International Journal of Science Academic Research
Vol. 05, Issue 04, pp.7281-7284, April, 2024
Available online at http://www.scienceijsar.com

ISSN: 2582-6425

Research Article

ANTIBACTERIAL OF ETHANOLIC AND VARIOUS FRACTIONS LEAVES EXTRACT OF *ARTEMISIA ANNUA* AND INVESTIGATION OF ITS FUNCTIONAL GROUPS USING FTIR TECHNIQUE

***Mays Hassan Obais Alkhalidi**

College of Biotechnology, Department of Genetic-Engineering, Al-Qasim Green University, Iraq

Received 10th February 2024; **Accepted** 25th March 2024; **Published online** 19th April 2024

Abstract

The annual short-day plant known as *Artemisia annua* is a member of the Asteraceae family of living plants. It has a stem that is upright and brownish or violet brown in colour. Despite the fact that it is possible for plants to reach a height of 200 centimetres when they are grown in cultivation, the plant itself does not have any hair and grows naturally between 30 and 100 centimetres tall. A. annua leaves are around three to five centimetres in length and are sliced deeply into two or three leaflets. These leaflets are divided into two or three leaflets. The present investigation focuses on determining the antibacterial capabilities of the fractions that are extracted from Artemisia annua leaves, as well as the bioactive components that are present in the extract. As part of the preparation for FTIR analysis, Peak (Wave number cm-^{l}), (Type of Intensity, Bond and Functional group assignment) were 675.09(Strong, C-Cl, alkyl halides), 692.44(Strong, C-Cl, alkyl halides), 738.74(Strong, =C–H, Alkenes), 813.96(Strong, =C–H, Alkenes), 974.05(Strong, =C–H, Alkenes), 1008.77(Strong, C-F, alkyl halides), 1049.28(Strong, C-F, alkyl halides), 1093.64(Strong, C-F, alkyl halides), 1232.51(Strong, C-F, alkyl halides), 1276.88(Strong, C-F, alkyl halides), 2922.16 (Strong, C-H, Alkane), 1276.88(Strong, C-F, alkyl halides), 1606.70(Bending, N-H, Amide), 1647.21(Variable, C=C, Alkene). The metabolites of Artemisia annua exhibited significant activity against Klebsiella pneumoniae (27.84± 0.45).

Keywords: *Artemisia annua* L., Antibacterial, Investigation, Functional Groups, FTIR Technique.

INTRODUCTION

The annual short-day plant known as Artemisia annua is a member of the Asteraceae family of living plants. It has a stem that is upright and brownish or violet brown in colour. Despite the fact that it is possible for plants to reach a height of 200 centimetres when they are grown in cultivation, the plant itself does not have any hair and grows naturally between 30 and 100 centimetres tall. A. annua leaves are around three to five centimetres in length and are sliced deeply into two or three leaflets. These leaflets are divided into two or three leaflets. The present investigation focuses on determining the antibacterial capabilities of the fractions that are extracted from Artemisia annua leaves, as well as the bioactive components that are present in the extract. Phenolic acids and flavonoids are two examples of compounds that have antioxidant action. Scientists are interested in these compounds because they have the potential to be utilised further. The pharmaceutical, cosmetic, and food industries could all potentially benefit from the discovery of these molecules once they have been refined [7]. There are an unlimited number of truly biologically active antioxidant phenolic compounds that can already be found in the well-known wormwood species. Meanwhile, these compounds include gallic acid, vanillic acid, caffeic acid, and apigenin [8]. Phenolic chemicals are found in a wide variety of plant species, and they have been linked to the prevention of a number of diseases in which oxidative stress plays a significant role. There is a positive correlation between the antioxidant capacity of wormwood and the amount of phenolic and flavonoid chemicals that it contains. The antioxidant, neuroprotective, hepatoprotective, anti-inflammatory, renoprotective, and gastroprotective actions of artemisia, as well as its digestive and antibacterial activities, are among the health benefits that artemisia provides [9]. Researchers are interested in the antibacterial activity of plants since there is a growing resistance among bacteria to treatments that are designed to destroy organisms that are resistant to antibacterial agents. Using the FTIR technique, the purpose of this study is to examine the antibacterial properties of ethanolic and different fractions of the leaves extract of Artemisia annua, as well as its functional groups.

Corresponding Author:** ***Mays Hassan Obais Alkhalidi
College of Biotechnology, Department of Genetic-Engineering, Al-Qasim Green University, Iraq.

MATERIALS AND METHODS

Extraction of Ethanol from Herbs Along with Samples In this particular investigation, the plant components that were utilised were the aerial parts and leaves of Artemisia annua L. plants native to Hillah City in Iraq. At the University of Babylon, the materials were identified according to their taxonomic classification. In a chamber that was completely dark and had no light, the plants were allowed to dry naturally at ambient temperature for eleven days. Once they had dried, they were then pulverised into a powder. At a temperature range of 3–6 degrees Celsius, the dried material, which weighed 1 gramme, was combined with 50 millilitres of 96% ethanol for a duration of 24 hours. Following the filtration of the sample via Whatman filter paper No. 4, the sample was concentrated at a temperature of 35 degrees Celsius. Using 10 millilitres of ethanol, the dried extract was recovered and then stored at a temperature of -18 degrees Celsius until it was used. For the extraction of each extract, three distinct replicates were carried out, and each experiment was carried out in duplicate.

Fourier transform infrared spectroscopy (FTIR) analysis of *Artemisia annua*

A number of data already derived from an FTIR instrument were experimentally run and laboratory processed using

mainly PC-based software in order to capture these FTIR spectra for each of the GLVs. This was actually done in order to record the FTIR spectra of both these types of GLVs. In order to experimentally prepare for FTIR analysis, a suitable small amount of laboratory crushed leaf samples was converted into pellets using KBr, and at the same time a suitable and thin layer was created by physically pressing the studied mixture. In order to collect valid and studied information about the transmission of infrared light, at the same time data was actually collected in the wave number range including 4000 cm−1 to 500 cm^{-1}. Here all experimental samples were subjected to three separate tests, with KBr pellets that were not actually treated serving as a control [10].

Antibacterial Activity

Finding the Lowest Effective Dosage (MIC)

Already standard strains of pathogenic bacteria such as Klebsiella pneumoniae, Escherichia coli, Streptococcus pyogenes, and Staphylococcus aureus have been experimentally screened. They were actually grown in a test tube containing 10 milliliters of experimentally sterilized nutrient broth for twenty-four hours at a temperature of 37 degrees Celsius. All plates were incubated at 37°C for only 24 h. Experimentally, in order to actually verify the shape of the bacteria, an optical microscope was used in the experiment. At the same time, the number of colonies actually belonging to each strain was dispersed in sterile saline and then laboratory adjusted to match the turbidity of the 0.5 McFarland standard, which was laboratory-produced on Mueller-Hinton agar and had a concentration of 1.5 x 108 colony-forming units per milliliter. [11].

Statistical Analysis

Experimentally, laboratory statistical analysis was performed using SPSS 19.0 with the aim of comparing the average values found. The confidence interval was either 95% or 99%. To be considered realistically statistically significant, the p-value must be less than 0.05.

RESULTS AND DISCUSSION

In this research, and based on the peak values present in the infrared spectrum that were actually present in the original samples studied [12, 13], the FTIR spectroscopy technique was actually used in the current study in order to identify these functional groups that were actually present. At the same time, FTIR analysis was performed, and the functional groups of the components were actually separated based on the peaks observed in the studied analysis. The experimentally obtained results revealed the presence of the following functional groups: free alcohol; Intermolecularly and intramolecularly bound alcohol; imine, oxime, ketone or alkene; Stretch phenol and amine. And stretch Amin. Peak (Wave number cm^{-1}), (Type of Intensity, Bond and Functional group assignment) were 675.09(Strong, C-Cl, alkyl halides), 692.44(Strong, C-Cl, alkyl halides), 738.74 (Strong, =C–H, Alkenes), 813.96 (Strong, =C–H, Alkenes), 974.05(Strong, =C–H, Alkenes), 1008.77(Strong, C-F, alkyl halides), 1049.28 (Strong, C-F, alkyl halides), 1093.64 (Strong, C-F, alkyl halides), 1232.51(Strong, C-F, alkyl halides), 1276.88(Strong, C-F, alkyl halides), 2922.16 (Strong, C-H, Alkane), 1276.88 (Strong, C-F, alkyl halides), 1606.70(Bending, N-H, Amide), 1647.21(Variable, C=C, Alkene). Laboratory examination by FTIR showed the presence of the following: free alcohol, alcohol bound between micromolecules, alkane, aromatic compounds, imine, oxime, ketone or alkene, and phenol. It became clear from the results obtained that the chlorophyll molecule is mainly responsible for the clear appearance of the bands in the infrared region. The main reason for this is because the chlorophyll molecule hides other components. The antibacterial activity of Artemisia annua leaf extracts was tested in vitro on four different types of microorganisms. According to (methanol, Ethyl acetate fraction, and Ethanol fraction) recorded 25.84±0.41, 19.00±0.28, and 27.84± 0.45 respectively in Klebsiella pneumoniae. While recorded 20.02±0.29, 15.07±0.24, and 25.43±0.41 for Escherichia coli. Record 24.19±0.38, 18.09±0.27, and 21.26±0.29 Streptococcus pyogenes. While 17.15±0.26, 24.15±0.39, and 26.81±0.38 Staphylococcus aureus in comparison with Rifambin 21.00±0.30 and Bacteracin 23.41±0.31.

Table 1. Fourier transform infrared spectroscopy (FTIR) peak values of solid analysis of *Artemisia annua* .

No.	Peak (Wave number cm^{-1})	Intensity	Corr. Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	675.09	67.825	1.011	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	692.44	69.075	1.098	Strong	C-Cl	Stretch	alkyl halides	600–800
3.	738.74	72.075	2.274	Strong	=C–H	Bending	Alkenes	650-1000
4.	813.96	76.441	2.172	Strong	=C–H	Bending	Alkenes	650-1000
5.	974.05	65.287	0.687	Strong	=C–H	Bending	Alkenes	650-1000
6.	1008.77	54.765	7.948	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1049.28	58.347	2.245	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1093.64	64.409	3.812	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1232.51	80.641	0.860	Strong	C-F	Stretch	alkyl halides	1000-1400
10.	1276.88	80.140	2.278	Strong	C-F	Stretch	alkyl halides	1000-1400
11.	1606.70	79.503	3.556	Bending	N-H	Stretch	Amide	1550-1640
12.	1647.21	79.220	4.828	Variable	C=C	Stretch	Alkene	1620–1680

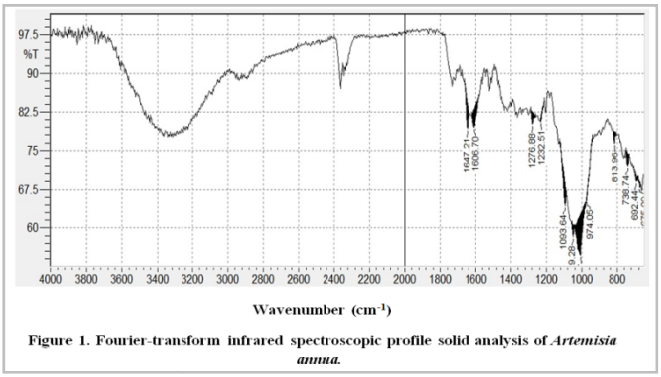

Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Artemisia annua.*

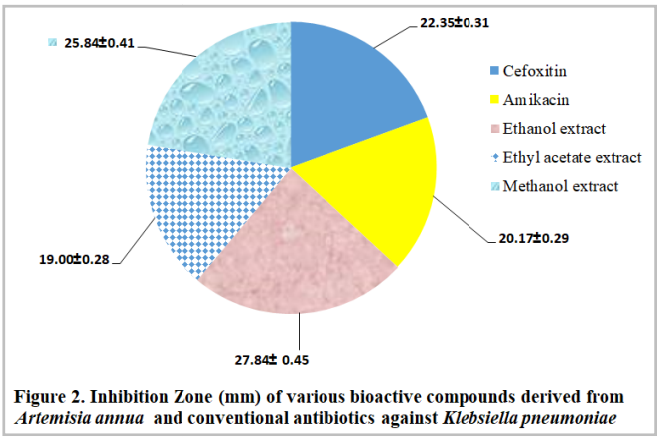

Figure 2. Inhibition Zone (mm) of various bioactive compounds derived from *Artemisia annua* and conventional antibiotics against *Klebsiella pneumoniae*

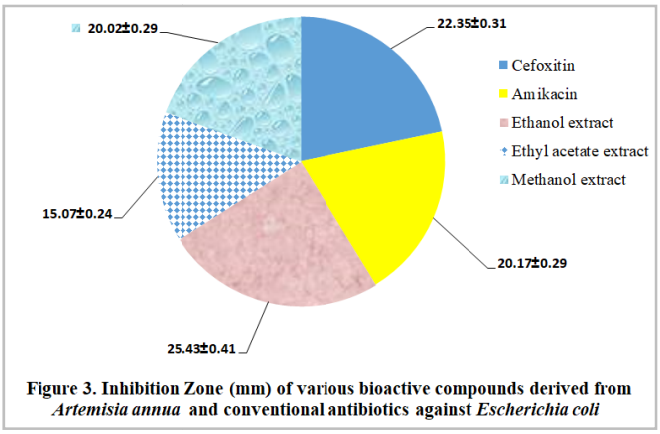

Figure 3. Inhibition Zone (mm) of various bioactive compounds derived from *Artemisia annua* and conventional antibiotics against *Escherichia coli*

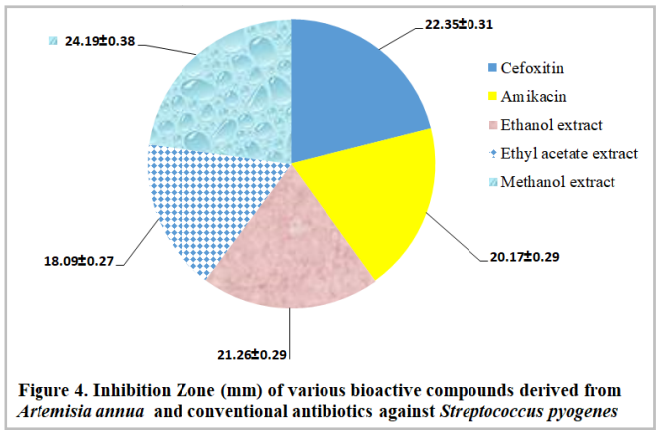

Figure 4. Inhibition Zone (mm) of various bioactive compounds derived from *Artemisia annua* and conventional antibiotics against *Streptococcus pyogenes*

The metabolites of Artemisia annua exhibited significant activity against Klebsiella pneumoniae (27.84± 0.45). Several different species of Artemisia have been demonstrated to produce metabolites that possess antibacterial properties. In addition, a tall species belonging to the genus Asteraceae was found to contain a significant amount of chlorogenic acid in the extract that was made from ethanol. Recent research has demonstrated that chlorogenic acid forms a link with the outer membrane, causes it to become disrupted, reduces the intracellular potential, and causes macromolecules to be released from the cytoplasm [15], all of which ultimately result in the death of the cell.

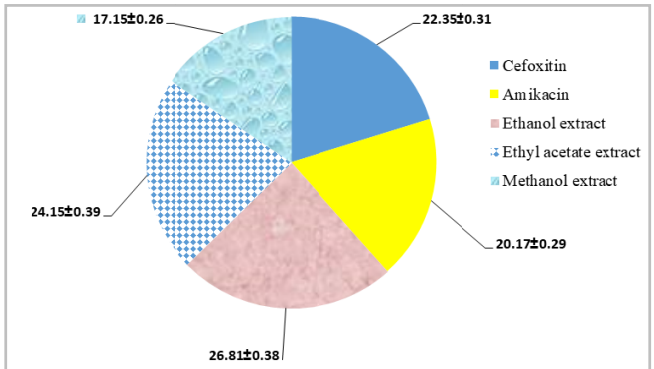

Figure 5. Inhibition Zone (mm) of various bioactive compounds derived from *Artemisia annua* and conventional antibiotics against *Staphylococcus aureus*

Conclusion

It was demonstrated that the Artemisia annua L. species, which is abundant in polyphenolic chemicals, primarily in the leaf, have antibacterial efficacy against Klebsiella pneumoniae, Escherichia coli, Streptococcus pyogenes, and Staphylococcus aureus. Within the range of 27.84 ±0.45, Artemisia annua demonstrated a noteworthy level of effectiveness against Klebsiella pneumoniae. Therefore, on the basis of our findings, the implementation of new therapeutic regimens for infectious diseases that are resistant to treatment based on the utilisation of extracts of Artemisia is a real potential and ought to be investigated further.

REFERENCES

1. Moacă, E, Pavel, I, Danciu, C. Romanian Wormwood (Artemisia absinthium L.): Physicochemical and Nutraceutical Screening. Molecules 2019, 24, 3087.
2. Ivanov, M, Gasic U, Stojkovic, D, Kostic, M. New Evidence for Artemisia absinthium L. Application in Gastrointestinal Ailments: Ethnopharmacology, Antimicrobial Capacity, Cytotoxicity, and Phenolic Profile. Evid. *Based Complement. Med.* 2021, 2021, 9961089.
3. Ahamad, J., Mir, S.R., Amin, S. A Pharmacognostic Review on Artemisia Absinthium. *Int. Res. J. Pharm*. 2019, 10, 25–31.
4. Mahmoudi, M., Ebrahimzadeh, M.A., Ansaroudi, F., Nabavi, S.F., Nabavi, S.M. Antidepressant and antioxidant activities of Artemisia absinthium L. at flowering stage. *Afr. J. Biotechnol.* 2009, 8, 7170–7175.
5. Batiha, G.E., Olatunde, A., El-Mleeh, A., Hetta, H.F., Al-Rejaie, S., Alghamdi, S., Zahoor, M., Magdy Beshbishy, A., Murata, T., Zaragoza-Bastida, A., et al. Bioactive Compounds, Pharmacological Actions, and Pharmacokinetics of Wormwood (Artemisia absinthium). *Antibiotics* 2020, 9, 353.
6. Mamatova, A, Korona-Glowniak, I. Phytochemical composition of wormwood (Artemisia gmelinii) extracts in respect of their antimicrobial activity. *BMC Complement.* Altern. Med. 2019, 19, 288.
7. Farzaneh, F., Ebrahim, H.S., Akbar, V. Investigating on Effect of Wormwood Extract on Reduction of Renal

Toxicity in Treated Rats by Azathioprine. *Biomed. Pharmacol. J.* 2015, 8, 291–299.
8. Kim, M.H., Seo, J.Y., Liu, K.H., Kim, J.S. Protective effect of Artemisia annua L. extract against galactose-induced oxidative stress in mice. PLoS ONE 2014, 9, e101486.
9. Fiamegos, Y, Kastritis, P, Exarchou, V. Antimicrobial and efflux pump inhibitory activity of caffeoylquinic acids from Artemisia absinthium against gram-positive pathogenic bacteria. PLoS ONE 2011, 6, e18127.
10. Hori R, Sugiyama J. A combined FTIR microscopy and principal component analysis on softwood cell walls. *Carbohydr Polym.* 2003;52:449–453.
11. Semeniuc, C, Rotar, A. Antibacterial activity and interactions of plant essential oil combinations against Grampositive and Gram-negative bacteria. *J. Food Drug Anal.* 2017, 25, 403–408.
12. Qian, W., Yang, M., Wang, T., Sun, Z., Liu, M., Zhang, J., Zeng, Q., Cai, C., Li, Y. Antibacterial Mechanism of Vanillic Acid on Physiological, Morphological, and Biofilm Properties of Carbapenem-Resistant Enterobacter hormaechei. *J. Food Prot*. 2020, 83, 576–583.
13. Sethupathy, S., Ananthi, S., Selvaraj, A., Shanmuganathan, B., Vigneshwari, L., Balamurugan, K., Mahalingam, S., Pandian, S.K. Vanillic acid from Actinidia deliciosa impedes virulence in Serratia marcescens by affecting S-layer, flagellin and fatty acid biosynthesis proteins. *Sci. Rep.* 2017, 7, 16328.
14. Yemis, G.P., Pagotto, F., Bach, S., Delaquis, P. Effect of vanillin, ethyl vanillin, and vanillic acid on the growth and heat resistance of Cronobacter species. *J. Food Prot.* 2011, 74, 2062–2069.
15. Mamatova, A.S., Korona-Glowniak, I., Skalicka-Wozniak, K., Jozefczyk, A., Wojtanowski, K.K., Baj, T., Sakipova, Z.B., Malm, A. Phytochemical composition of wormwood (Artemisia gmelinii) extracts in respect of their antimicrobial activity. BMC Complement. *Altern. Med.* 2019, 19, 288.

****