder form, stored in a sterile plastic container and kept in a dark place until the extraction process is performed. For the *P. lanceolata* plant, only the leaves are used, while for the *H. stoechas* plant, the leaves, stem and flowers are used together in powder form [21].

**2.3 Plant extraction:** The powdered forms of *P. lanceolata* or *H. stoechas* were extracted by the following methods:

**2.4 Soxhlet extraction:** 10 grams of dry plant powder (*P. lanceolata* or *H. stoechas*) was placed in a conical flask containing 200 ml of 70% ethyl alcohol plugged with cotton and then kept on a hot plate using the soxhlet extraction technique for 9 hours (Figure 2). The extract is filtered and then the filtrate is evaporated using a rotary vacuum evaporator under reduced pressure to obtain the crude extract as described in [22] with some modification.

**Figure 2***:* Using soxhlet extraction technique

**2.5 Aqueous decoction preparation:** To prepare the decoction, 20 grams of plant powder (*P. lanceolata* and *H. stoechas*) are soaked in 50 ml of distilled water (DW), boiled at 100°C in a 200 ml Erlenmeyer flask, and allowed to boil for 10 minutes. The extract is cooled and filtered through Whatmann No.4 filter paper to get pure extract which was stored in the refrigerator in a clean, sterilized bottle until use. The concentration of the aqueous decoction (20 g/50 ml dw) was about 4 x $10^5$ ppm [22].

**2.6 Preparation media to determine the antibacterial activities of studied plants:** Gram-negative bacterial *Escherichia coli* and *Pseudomonas aeruginosa* were used. Bacteria were obtained from a laboratory. Swab samples were collected from patients at the Specialized Clinic laboratory in the City of Sirte, Libya. Both bacteria were cultured and prepared for microbiological identifications. The clinical isolates were identified by standard morphological, cultural and biochemical profile in the Specialized Clinic laboratory.

After microbiological identifications, both bacteria were grown on Nutrient Agar at 37°C for 24 h in the laboratory of the Faculty of Science at Sirte University. The bacteria were inoculated onto Nutrient and MacConkey agar to isolate *P. aeruginosa*.

According to previously published research, with some modifications [23, 24, 25]. The alcoholic crude extract was considered to be 100% basic concentration. From each plant extract, a basic stock solution was prepared by dissolving 200 mg of the crude extract in 1 ml of distilled and sterile water.

The antibacterial effect of the plant extract was evaluated using the disk inhibition zone method, and the antimicrobial activity was measured based on the diameter of the inhibition zone formed around the disk.

Antibiotic susceptibility testing was performed using the (Bauer & Kirby) Disc diffusion assay method using the cylindrical plate method or cup and plate method (well diffusion). Antibiotic Amikacin disc (AK) 6mm (bioanalyse) was used at a concentration of 30µg/disc as a positive control or antimicrobial (antimicrobial susceptibility test tablets).

The disc diffusion assay was used and the discs with a diameter of 6 mm from the filter papers were immersed in both ethyl alcohol and aqueous decoction extracts of *P. lanceolata* and *H. stoechas* plants, as well as in sterile distilled water, negative control, for an hour, then left it to dry. Antibiotic Amikacin disc (AK) 6mm (bioanalyse) was used at a concentration of 30µg/disc as a positive control or antimicrobial (antimicrobial susceptibility test tablets). Bacterial colonies were isolated from the stock bacteria plate into a new petri dish containing nutrient agar culture medium to obtain separate and pure colonies.

After that one bacterial colony was taken from a stock that was isolated the day before using a sterile cotton swab. The bacteria used in the study were spread and distributed well on the solidified medium prepared in advance in a petri dish and left for about 15-30 minutes. The Nutrient agar plates were divided into four equal portions. Each portion has added the Amikacin antibiotic disc as the positive control and sterile distilled water as the negative control, in addition to the extract of the two plants *P. lanceolata* and *H. stoechas*.

Furthermore, the nutrient agar plates were divided into four equal portions. Then with the help of a sterile borer, it made three cavities one in each of three portions, a diameter of 6 mm. Then two cavities were filled with extract plants solution, and one cavity was added sterile distilled water for negative control. In portion four antibiotic disc was added as positive control. Slowly the plates were incubated at a temperature of 37ºC for 24 hours. For the cylindrical plate method or cup and plate method, wells 6 mm in diameter were made on the agar in a sterile way in which 100 µL of the extract was carefully dispensed into each well [25] .

## 3. Results:

In this study, the antibacterial activity of ethyl alcohol and aqueous decoction extracts of the *P. lanceolata* and *H. stoechas* against bacterium *E. coli* and *P. aeruginosa* were performed using the (Bauer & Kirby) Disc diffusion assay method and using cylindrical plate method or cup and plate method.

The results of the current study showed that ethyl alcohol (Figures 3 and 4), and aqueous detection (Figures 5 and 6) of *P. lanceolata* extract and *H. stoechas* extract had no inhibitory antimicrobial effects on the growth of both bacteria *E. coli* (Figure 3 and 5) and *P. aeruginosa* (Figures 4 and 6) using Disc diffusion assay method. Moreover, in Figures 7 and 8 use the cylindrical plate method or cup and plate method. The antibiotic used as a control showed a clear effect when concentrated (30µg/disc) on the two types of bacteria used in this study.

In summary, this study showed no inhibition zone with the concentration and amount of plants *P. lanceolata* and *H. stoechas* against *E. coli* and *P. aeruginosa* bacteria. This study showed that there was no inhibition zone at the concentration used and indicated in the material and method, therefore no more low concentrations were used.

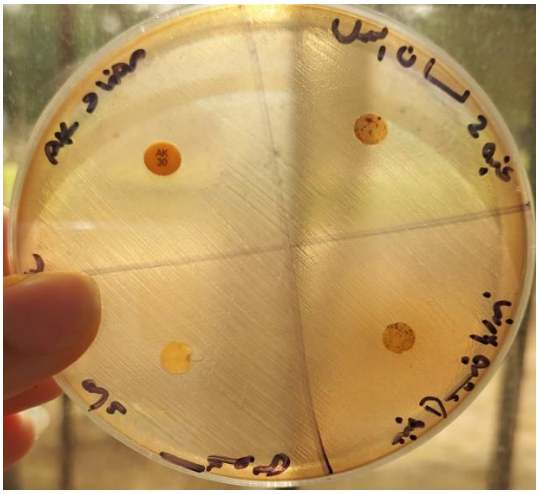

**Figure 3**: Antimicrobial effect of *P. lanceolata* and *H. stoechas* alcoholic extracts on the growth of *E. coli* bacteria (disc diffusion and control).

**Figure 4**: Antimicrobial effect of *P. lanceolata* and *H. stoechas* alcoholic extracts on the growth of P. *aeruginosa* bacteria (disc diffusion and control).

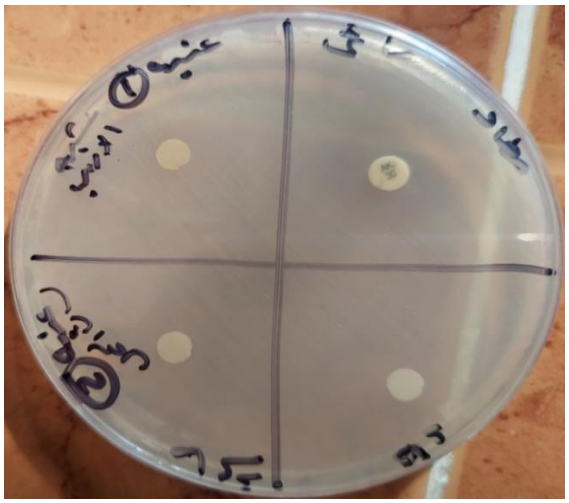

**Figure 5:** Antimicrobial effect of *P. lanceolata* and *H. stoechas* aqueous extracts detection on the growth of *E. coli* bacteria (disc diffusion and control).

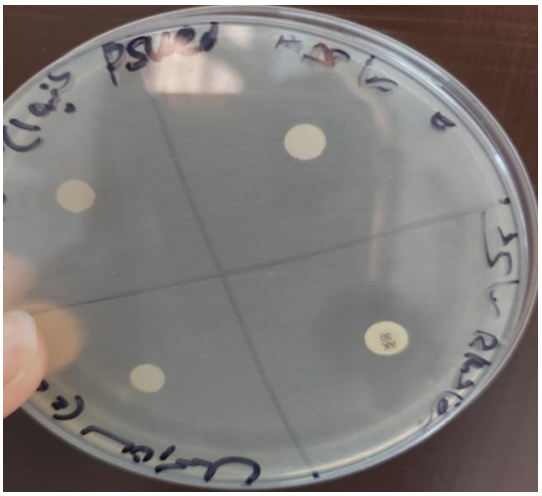

**Figure 6**: Antimicrobial effect of *P. lanceolata* and *H. stoechas* aqueous extracts detection on the growth of *P. aeruginosa* bacteria (disc diffusion and control ).

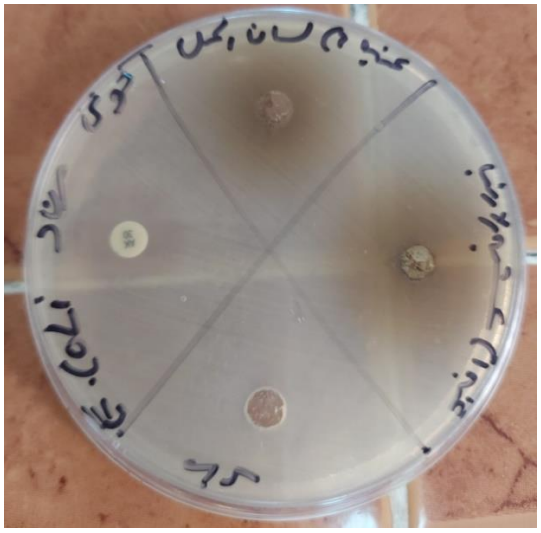

**Figure 7:** Antimicrobial effect of *P. lanceolata* and *H. stoechas* aqueous extracts detection on the growth of *E. coli* bacteria (well diffusion and control).

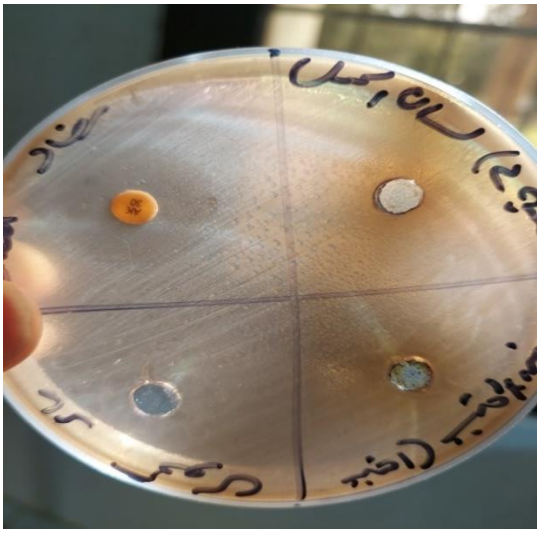

**Figure 8**: Antimicrobial effect of *P. lanceolata* and *H. stoechas* aqueous extract detection on the growth of *P. aeruginosa* bacteria (well diffusion and control ) .

## 4. Discussion:

It is necessary to determine the antimicrobial activity of selected medicinal plants as a very important source of drugs. Moreover, although the increasing interest in the medical effects of extracts herbal, there exists a lack of scientific data on the biological activities and the nature of the active compounds of some plant extracts.

In this study, the concentration and amount of plants *P. lanceolata* and *H. stoechas* collected from region of Libya, and extract used of the ethanolic and aqueous decoction extract, as described in the material and methods, showed no antimicrobial activities against *E. coli* and *P. aeruginosa*.

It has been shown that *P. lanceolata* aqueous extract has low or moderate antimicrobial activities against *S. aureus*, *S. epidermidis*, *P. vulgaris*, and *S. arcescens*. However, the methanolic extract of *P. lanceolata* showed no significant antibacterial activity against the mentioned bacteria [26]. In another study, the *P. lanceolata* root extracts showed suitable antibacterial effects against *Proteus vulgaris*, *Bacillus cereus*, and *Salmonella paratyphi* [27]. In a study by Abbasi *et al* (2022) [28] water, ether, and chloroform extract were used to detect the antibacterial potentials of *P. lanceolata* and *P. major* extracts against *P. aeruginosa.* The results indicated a remarkable antibacterial activity of the crude extracts compared to other fractionated plant extracts. and using water extract shows an inhibition

zone against *P. aeruginosa* but by using the Petroleum ether and chloroform did not show any inhibition activity.

Despite several investigations on the antimicrobial activity profiles of different *Helichrysum* species, the effect on *E. coli* O157:H7 has not until now been evaluated in detail [29]. It has been observed in the study by Rios 1991[30] where the use of *H. stoechas* herb no activity was observed against Gram-negative bacteria. In this study, antibacterial effects were not observed for *P. lanceolata*. This result was similar finding to the study by Orhan *et al*, (2002) [31], the antibacterial activity of an ethanolic extract from *P. lanceolata* was investigated by agar diffusion and microdilution methods using *E. coli* and *P. aeruginosa* and showed no antibacterial effects were observed for *P. lanceolata.* Moreover, a study by Molnár *et al*. 1989 [32] showed only weak antibacterial effects on *E. coli* regarding single compounds of *P. lanceolata* exerted. The methods for drying plant material or grinding the plants with a blender to obtain the plant in powder form may cause a loss of value products of the plants [33]. A study by Tamura and Nishibe (2002) [34] reported that phytochemicals in *P. lanceolata* are sensitive to drying treatments.

There have been more studies using different *Helichrysum* species for their antimicrobial activity, especially of methanol extracts against a wide range of test microorganisms including E. *coli* and *P. aeruginosa*, which reported that the methanolic extracts had antibacterial activity against *P. aeruginosa*, and *S. aureus*. However, no activity was found against *E. coli* [35]. Some other different studies used different species such as a study by Sobhy and El-Feky, 2007 [17] determined that the antimicrobial activity of the ethanol extract of *H. stoechas* (L.) D.C. collected from the Green Mountain region of Libya, had potent inhibition activity on gram-negative bacteria such as *Klebsiella pneumonae*, *Enterobacter coleacae*, *P. aeruginosa* and *E. coli*. In contrast, had moderate antimicrobial activity on *S. aureus*, however, it did not affect on *S. aureus*, *Staphylococcus epidermis* and *Staphylococcus citrus*. Furthermore, the observation that certain organisms tolerated essential oils but were susceptible to an ethanol extract or, on the contrary, tolerated alcoholic extracts but were susceptible to essential oils, strongly supports the traditional medicinal uses of *H. stoechas* as a complete crude extract. In addition, it has been shown that Gram-positive bacteria were more sensitive to essential oils from herbs and inflorescences in comparison with Gram-negative bacteria [36].

Because of the different *Helichrysum* species and *Plantago* species used and the different methods used in many studies for the evaluation of antimicrobial activities, it is difficult to compare the results of different studies. Most studies approved that the herbs that are used as traditional medicinal are safe and effective, but up to now, studies have not been completed on the toxicity of these herbal extracts or their antibiotic activity.

Bacteria have the genetic ability to transmit and get resistance to antibiotics, which are used as therapeutic factors [37]. Bacterial strains are multi-resistant; this problem is increasing, and the outlook for the use of antimicrobial antibiotics in the future is still unclear. Developing research to better understand the genetic mechanisms of resistance and continuing studies to develop new antibiotics, especially natural ones, to offer appropriate and efficient antimicrobial antibiotics.

It has been reported that ineffective of the methanol extract from *Helichrysum compactum* Boiss against *E. coli* O157:H7 [38]. This may be because of the low flavonoid content of *H. compactum* as described by Süzgeç *et al*, 2005 [39]. This may be a similar reason there are no effective in this study or due to aggressive bacteria taken from patients. However, further purification and testing should be performed on the active roots and leaves or all plants in both herbs, ethanol extract, or other extracts to identify the major active ingredient(s) responsible for its antimicrobial activity.

## 5. Conclusion:

The ethanol and water extracts from the leaves of *P. lanceolata* collected from Sirte City and dry *H. stoechas* showed an inefficacious effect against *E. coli* and *P. aeruginosa* in the present study. The difference in the antimicrobial features of specific species may be due to the geographical region of the plant growth or age of the plant, the organic solvent used or the method of extract, and the existence of various antibacterial secondary metabolites. However, further studies, particularly in bacterial infection are needed to establish conclusive evidence of the effectiveness of herbs used in this study extract against urinary tract infections, either alone or in combination with conventional therapies.

## Conflict of interest

The author declares that there is no conflict of interest.

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