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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF AERIAL PARTS OF THE *ACHILLEA MILLEFOLIUM*

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Abstract

The work presented in this paper consists of "Phytochemical analysis and antimicrobial activity of aerial parts of the Achillea millefolium". Achillea millefolium is widely recognized for its many uses and lengthy history in conventional medicine worldwide. Since ancient times, the herb has been used to treat a variety of illnesses in Balochistan. Methanol was used at room temperature to extract the aerial portion of Achillea millefolium for this study. A qualitative phytochemical analysis and biological tests (antimicrobial activity against four gram-positive and gram-negative bacteria) were conducted prior to the extract. The results illustrated that the plant extract shows fine acti-bacterial properties and is rich in secondary metabolites.

INTRODUCTION

Achillea millefolium (yarrow) is regarded as the *Asteraceae* family's most significant genera due to its therapeutic uses. Certain parts of plants are specifically home to secondary metabolites. In the *Achillea* genus, flavonoids have been found in the majority of species. For example, the aerial parts of *Achillea sintenisii* contain phenolic compounds, and the leaf extract of *Achillea millefolium* contains both flavonoids and phenolic compounds. The different amounts of secondary metabolites that plants contain may be caused by differences in their growing environments (1). This genus is rich in essential oils, terpenes, flavonoids, phenolic acids, fatty acids, amino acids and inulin. It also showed high cytotoxic potential due to the presence of high content of phenolic compounds. The main phytochemical compounds that have been isolated from *Achillea millefolium* contain essential oils and Flavonoid derivate including Rpigenin, Rutin, Lutein, and Campherol (2). Naturally, bioactive Phenolic compounds are thought to be the most potent antioxidants, preventing or delaying a wide range of illnesses, including cancer, Alzheimer's,

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high blood pressure, and inflammation. Numerous studies have demonstrated *Achillea millefolium*'s anticancer activity in various cancer cell lines, which may be related to phenolic compounds. One study examined the mechanism of action of Casticin, which was isolated from the same plant and causes apoptosis and G2/M arrest in cancer cells by downregulating cyclin A and inducing p21 (cyclin-dependent kinase inhibitor) (3). *Achillea millefolium* (yarrow), has long been used in folk medicine to treat headaches, skin irritation, dyspepsia, and hepatobiliary problems. Previous investigations have demonstrated the antibacterial, anti-inflammatory, antispasmodic, anti 1 helminthic, and disinfectant activities of *Achillea millefolium*. Yarrow extracts were also efficient against a variety of bacterial and fungal species, and in some situations, they were even more potent than penicillin-family drugs. According to information from the literature, phenolic compounds and essential oils are the primary secondary metabolites in *Achillea millefolium* that are responsible for its biological activities (4) .Due to its lengthy history used for treatment of many disease, from those in Europe to those in Asia, *Achillea millefolium* is the plant that best represents the genus. Various herbal remedies, both liquid and solid, are still produced to treat minor skin conditions, urinary tract problems, digestive problems, and appetite problems. It has been demonstrated that phenols, flavonoids, monoterpenes, sesquiterpenes, and sesquiterpenoids are the key active compounds. The chemical makeup of *Achillea millefolium* has been studied extensively since the early 1900s, and more than 120 different compounds have been found. Numerous nations, notably Italy, have conducted studies on the chemical makeup of extracts made from *Achillea millefolium*, including its essential oils (5).

In pharmacologically point of view, one of the significant classes of active constituents that exhibit potential biological activities of *Achillea millefolium* are the phenolic compounds, particularly flavonoids. millefolium extracts such as antidiabetic, gastro protective, antioxidant and antibacterial effects. In a similar vein, yarrow essential oil finds extensive application in contemporary pharmaceutical, food, and cosmetic products. The phytochemical profiles of *Achillea millefolium* because of the existence of several chemotypes, millefolium essential oil is extremely complex. (2).

Moreover, by lowering the risk of numerous illnesses and preserving both physical and mental health, yarrow can also be a useful functional food that can be included in a diet as tea. *Achillea millefolium* is said to possess a wide range of pharmacological characteristics. It contains a variety of chemical components, including terpenes, in its essential oil and sesquiterpenes (6).

Methods and Materials

Collection and Identification

The aerial parts of the *Achillea millefolium* were collected from Bashore area and were identified.

Extraction procedure

The plant material was dried under shade for two weeks and was ground to powder (1.2 kg). It was then soaked in methanol (3 L) at room temperature for 7 days (thrice). The solvent was filtered through a fine cloth. The filtrate was condensed on rotary evaporator. Then combined concentrated extract (07 gm) was obtained.

Phytochemical Qualitative Analysis

The following standard techniques were used to evaluate the presence of phytochemicals in the methanolic extract of the plant's aerial portion. (7).

Molish Test: Several drops of α-naphthol solution were combined with the extract. Concentrated sulfuric acid was added from the walls of the tube. The presence of carbohydrates was indicated by the appearance of a reddish-brown ring. A few drops of Molish reagent (α naphthol) were combined with methanolic plant extract and conc. H_2SO_4, was added along the wall of tube. Ultimately, the presence of carbohydrates was indicated by the formation of a violet ring (8).

Frontier in Medical & Health Research

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Fehling Solution Test: After dissolving 0–25 g of methanolic extract in boiling distilled water, 3 ml of each Fehling solution (solutions A and B) was added. The mixture was heated in water bath. The presence of carbohydrates was confirmed by the formation of a reddish-brown precipitate. Carbohydrates are made up of mono-saccharides. Monosaccharide units are produced when di, oligo, and polysaccharides hydrolyse in the presence of mineral acid. These substances react to different colour reaction and identification tests and are optically active. It consists of two solutions that are combined in situ and is typically used to reduce sugar. Fehling solution B is made up of sodium potassium tartrate, while Fehling solution A contains 0–5% copper sulphate. Two millilitres of methanolic extract were added to an equal volume of Fehling A and Fehling B solution (1 millilitre each), and the mixture was then boiled for five minutes on a water bath. Since there was no precipitate, there was no reducing sugar present.

Bendict Test: In the test tube, an equal volume of Benedict's reagent and extract were combined, and the mixture was heated for five minutes on a boiling water bath. The presence of carbohydrates was indicated by the red colour appearance. It is primarily made up of sodium hydroxide and copper sulphate and is used to reduce sugars. One millilitre of Benedict solution was added to the methanolic extract and brought to near boiling. Since there was no precipitate, there was no reducing sugar present.

Biuret Test: A few drops of copper sulphate ($CuSO_4$) solution were added to three millilitres of extract containing four percent NaOH. The presence of proteins was indicated by their reddish or pink appearance.

Lead Acetate Test Solution: A small amount of extract was mixed with a lead acetate solution, a yellow precipitate formed, indicating the presence of flavonoids.

Test for Carboxylic Acid: Lead acetate solution was added to a small amount of extract, a yellow precipitate formed, indicating the presence of flavonoids.

Test for Saponins: In this test, 10–20 millilitres of water were mixed with 0.5–5 grams of extract, and the mixture was shaken for a few minutes. The presence of saponins was indicated by frothing formations that lasted for 60–120 seconds.

Test for Alkaloids: 2 mg of the methanol extract was taken in a test tube; a few drops of Hager's reagent were added. Formation of yellow precipitate confirms the presence of alkaloids.

Test for steroids: Chloroform was used to extract two millilitres of methanolic extract for this test. A few drops of conc. Sulfuric acid were added from the test tube's side wall.

Ferric chloride test

Two millilitres of water were mixed with two to three drops of $FeCl_3$ and then added to two millilitres of methanolic extract to conduct the test. The presence of tannins was indicated by the greenish precipitate that formed.

Chemical test for terpenoid

This test involved mixing a methanolic extract solution with two millilitres of acetyl chloride. Carefully add two to three drops of sulfuric acid to create a layer. Terpenoids were present because of the interface's deep red colour.

Lead acetate test

In the Lead Acetate test, one millilitre of a 10% lead acetate solution was added to the methanolic extract, and the results were recorded. Flavonoids were present because a yellow precipitate formed.

Determination of Antimicrobial Activity

Agar-well diffusion assay: Media for bacterial culture: The medium was steamed for 30 minutes, neutralized for 30 minutes at 37°C, and then filtered. The medium was sterilized for 20 minutes at 121 °C and 15 pounds.

Frontier in Medical & Health Research
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Method: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Klebsiella pneumonia* were all suspended in sterile normal saline for 24 hours. After labelling each medium plate, a sterile cotton swab was used to inoculate it.

Results and Discussion

Table 1: Phytochemical analysis

S.N.	Phyto-chemical analysis	Reagents used	Interference	Outcome
1.	Carbohydrate test	1. Molish's Reagent 2. Benedict's Reagent 3. Fehling's Reagent	1. Creation of violet rings 2. No formation of orange red ppt 3. No formation of red ppt.	1. Positive 2. Negative 3. negative
2.	Steroid test	Liebermann Burchard Reagent	Creation of blue ring at the junction of two liquids	positive
3.	Alkaloid test	Hager's Reagent	Formation of yellow colour ppt.	positive
4.	Saponin test	Froth test	Formations of frothing.	positive
5.	Tannin test	Ferric chloride Reagent	Formation of greenish ppt.	positive
6.	Flavonoid test	Lead Acetate Test	Formation of yellow colour.	positive
7.	Terpenoid test	Acetyl chloride test	Formation of deep red coloration of the interface.	positive
8.	Protein test		No formation of ppt.	positive

Biological assays

Antibacterial Activity

The antibiotics treat bacterial infections. When it comes to treating various illnesses, antibiotics are essential. Microorganisms such as fungi, bacteria or protozoan are inhibited by a substance called an anti-microbial. Micro biocidal are antimicrobial medications that either stop microorganisms from growing or eradicate them. Due to the problem of creating a large number of drug-resistant microorganisms, efforts are currently being made to find alternative drug sources. Nowadays, finding novel antibiotics has become essential. (9).

The inhibition zone of the methanolic extract ranged from 7 to 20 mm for four Gram-positive bacteria and from 7 to 13 mm for two Gram-negative bacteria. Aerial part extract was made using the percolation method (10). By measuring the diameter of the growth inhibition zone, the effects of varying extract concentrations on Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus saprophyticus were assessed using the disk diffusion and well diffusion methods (11). Propylene glycol and gentamicin served as the negative and positive controls, respectively (12). In both techniques, the methanolic extract of the aerial parts had a positive inhibitory effect on S. aureus, S. epidermidis, and S. saprophyticus growth, particularly in the well diffusion. The strains of S. saprophyticus and S. epidermidis

Frontier in Medical & Health Research
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exhibited the highest susceptibility to the extract (89 percent) in the well diffusion method.

Table 2: Gram-Positive Bacteria.

One of inhibition (mm)	Gram-positive Bacteria
Methanolic Extract	
22	*Staphylococcus aureus*
15	*Staphylococcus AB 188*
20	*Staphylococcus epidermidis*
12	*Streptococcus pyogenes*

Table 3: Gram-Negative Bacteria

Zone of inhibition (mm)	Gram-negative Bacteria
Methanolic Extract	
11	*Shigella boydii*
12	*Salmonella typhi*

Conclusion

Accordingto the phytochemical analysis, the plant extract from *Achillea millifolium* contains a variety of phytochemicals, including alkaloids, flavonoids, phenolic compounds, cardiac glycoside, and reducing sugars. Methanolic extract of aerial parts of *Achillea millifolium* This plant has long been used to treat abdominal infections, and *Achillea millifolium* showed a strong inhibitory effect on *S. aureus* growth, particularly in the well diffusion method. Studies on *Achillea millifolium* revealed that therapeutically active substance can be isolated from this plant.

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Authors contributions

Collected data and design the experiments; Kamran Ali[1], Bilal Ahmed[1], Shazia Iqbal[2] performed analysis and experiments; Kamran Ali[1], Bilal Ahmed[1], Shazia Iqbal[2], Fazila Khan[5], Asia Ali Mohsin[3] Inserted data and wrote paper; Kamran Ali[1], Shazia Iqbal[2], Asia Ali Mohsin[3], Samra Farooq[4], Mahrukh Naseem[6] contributed material and analysis tools; Shazia Iqbal[1], Bilal Ahmed[1], Asia Ali Mohsin[3], Samra Farooq[4], Yousaf Khan[7], Saba Gul[8]

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