Journal of Current Medical Research and Opinion
Received 31-12-2023| Revised 30-12-2023| Accepted 12-01-2024| Published Online 14-01-2024

DOI: https://doi.org/10.52845/CMRO/2024/7-1-7T ISSN (O) 2589-8779 | (P) 2589-8760

CMRO 07 (01), 2033-2039 (2024)

Original Articles

Spectrum Analysis of Bioactive Compounds of *Achillea millefolium* Flower-Heads Using FTIR Technique and Investigation of Its Fractions and Crude Flowers Extract as Anti-Bacterial Activity

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Abstract:

Background: Plants produce chemicals known as secondary metabolites, which are molecules that provide them an advantage in their own habitat or environment. These little compounds bring about a vast variety of impacts, not only on the plant itself but also on other species that are alive. They either support the growth of perennial plants or indicate the behavior of deciduous plants, and they cause flowering, fruit set, and abscission. In addition to their antibacterial properties, they can either act as attractants or, on the other hand, behave as repellents. A total of more than 50,000 secondary metabolites have been identified within the realm of plant study. Secondary plant metabolites are responsible for the activities of medicinal plants as well as a significant number of modern pharmaceuticals.

Objective: Using the Fourier transform infrared spectroscopy (FTIR) technology, the objectives of this study are to analyze the bioactive compounds of Achillea millefolium and to evaluate the anti-bacterial activity of the plant.

Materials and Methods: For the purpose of analyzing chemical characteristics, the Fourier transform infrared (FTIR) method employs infrared light to scan test samples. We used Mueller-Hinton agar plates to conduct a screening to determine whether or not the extracts of Achillea millefolium flowers possessed antibacterial properties. Phytochemicals and the solvents that were employed for each extract served as the controls for this experiment.

Results: The major constituents were FT-IR Peak values (Wave number cm^{-1}): 958.62 (Strong), 985.62 (Strong), 918.12 (Strong), 1012.63 (Strong), 1026.13 (Strong), 1093.64 (Strong), 1242.16 (Bending), 1255.66 (Medium), and 1606.70 (Bending) with Functional group assignment Alkenes, Alkenes, alkyl halides, alkyl halides, alkyl halides, alkyl halides, alkyl halides, Aromatic, and Amide respectively. The metabolites of *Achillea millefolium* exhibited significant activity against *Staph. aureus* (20.39±0.31).

Conclusion: *A. millefolium* can play a significant role as antibacterial activity source. A. millefolium suggest they be used as natural drugs in clinical settings.

Keywords: *Achillea millefolium*, Spectrum Analysis, Secondary metabolites, Anti-Bacterial Activity.

Introduction:

There are four primary categories that can be used to categorize secondary metabolites found in plants. These categories are terpenoids, phenolic compounds, alkaloids, and chemicals that contain sulfur. Phytochemicals have the potential to inhibit the growth of microorganisms, serve as attractants or repellents, or act as deterrents against herbivores. Since more than three thousand years ago, the Achillea millefolium plant, which is also known as milfoili or yarrow, has been utilized for therapeutic purposes in both conventional and alternative medicine [2]. It is the most well-known and extensively distributed species in the Asteraceae family. The principal phytochemical compounds that are extracted from *A. millefolium* are the essential oil and derivatives of flavonoids, particularly apigenin, rutin, lutein, and camphor. The essential oil of A. millefolium contains a combination of monoterpenes and sesquiterpenes, with monoterpenes accounting for around 90 percent of the total percentage [3]. Variations in essential oil content may be the result of environmental factors such as temperature, sunshine, relative humidity, and irradiance, as well as ecotype, chemotype, phenophases, altitude, and other physiological and environmental factors that influence plant growth. Furthermore, the genetic background ought to be taken into consideration in order to modify the chemical composition of secondary metabolites in plants [4]. Along with flavonoids such as aglycones, flavones, and flavonols such as O-glycosides, A. millefolium also contains flavonoids themselves. The flowering tops of the Achillea millefolium plant, which contain the essential oil, are generally considered to be the most effective sections of the plant. These flowering tops are widely utilized for the treatment of a variety of conditions, including influenza, hemorrhage, dysmenorrhea, and diarrhea. Achillea millefolium extracts and oils can be found in a variety of forms, the most common of which are essential oil, infusion, alcohol extract, decoction, hydroalcoholic extract, and aqueous extract [5]. Additionally, there is a relationship between the antioxidant activity and the phenolic compounds. It is possible that the antioxidant action of essential oil is due to the relatively high concentrations of the phenolic compounds, specifically carvacrol and thymol, that are present in Essential Oil [6]. There are a wide range of pharmacological activities that are thought to be possessed by Achillea millefolium, including analgesic, anti-inflammatory, antidiabetic, cholagogue, spasmolytic, anticancer, antioxidant, antifungal, and antiseptic properties [7]. Several chemical components, including essential oils, sesquiterpenes, phenolic compounds, and others, are responsible for the liver-protective activities. Essential oils, sesquiterpenes, and phenolic compounds are among the chemical compounds that are responsible for these actions [8]. In addition, previous research suggests that A. millefolium has the ability to serve as an antiulcer agent. It is possible that flavonoids, notably Quercetin and Apigenin, are responsible for the attenuation of ileum contractions that has been seen after the addition of the extract [9]. Using the Fourier transform infrared spectroscopy (FTIR) technology, the aims of this study are to analyze the bioactive compounds of *Achillea millefolium* and to evaluate the anti-bacterial activity of the plant.

Materials and Methods:

Plant Collection and Preparation of *Achillea millefolium* flower-heads Extract

The flower heads of *Achillea millefolium* were obtained from the hilla city in Iraq. When the flower-heads of *Achillea millefolium* were pulverized, they were macerated in methanol at a concentration of 90% and then filtered. A crude extract was obtained by first filtering the extract and then concentrating it using a Boeco Rotary Evaporator RVO 400 SD, which was manufactured by Boeco in Germany. In a separating funnel, the crude extract was dissolved in the least amount of deionized distilled water, and then it was combined with hexane. Following a vigorous shaking of the mixture, it was laid aside for a day to allow it to be settled [10]. A mixture of dichloromethane (DCM) was used to re-mix the residual after the hexane fractions of the settling extract were separated as distinct components. The exact same method was carried out in order to gather the DCM fractions.

The EA fraction was obtained by treating the residual from the DCM fraction with ethyl acetate (EA), giving rise to the EA fraction. After all is said and done, the separation will result in the ethyl acetate fraction, while the remaining component will be an aqueous/water fraction.

Fourier transform infrared spectroscopy (FTIR) analysis of *Achillea millefolium*

The FTIR spectra of native and defatted GLVs were recorded using an FTIR instrument (Model/Make: IFS 25, Bruker, Germany), with the operation of the instrument and the processing of the data being handled by a PC-based software. In preparation for FTIR analysis, a small quantity of powdered leaf samples were transformed into pellets by means of KBr, and a thin film was created by means of the application of pressure. Over a wave number range that extended from 4000 cm−1 to 500 cm−1, the data pertaining to the transmittance of infrared light was gathered. Three separate analyses were performed on each of the samples [11], with plain KBr pellets serving as the control. A comparison was made between the spectral data and a reference in order to determine the functional groups that were present in the sample.

Screening for the antibacterial activity of the *Achillea millefolium* flowers extracts

At a temperature of 37 degrees Celsius, the cultures of bacteria were cultured in Brain Heart Infusion liquid medium. Following the completion of a growth period of six hours, each microorganism was injected onto the surface of Mueller-Hinton agar plates at a concentration of 106 cells per milliliter. After that, filter paper discs with a diameter of six millimeters were placed on the surface of each inoculation plate. These discs were saturated with either extract or phytochemicals, having a volume of fifty microliters. In order to assess the effectiveness of the process, each extract was concurrently introduced into a hole that was created in new plates, with a volume of fifty microliters. One day was spent incubating the plates at 37 degrees Celsius. It was feasible to observe the inhibitory zone after this length of time had passed [12]. In general, it was determined that cultivated bacteria with halos that were around 7 millimeters in diameter or larger were vulnerable to either the extract that was examined or the phytochemical. When necessary, DMSO and Tween 80 at a concentration of 2% were utilized in order to dissolve the extracts in the culture medium. In the preliminary investigations, the solvents that were employed for each extract and the phytochemicals served as the controls, and they did not exhibit any instances of inhibition.

Statistical analysis

The GraphPad Prism 5 Statistical Package (GraphPad Software, USA) was utilized in order to carry out the various statistical analyses. One-way analysis of variance (ANOVA) was used to assess the data, and then the Bonferroni test was performed on the results. For triplicate determinations, the results of the in vitro IC50 were represented as the mean plus or minus the standard error of the mean. The activities of free radical scavenging were indicated as a percentage, while the quantification of phytochemicals was expressed as the mean plus or minus the associated standard deviation. For statistical significance, a p-value of less than 0.05 was evaluated.

Results and Discussion:

The major constituents were FT-IR Peak values (Wave number cm-^{l}): 958.62 (Strong), 985.62 (Strong), 918.12 (Strong), 1012.63 (Strong), 1026.13 (Strong), 1093.64 (Strong), 1242.16 (Bending), 1255.66 (Medium), and 1606.70 (Bending) with Functional group assignment Alkenes, Alkenes, alkyl halides, alkyl halides, alkyl halides, alkyl halides, alkyl halides, Aromatic, and Amide respectively.

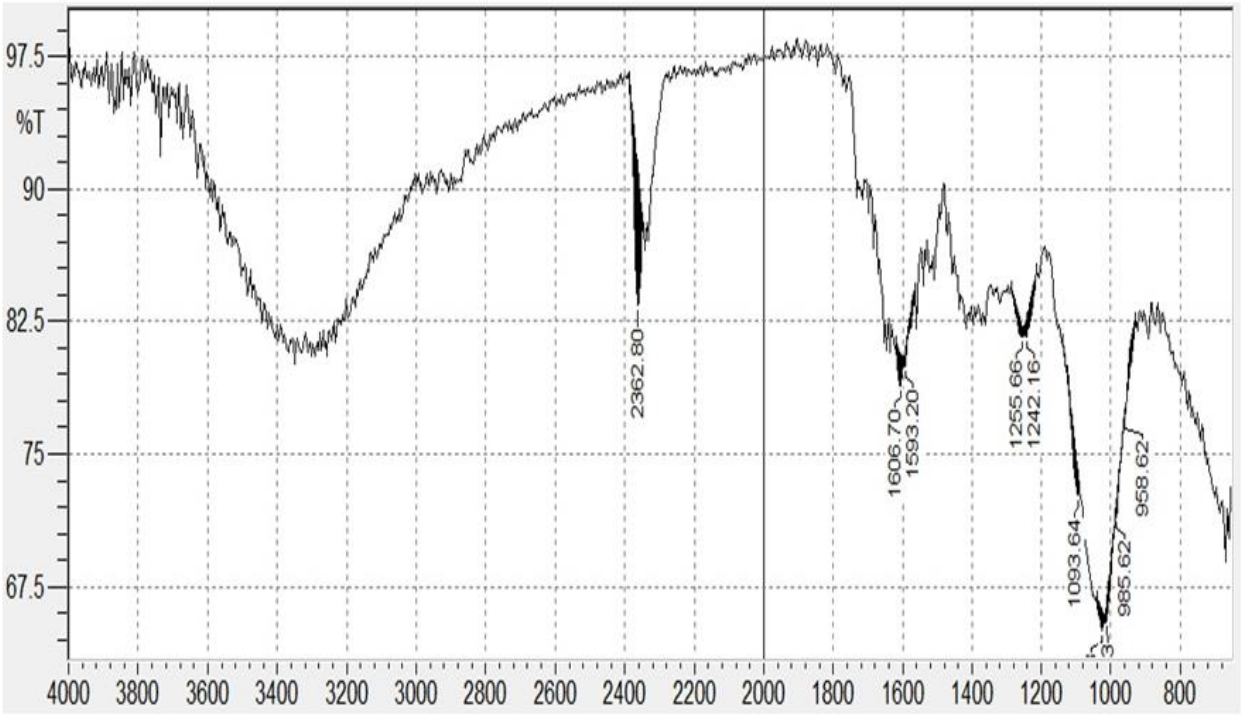

Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Achillea millefolium*.

Table 1. FT-IR peak values of solid analysis of *Achillea millefolium*.

No	Peak (Wave number cm-1)	Intensity	Corr. Intensity	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	958.62	77.147	0.325	3.099	0.009	Strong	=C–H	Bending	Alkenes	650-1000
2.	985.62	71.466	0.414	3.506	0.049	Strong	=C–H	Bending	Alkenes	650-1000
3.	1012.63	65.604	0.851	4.842	0.127	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1026.13	65.029	1.155	4.236	0.094	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1093.64	72.698	1.021	5.595	0.157	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1242.16	81.648	1.051	2.960	0.141	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1255.66	81.607	0.580	2.880	0.085	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1593.20	79.968	0.777	2.221	0.065	Medium	C=C	Stretch	Aromatic	1400-1600
9.	1606.70	78.913	1.794	2.100	0.124	Bending	N-H	Stretch	Amide	1550-1640
10.	2362.80	83.382	7.728	2.083	0.691	Unknown	-	-	-	-

In *vitro* antimicrobial activity of *Achillea millefolium* flowers extracts on four microorganisms

Figures 1, 2, and 3 illustrate the zone of inhibition (measured in millimeters) of three different harmful bacteria against a variety of bioactive chemicals and conventional antibiotics produced from plants. According to the type of extract (methanol, Ethyl acetate fraction, and Ethanol fraction) recorded 18.36±0.30, 17.34±0.29, and 16.23± 0.28 respectively in *Enterococcus faecalis*. While recorded 20.00±0.31, 20.39±0.31, and 19.52±0.30 for *Staph. aureus*. At the same time record 16.83±0.27, 20.46±0.31, and 19.99±0.29 *Streptococcus pyogenes*. in comparison with Amikacin 18.10±0.30 and Azithromycin 25.83±0.34. The metabolites of *Achillea millefolium* exhibited significant activity against Staph. aureus (20.39±0.31). When it comes to antibacterial activity, yarrow extracts, both aqueous and ethanolic, have been shown to be effective against a variety of microorganisms. These microorganisms include those that cause skin infections, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*, as well as those that are associated with gastrointestinal diseases, such as *Salmonella thypi* and *Escherichia coli*. The majority of the chemical substances that are produced by plants come from their secondary metabolism, which is primarily associated with defensive mechanisms or interactions with the surrounding environment [13]. The food, cosmetic, and pharmaceutical industries are showing a growing interest in essential oils (EOs) among plant extracts. This is due to the fact that essential oils are reasonably safe, they are widely accepted by consumers, and they can be exploited for possible multi-purpose functional applications. It is believed that these oils will play a part in the mechanisms that plants use to defend themselves against phytopathogenic bacteria [14]. Because of its medical properties, the *Achillea millefolium* L. plant, sometimes known as yarrow, has been utilized in the pharmaceutical industry for the manufacturing of herbal medications. The stem, leaves, and flowers of this plant have all been utilized. In addition to its application in the pharmaceutical sector, the plant is also utilized in the cosmetic industry for the creation of photoprotective products, in the food industry as an ingredient for the production of liqueurs and flavorings, and in landscaping as a decorative plant. The height of the yarrow plant ranges from 30 to 50 centimeters. It is a perennial plant that is dark green in color, pungent, and has stems that are rhizomatous and robust [15]. The leaves are plentiful, the leaves are tall and pinnate, and the flowers are either pink or white. Along highways, in fields, and in pastures, the plant can be seen growing natively. When it is farmed, the flowering season occurs in the spring. A wide variety of conditions, including but not limited to hemorrhage, ulcers, diarrhea, cancer, tumors, warts, leukorrhea, influenza, pneumonia, and others, have been successfully treated with yarrow. Additionally, it is regarded to be an abortive, contraceptive, anti-hematic, healing, analgesic, anti-inflammatory, antipyretic, anthelmintic, antibacterial, antifungal, anticancer, antioxidant, and antioedematous product.

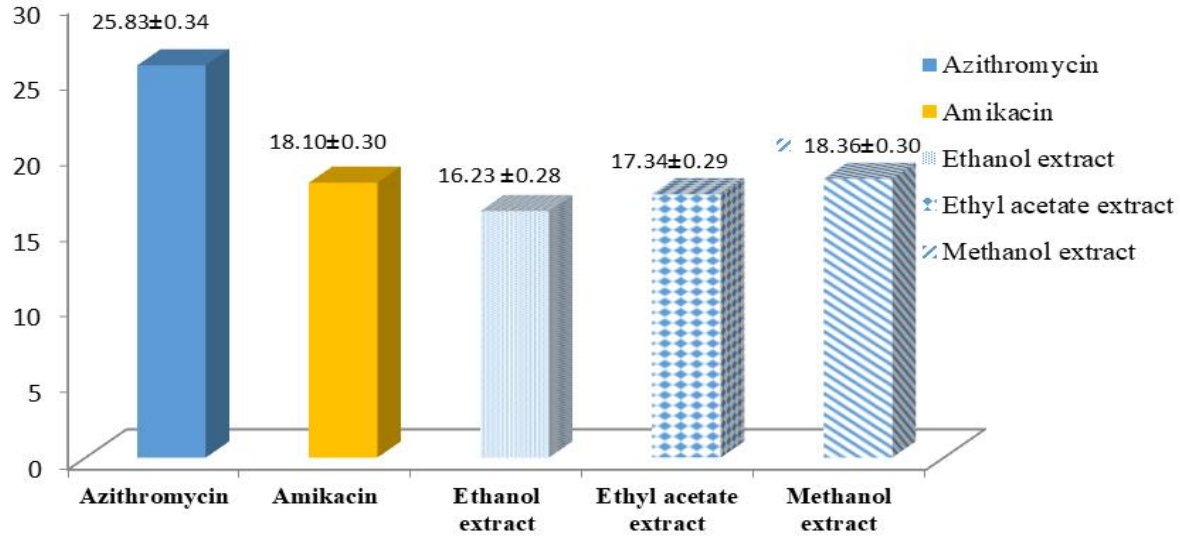

Figure 2. In *vitro* antimicrobial activity of *Achillea millefolium* secondary metabolites and conventional antibiotics against *Enterococcus faecalis*

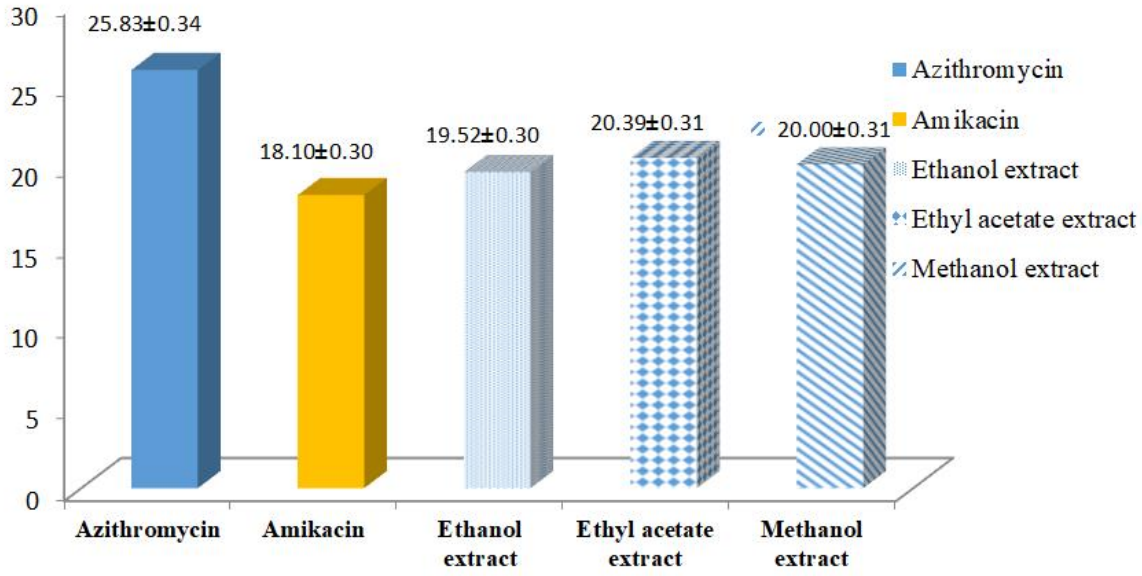

Figure 3. In *vitro* antimicrobial activity of *Achillea millefolium* secondary metabolites and conventional antibiotics against *Staph. aureus*

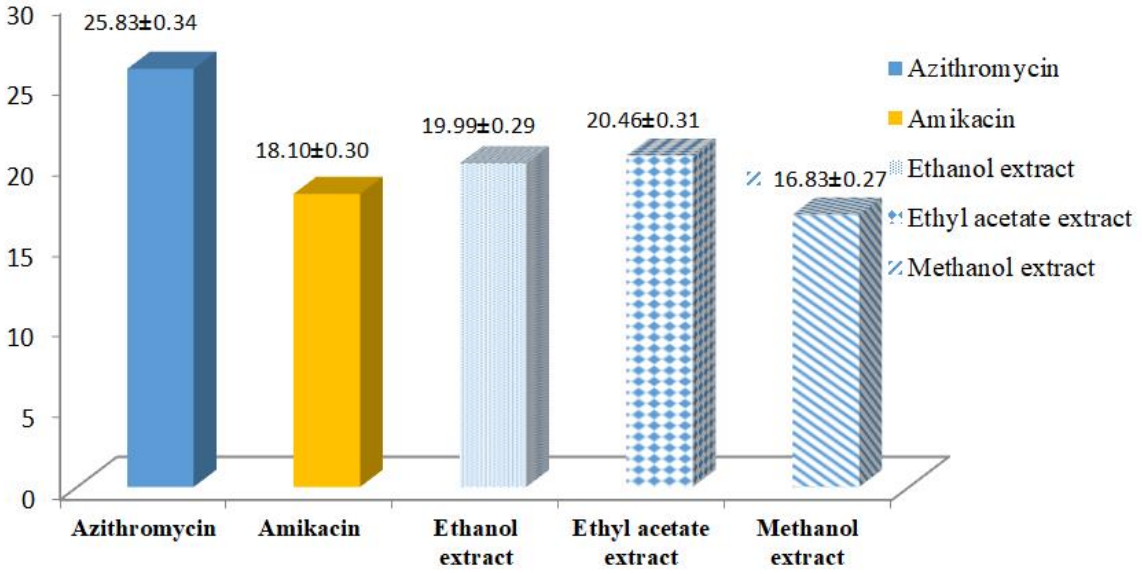

Figure 4. In *vitro* antimicrobial activity of *Achillea millefolium* secondary metabolites and conventional antibiotics against *Streptococcus pyogenes*

Conclusion:

In the past, all of the herb-drug interactions that were connected with *Achillea millefolium* were thoroughly explained. When it comes to *Achillea millefolium* CYP, the most significant interactions occur with medications that have a limited therapeutic index. Additionally, activity is compatible with its application that is less widespread. According to the available research, *Achillea millefolium* has the potential to play a significant role as a source of antibacterial activity. There is evidence that suggests *Achillea millefolium* could be utilized as natural pharmaceuticals in clinical settings.

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