AUCTORES
Globalize your Research

Journal of Nutrition and Food Processing

Sara Thamer Hadi *

Open Access

Research Article

Investigation into the impact of certain leaf extracts derived from Inula viscosa L. on various strains of pathogenic bacteria in poultry

Hasan Y Hoseen [1], Dr. Tawafik Dalla [2], Dr. Fahim Abd Al Aziz [3], Samer Younes [4*]

[1]Postgraduate Student (PhD), Department of Animal Production, Faculty of Agriculture, Tishreen University, Lattakia - Syria.

[2]Professor, Department of Animal Production, Faculty of Agriculture, Tishreen University, Lattakia, Syria.

[3]Professor, Department of Laboratory Medicine, Faculty of Human Medicine, Tartous University, Syria.

[4]Bachelor degree student, Department of Pharmacy, Tartous University, Syria.

***Corresponding author:** Samer Younes, Department of Pharmacy Tartous University Syria.

Received date: June 25, 2024; **Accepted date:** July 17, 2024; **Published date:** August 05, 2024

Citation: Hasan Y Hoseen, Tawafik Dalla, Fahim A. Al Aziz and Samer Younes, (2024), Investigation into the impact of certain leaf extracts derived from Inula viscosa L. on various strains of pathogenic bacteria in poultry, *J. Nutrition and Food Processing*, 7(9); **DOI:10.31579/2637-8914/241**

Abstract:

The study was designed to investigate the efficacy of acetone, ethanol, hexane, diethyl ether, and aqueous extracts from clay plants against four pathogenic bacteria commonly found in poultry: Escherichia coli, Streptococcus aureus, Pseudomonas aeruginosa, and Clostridium perfringens. The research took place in the microbiology and drug laboratories of the Faculty of Pharmacy at the University of Tartous, as well as the microbiology laboratory at the Faculty of Agricultural Engineering at Tishreen University. Tayon plant leaves were harvested in October and November of 2021 from the Safita region, dried, and stored for future use.

The susceptibility tests were carried out on the four bacteria under investigation using the five different extracts. The findings revealed that Escherichia coli, Streptococcus aureus, Pseudomonas aeruginosa, and Clostridium perfringens were all impacted by the extracts, except for Escherichia coli which displayed resistance to acetone, ethanol, hexane, and diethyl ether extracts, but not to the aqueous extract. Pseudomonas aeruginosa also exhibited resistance to the diethyl ether extract at concentrations of 20 and 40 µl, with no inhibition zone observed.

At a concentration of 80 µl, the largest average diameter of the bacterial growth inhibition zone was observed for the acetone extract (4.82, 24.95, 15.41, and 22.14 mm), ethanol (9.32, 28.11, 22.98, and 29.24 mm), hexane (6.42, 18.91, 13.23, and 17.47 mm), diethyl ether (9.33, 21.22, 9.56, and 18.75 mm), and aqueous extract (23.45, 26.22, 15.71, and 24.12 mm), respectively, in comparison to concentrations of 20 and 40 microliters and the control.

These results suggest that the plant leaf extracts possess antimicrobial properties against the pathogenic bacterial strains tested, indicating their potential as natural sources of antibiotics in the future.

Key words: flaxseed; Linum usitatissimum L; alpha-linolenic acid; omega-3 fatty acids; lignans; secoisolariciresinol diglucoside; dietary fibre, cardiovascular health; cancer; gut health; brain function; menopause

Introduction

Numerous medicinal plants are of significant interest due to their potential as sources of natural products. These plants have been explored as alternative therapies for various pathogens and as food preservatives, indicating the presence of active microbiological components (Panovska et al., 2005). The threat posed by germs and fungi to health, along with their role in weakening immunity, underscores the critical need for effective and affordable antimicrobial agents (Rasooli and Mirmostafa, 2003). Conversely, the overuse of antibiotics has led to the development of resistance mechanisms by bacteria and fungi against both existing and novel antimicrobial agents (Pareke and Chanda, 2007), necessitating the discovery of new treatments. Plants have historically been and continue to be primary sources of such treatments, with an estimated 50% of

medicinal products in Europe and the United States derived from natural sources, including plants and their derivatives (Cordell, 2002; Newman et al., 2003). Medicinal and aromatic plants have long been utilized in the treatment of various ailments, with their medicinal properties often discovered through experimentation, and a significant portion of the population relying on them for traditional medicinal practices (Stanley and Luz, 2003; Mathias et al., 1996). The extraction of plant-based compounds has played a crucial role in the development of many commonly used drugs today (Simpson and Ogorzaly, 2001). These plants produce a wide array of biologically active molecules that exhibit antibacterial properties and inhibit the growth of microbial pathogens (Oskay et al., 2009). Inula viscosa L., found in various coastal and inland regions of Syria and belonging to the Compositae Family, has been recognized for its medicinal properties for an extended period (Pelletier, 1992). Research has demonstrated that extracts of Inula viscosa L. possess antioxidant properties (Chevolleau et al., 1992) and contain chemical compounds such as triterpenoids (Grande et al., 1985), flavonoids (Ravi Kant, 2010), and sesquiterpene lactones (Grand, 1992) that are essential in the treatment of numerous diseases.

Prior research on extracts from Inula viscosa L has demonstrated effectiveness against both gram-negative bacteria like Bacilli coli and aeruginosa, as well as gram-positive bacteria such as Staphylococcus (Chourasia, 1987; Ali-winter, 1998). Inula viscosa L extracts are rich in various active compounds, including sterols, bioflavonoids, and tomentosin, each possessing unique bioantibiotic properties (Wichtlm, 2004; Bisset, 1994).

In addition to its antibacterial properties, Inula viscosa L is utilized for various purposes such as muscle relaxation (Kaileh et al., 2007; Talib and Mahasneh, 2010; Hudaid et al., 2008), as an antitumor agent (Talib et al., 2010), an antioxidant (Schinella et al., 2002), for diabetes treatment (Yaniv et al., 2010), and as an anthelmintic (Oka, 2001; Talib and Mahasneh, 2010; Afifi-Yazar et al., 2011).

The importance of the research:

The research conducted in this study is of great significance as it focuses on the utilization of extracts derived from Inula viscosa L. (aerial parts) to effectively combat certain types of intestinal bacteria in poultry. Furthermore, the study aims to investigate the potential advantages of incorporating the leaves of Inula viscosa L., which contain safer compounds, in order to enhance the health of both birds and consumers. These compounds have been discovered to not only improve productivity and promote weight gain in birds, but also stimulate their immune system. Consequently, the birds exhibit heightened resistance to diseases, resulting in decreased mortality rates. This positive impact on the health and well-being of the birds also carries economic implications, as it enhances the feasibility of poultry production. The primary objective of this research is to validate these findings and provide scientific recommendations that can contribute to the ongoing efforts in combating specific diseases.

Research Objectives:

The main aim of this study is to assess the efficacy of Inula viscosa L. in suppressing the proliferation of bacteria that lead to intestinal infections in poultry.

Materials and Methods:

Sample Collection:

Inula viscosa L. plants were collected from the Safita region in Tartous Governorate during October and November 2021. The leaves were carefully harvested and washed with distilled water. Subsequently, they were dried in the shade for a period of two weeks. Once dried, the leaves were ground using a mortar and pestle, and the resulting powder was stored in bags at a temperature of 4°C until the extraction process was conducted the following day.

Testing Locations:

The experiments were carried out at the Microbiology Laboratory in the Faculty of Agricultural Engineering at Tishreen University, as well as the Laboratory of Pharmacognosy and Microbiology in the Faculty of Pharmacy at Tartous University.

Extraction:

To obtain various extracts of the solvent, a 10 g portion of dry powder was placed in a 500 ml container. Subsequently, 200 ml of the solvent was added to produce five distinct extracts: estone, ethanol, hexane, diethyliter, and aqueous extract. The mixture was then filtered using filter papers. Following this, the extract underwent a drying process using a Rotary Evaporator to evaporate the solvent. The resulting dry extracts were carefully stored in a freezer at a temperature below -20 °C until the microbial activity testing was conducted.

Pathogenic germs:

Three different types of pathogenic germs were utilized in the study. Firstly, Clostridum perefrings, an anaerobic, gram-positive, sporident bacterium commonly found in various environments (Johansson, 2006). Secondly, Escherichia coli, a gram-negative, aerobic, and mobile bacterium (Johnson et al., 2008; Oakley et al., 2014). Lastly, Streptococcus aureus, a gram-positive, non-motile, non-sporogenic, non-capsular constituent, anaerobic bacterium (Kloos and Bannermarr, 1994). Additionally, Pseudomonas auroginosa, a gram-negative, motile, aerobic bacterium, was also included in the study (Krylov, 2014).

The effect of the extracts on the pathogenic germs:

The impact of the inula viscosa leaf extracts on pathogenic germs was assessed through a tablet propagation method, as described by Sengul et al. (2009). Each extract, dissolved in a 5% sulfoxide Dimethyl (DMSO) solution, was absorbed onto 6 mm filtration tablets using three different concentrations (20, 40, and 80 µL). These tablets were then allowed to dry at room temperature. As a control, filtration tablets impregnated with a 5% sulfoxide Dimethyl (DMSO) solution were used without the addition of any of the four extracts.

For each of the three pathogenic microbial species, a bacterial suspension was prepared. A cotton swab was used to transfer 0.5 ml of the suspension onto the surface of Mueller Hinton agar medium. After 20 minutes, the tablets impregnated with the extracts were carefully placed on the culture medium using sterile forceps. The plates were then incubated at 37 °C for a period of 24 to 48 hours.

Measurement of inhibitory zones:

Once the incubation process has concluded, the presence of a distinct region devoid of any bacterial growth surrounding the filtration tablets serves as undeniable proof of germ inhibition and the efficacy of Inula viscosa leaf extracts. The potency of the extract is assessed based on the diameter of the surrounding area influenced by its effects. A larger diameter indicates a more effective extract. To measure the inhibition diameters, a graduated ruler is employed, and four samples of each extract are utilized in every experiment.

Results and discussion

The impact of the five extracts on the four types of bacteria varied depending on the specific extract and its concentration, as well as the bacterial strain. Table (1) reveals that the acetone extract had no effect on E. Coli bacteria at concentrations of 20 and 40 µL. However, at a concentration of 80 µL, it resulted in the largest average diameter of the bacterial growth area, with an inhibition diameter of 4.82 mm.

On the other hand, S. Aureus, P. AurGinosa, and C. Perferses were all affected by the acetone extract at all three concentrations used, with the highest average diameter of the bacterial growth area observed at a concentration of 80 µL. The inhibition diameters for these bacteria were

24.95 mm, 15.41 mm, and 22.14 mm, respectively, compared to the control.

In the case of the ethanolic extract of Inula viscosa, the inhibition area diameter for E. coli was 9.32 mm at a concentration of 80 µL. For S. aureus, P. auroginosa, and C. perfringes, the inhibition areas were 28.11 mm, 22.98 mm, and 29.24 mm, respectively, surpassing the 20 and 40 µL concentrations as well as the control.

When the hexane extract was used at concentrations of 20, 40, and 80 µL, it affected all four bacteria except for E. coli. The concentrations of 20 and 40 µL did not have any impact on E. coli, and there was no inhibition area observed around the tablet. However, at a concentration of 80 µL, it resulted in the highest average inhibition diameter for all four bacteria, with inhibition diameters of 6.42 mm, 18.91 mm, 13.23 mm, and 17.47 mm, respectively, compared to the control treatment of DMSO 5%.

In the case of the diethyleter extract, the concentration of 80 µL had the greatest effect on the average diameter of the bacterial growth inhibition area, surpassing the 20 and 40 µL concentrations. The average diameter of the bacterial growth inhibition area for E. coli, S. aureus, P. auroginosa, and C. perfringes was 9.33 mm, 21.22 mm, 9.56 mm.

Clostridium Perfringes	*Pseudomonas auroginosa*	*Streptococcus aureus*	*Escherichia coli*	**Germs** / **Extracts**	
18.84	10.12	***21.33	R**	20*	Acetone
20.81	11.30	23.12	R	40	
22.14	15.41	24.95	4.82	80	
24.02	18.97	23.67	R	20	Ethanol
25.87	20.14	25.45	R	40	
29.24	22.98	28.11	9.32	80	
13.31	9.32	15.24	R	20	Hexane
14.81	11.01	17.32	R	40	
17.47	13.23	18.91	6.42	80	
14.11	R	17.82	R	20	Diethyl ether
16.21	R	19.21	R	40	
18.75	9.56	21.22	9.33	80	
20.83	11.21	22.51	17.11	20	Aqueous extract
22.91	12.31	24.12	21.24	40	
24.12	15.71	26.22	23.45	80	
R	R	R	R	%5	control**** (DMSO)

*Concentrations of extract used in microliters,** R= Resistance,*** Average diameter of growth inhibition ring in mm ,****) Control (DMSO): addition sulfoxide Dimethyl %5 Without adding any of the five extracts used.

Table 1: The mean effectiveness of allium plant extracts in hindering the growth of the tested bacteria was evaluated.

The data presented in Table (1) demonstrates that the aqueous extracts exhibited an impact on both Gram-positive and Gram-negative pathogenic bacteria across all three concentrations tested, with no observed resistance. Notably, the concentration of 80 µL resulted in the most significant effect on the average diameter of the bacterial growth inhibition zone, surpassing the effects of the 20 µL and 40 µL concentrations. For instance, the average diameter of the bacterial growth inhibition zone was measured at 23.45 mm for Escherichia coli, 26.22 mm for Streptococcus aureus spores, 15.71 mm for Pseudomonas aeruginosa, and 24.12 mm for Clostridium perfringens spores in comparison to the control.

This variation in average inhibition diameters may be attributed to factors such as the type of extract, its concentration, and the specific strains of bacteria utilized. Additionally, the timing of the collection of inula viscosa leaves may also play a role in this discrepancy, as leaves harvested in June and September are known to contain higher levels of active compounds compared to other months of the year (Wang et al., 2004).

It should be noted that gram-negative bacteria exhibited less sensitivity to gram-positive bacteria. This difference in sensitivity may be attributed to the variation in the composition of their cell walls. Additionally, gram-positive bacteria and fungi were found to be more susceptible to plant extracts compared to gram-negative bacteria. The effectiveness of these extracts in inhibiting germ growth could be attributed to the presence of flavonoids and terpenes in Inula viscosa, as mentioned by Nostro et al. (2000). Furthermore, saponins, including citrol, which is a chemical compound found in Inula viscosa, were found to cause damage to cell membranes (Liu et al., 2014).

Our findings align with previous studies that have demonstrated the efficacy of ethanolic and aqueous extracts of Inula viscosa against Escherichia coli and Streptococcus aureus. These extracts exhibited both positive and negative antibacterial activity, with the aqueous and ethanolic extracts inhibiting the growth of Streptococcus aureus by 10 and 14.9 mm, respectively, and E. coli by 6 and 6 mm, respectively (Ali-Shtayeh et al., 1998). On the other hand, the mixture of ethylacetate and methanol extracted from Inula viscosa showed inhibitory effects only against Streptococcus aureus, with an inhibition area diameter of 25 mm. No effect was observed on Pseudomonas auroginosa and Escherichia coli (Smadi and Hamed, 2011). Another study reported that the ethanolic extract of Inula viscosa exhibited inhibitory effects on Escherichia coli, Pseudomonas auroginosa, and Streptococcus aureus, with average inhibition diameters of 6, 22, and 14 mm, respectively (Oskay et al., 2009).

Conclusions

The research findings indicated that the extracts derived from the leaves of Inula viscosa possess anti-bacterial properties against both Gram-

negative and Gram-positive pathogenic bacteria. Notably, the gram-positive bacteria exhibited greater susceptibility compared to gram-negative bacteria. For instance, E. coli bacteria displayed resistance at concentrations of 20 and 40 µl against methanol, chloroform, and dichloromethane extracts. The most significant inhibition zone was observed with the methanolic extract at a concentration of 80 µl against S. aureus bacteria, resulting in an inhibition zone of 33.55 mm, followed by C. perfringes with an inhibition zone of 30.74 mm, in comparison with the control group.

Recommendations:

It is recommended to incorporate the extracts of Inula viscosa leaves into poultry feed due to their potential in mitigating the transmission of intestinal diseases, which can have a positive impact on poultry health.

References

1. Ahmad I and Beg ZA. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens. J. Ethnopharmacol. 74: 113-123.
2. Ahmad Tajehmiri, Fahimeh Issapour, Mina Nasiri Moslem, Maryam Tavakoli Lakeh, and Masoud Hassani Kolavani. (2014). In vitro antimicrobial activity of Artemisia annua leaf extracts against pathogenic bacteria, j. advanced studies in biology, 6(3): 93-97.
3. Ali-shtayeh m. S., yaghmour r. M. R., faidi y. R., salem k., and al nuri m. A. (1998). Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. J. Ethnopharmacol. Vol. 60,265-271.
4. Chevolleau S,Deadl And Ucciani. (1992). Determination De I‘activite‘ Antioxydante D‘extraits Ve‘ge‘taux .Revue Francaise De Corps Gras 39.3-8.
5. Darsanaki R.K. and Parsa Lisar., M. (2014). Antimicrobial Potential of Root, Stalk and Leaves of Rheum Ribes. J. Rep. Pharm. Sci. 3(1): 10-13.
6. Grande, M.; Piera, F.; Cuenca, A.; Torres, P.; Bellido, I.S. (1985). Flavonoids From Inula Viscosa. Planta Med. 39, 414–419.
7. Grande, M.; Torres, P.; Piera, F.; Bellido, I.S. (1992). Triterpenoids From Dittrichia Viscosa.Phytochemistry 31, 1826–1828.
8. Liu. L. L., J. H. He, H. B. Xie, Y. S. Yang, J. C. Li, and Y. Zou. (2014). “Resveratrol induces antioxidant and heat shock protein mRNA expression in response to heat stress in black-boned chickens,” Poultry Science, vol. 93, no. 1, pp. 54–62.
9. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolke, R.H., (1995). Manual of Clinical Microbiology, vol. 6th ed. ASM, Washington, DC.
10. Nostro, A., Germano, M.P., D'Angelo, V., Marino, A. and Cannatelli, M.A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology, 30: 379-384.
11. OSKAY M.; OSKAY D. and KALYONCU F. (2009). Activity of Some Plant Extracts Against Multi-Drug Resistant Human Pathogens. Iranian Journal of Pharmaceutical Research. Vol. 8, N°. 4. pp. 293-300.
12. Rasooli, I. and Mirmostafa, S.A. (2003). Bacterial susceptibility to and chemical composition of essential oils from Thymus kotschyanus and Thymus persicus. Journal of Agricultural and Food Chemistry, 51 (8): 2200-2205.
13. Ravi Kant U, Pratibha D, And Shoeb A. (2010). Screening Of Antibacterial Activity Of Six Plant Essential Oils Against Pathogenic Bacterial Strains. Asian Journal Of Medical Sciences 2(3):152-158.
14. SENGUL M.; YILDIZ H.; GUNGOR N.; BULENT C.; ESER Z. and ERCISLI S. (2009). Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pak. J. Pharm. Sci., Vol. 22, N°. 1, pp. 102-106.
15. SMADI I. D. and HAMED O. (2011). Studies Toward Isolation and Identification of Bioactive Substances from Medicinal Plants. Degree of Master of Science in Chemistry, Faculty of Graduate Studies, An- Najah National University, Palestine.
16. Spcies,M.Nigam, R.Pezzani, H.Prasad Devokota, S.Plygun, B. Salhi, and W. Setezer, (2019). Bioactive Compounds and Health Benefits of Artemisia , Journals Sagepub , 14(7).
17. Talib, W.; Mahasneh, A. (2010). Antimicrobial, Cytotoxicity and Phytochemical Screening of Jordanian Plants Used in Traditional Medicine. Molecules, 15, 1811–1824.
18. WANG W.; BEN-DANIEL B. H. and COHEN Y. Control of Plant Diseases by Extracts of Inula viscose. Phytopathology, Vol. 94, N.
19. Pareke, J.; Chanda, S. (2007). In vitro screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. Afr. J. Microbiol. Res.1, 92–99.
20. Cordell, G. (2002). Natural products in drug discovery—Creating a new vision. Phytochem. Rev. 1, 261–273.
21. Newman, D.; Cragg, G.; Snader, K. (2003). Natural products as sources of new drugs over the period 1981–2002. J. Nat. Prod. 66, 1022–1037.
22. Kloos, W,E.and T.L Bannerman (1994). Update on clinical significance of coagulase – negative Staphylococci. clin microbial Rev7:117- 140.
23. Krylov, N. Victor. (2014). Chapter Five - Bacteriophages of Pseudomonas aeruginosa: Long-Term Prospects for Use in Phage Therapy, Advances in Virus Research, Volume 88, Pages 227-278.
24. Oakley, B.B.; Lillehoj, H.S.; Kogut, M.H.; Kim, W.K.; Mauer, J.J.; Pedroso, A.; Lee, M.D.; Collett, S.R.; Johnson, T.J.; Cox, N.A. (2014). The chicken gastrointestinal microbiom. FEMS Microb. Lett. 360, 100–112.
25. Johnson, T.J.; Wannemuehler, Y.; Doetkott, C.; Johnson, S.J.; Rosenberger, S.C.; Nolan, L.K. (2008). Identification of minimal predictors of avian pathogenic Escherichia coli virulence used for rapid diagnostic tool. J. Clin. Microbiol. 46, 3987–3996.

DOI:10.31579/2637-8914/241