

Antimicrobial Activities of Herbal Plant-Dried Chamomile Flowers (Matricaria Chamomilla) Against Different Bacterial Strains

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ABSTRACT

The well-known medical herb chamomile (*Matricaria chamomilla*), often known as German chamomile, has long been utilized for its antibacterial, anti-inflammatory, and antioxidant qualities. The dried flowers of chamomile include a variety of terpenoids and flavonoids that enhance its medicinal properties. Numerous human conditions, including as hay fever, inflammation, muscular spasms, menstrual problems, sleeplessness, ulcers, wounds, gastrointestinal disorders, rheumatic pain, and hemorrhoids, are frequently treated with chamomile products. *Yersinia kiritensenii*, *Staphylococcus aureus*, *Salmonella*, *Micrococcus Luteus*, *Staphylococcus saprophyticus*, and *Serratia rubidaea* were among the bacterial strains against which the antibacterial properties of an aqueous extract of dried chamomile flowers were to be ascertained. During this investigation, the following tests were performed: phytochemical analysis, antibacterial activity, antibiotic susceptibility test (disc diffusion method), and Minimum Inhibitory Concentration (MIC). Numerous phytochemicals, including alkaloids, tannins, coumarins, phenols, flavonoids, glycosides, proteins, and amino acids, have been shown to be present in this herbal extract during phytochemical examination. The study's findings demonstrated that chamomile extract has antibacterial qualities and can be utilized as an ingredient in a variety of antimicrobial medication formulations.

Keywords: *Matricaria chamomilla*, aqueous extract, Antibacterial activity, Anti-Susceptibility Test.

I. INTRODUCTION

For thousands of years, people have been aware of how plants affect human health. Since at least 5000 years ago, herbs have played a significant role in both conventional and alternative medical practices. The longevity of herbal remedies may be attributed to their propensity to act gradually and typically with few harmful side effects [1]. Even if the pharmaceutical industry has developed significantly and modern medicine is now able to cure many frequently dangerous ailments, natural medicines and, most importantly, medicinal plants have long been the mainstay of our grandparents' treatment. In recognition of our ancestors' empirical knowledge, traditional herbal medicine benefits almost 80% of the world's population [2]. Products made from plants and herbs have long been used safely as natural remedies for a variety of illnesses. Utilizing medicinal plants reduces the need for antibiotics, which prevents resistant germs from emerging and spreading quickly. [3]. German chamomile (*Matricaria chamomilla* L.), a significant medicinal plant indigenous to southern and eastern Europe, is one example of this. In addition, it grows in Brazil, Russia, Yugoslavia, France, Germany, and Hungary. These plants can be found in North Africa, Asia, North and South America, Australia, and New Zealand [4]. German Chamomile belongs to Asteraceae family [3]. Internally, it is primarily used as a tisane (infuse 1 tablespoonful of the drug in 1 L of cold water without heating) to treat stomach pain, slow digestion, diarrhea, and nausea; less frequently, but very successfully, it is used to treat urinary tract inflammation and painful menstruation [5]. Furthermore, topical health and cosmetic treatments frequently contain it due to its anti-inflammatory, antioxidant, and skin-calming properties [6]. Additionally, it is utilized in a number of food, cosmetic, and pharmaceutical industries [2] One of the main categories of cultivated medicinal herbs is *M. chamomilla*. It includes a wide range of active and therapeutically intriguing chemical classes. The most

significant components are thought to include polyacetylenes, coumarins, flavonoids, and sesquiterpenes. The annual plant known as true chamomile has slender, spindle-shaped roots that only pierce the ground flat. The tall, heavily ramified, branching stem reaches a height of 10 to 80 cm. The flower heads are pedunculate, positioned independently, and range in diameter from 10 to 30 mm [5]. One of the most significant aspects of *Matricaria chamomilla* is its blossoms, which are employed in a variety of medicinal remedies, including carminative and antipyretic ones. Flowers from *Matricaria chamomilla* may be used as raw materials to make a novel medication that treats urinary tract infections in people [7]. The purpose of this study was to screen for phytochemicals and evaluate the antibacterial efficacy of chamomile flowers against several bacterial strains (*Salmonella*, *Micrococcus Luteus*, *Staphylococcus saprophyticus*, *Yersinia kritensenii*, *Staphylococcus aureus*, *Serratia rubidaea*).

II. MATERIALS AND METHODS

Plant Materials: The Plant was obtained from local market in Mohali and was in the form of dried flowers.

A) Plant Extraction

Aqueous Extract: To make the extract, 25 grams of weighed chamomile flowers were dipped in 150 milliliters of distilled water for a whole day, shaking occasionally at room temperature. The mixture was then filtered and put in a reagent bottle or a clean conical flask. The extract was obtained by filtering it with what mann No. 1 filter paper. After a while, the residues were gathered and stored at 4 °C to conduct phytochemical tests [8].

B) Phytochemical Analysis

A phytochemical test was used to determine whether the aqueous extract of chamomile flowers contained bioactive compounds.

Flavanoids Estimation: A few drops of 10% lead acetate solution was added to one milliliter of the

extract in a test tube. When flavonoids are present, yellow precipitates are formed.

Coumarin Detection: 1-2 ml of the extract, 10% NaOH, and chloroform were placed into a test tube. When coumarins were present, a yellow color was produced as an indicator.

Glycoside Detection: Five milliliters of the extract were measured with a measuring cylinder before being moved to a test tube. Then, two milliliters of glacial acetic acid, concentrated H₂SO₄, and a drop of 5% FeCl₃ solution were added, and the presence of glycosides was indicated by a yellow appearance.

Phenolic compound Detection:

Ferric chloride test: Take Extract aqueous solution and a few drops 5% ferric chloride sol. in a test tube. The production of a dark green or blue-black color indicated the presence of phenolic chemicals.

Protein & amino acid Detection:

Ninhydrin test: Two milliliters of chamomile extract combined with two drops of ninhydrin solution. The presence of amino acids is indicated by the solution turning purple.

Xanthoprotic test: A test tube was filled with chamomile extract and a few drops of concentrated nitric acid, indicated by the yellow appearance of the solution.

Carbohydrate Detection

A dry test tube containing two milliliters of chamomile extract, a few resorcinol crystals, and an equal volume of concentrated hydrochloric acid is heated for a few minutes. The presence of carbohydrates is indicated by a rose-colored appearance.

Alkaloid Detection: One or two drops of Mayer's reagent should be added to the test tube's sides, along with a few milliliters of chamomile extract. Alkaloids are present when a creamy white or yellow precipitate appears.

Phytosterol Detection

A few drops of concentrated H₂SO₄ and a few milliliters of chamomile extract were added. (gently

shaken and left to stand). The presence of phytosterols was indicated by a red appearance (in the lower layer).

Lignin Detection

A few milliliters of Gallic acid and chamomile extract were placed in a dry test tube, which can be detected by their olive green appearance.

Anthraquinone Detection

Borntrager's Test: A few milliliters of chamomile extract were added to 10 mL of 10% ammonia solution and shaken vigorously for 30 s. Anthraquinones were identified using pink, violet, or crimson solutions [9].

C) Antibacterial Activity

To test the antibacterial activity, the disc diffusion method was employed. *Salmonella*, *Micrococcus luteus*, *Staphylococcus saprophyticus*, *Yersinia kristensenii*, *Staphylococcus aureus*, and *Serratia rubidaea* were the bacteria used in this investigation. Mueller Hinton Agar (MHA) was used. Mueller-Hinton agar (MHA) plates were made by adding 20 milliliters of molten media on sterilized Petriplates. After then, the media was given time to solidify. A glass spreader was used to distribute the bacterial cultures once the media had solidified. Several doses of chamomile plant extract (200, 100, 50, and 25 mg/3 ml) were pipetted onto sterile paper discs (6 mm in diameter) and applied to the surface of agar plates that had been inoculated. Plates were kept for incubation for 24 hours at 37 °C. The diameter of the inhibitory zone (mm) that the extract created around the disc was used to express the antibacterial activity. After that, the plates were examined for zones of inhibition, which were then measured and noted using a millimeter ruler. [10,11,12].

D) Antibiotic Susceptibility Test (AST)

The disc diffusion approach is applied to AST. A glass spreader was used to apply 100µl of the bacterial strains (*Staphylococcus saprophyticus* and *Salmonella typhii*) to each plate in the MHA medium. The discs were placed on the MHA media that contained the bacteria after being dipped in several quantities of aqueous chamomile extract and allowed to diffuse throughout the disc. As a positive control, 10µg/disc of

doxycycline is utilized. The plates were incubated at 37°C for 24 hours. Following incubation, zones of inhibition were measured in millimeters (mm) using a transparent ruler.

E) Minimum Inhibitory Concentration (MIC)

The agar-disc diffusion method was used to find the aqueous chamomile extract's Minimum Inhibitory Concentration (MIC). The lowest concentration at which a distinct zone of microbial growth inhibition would be seen is known as the minimum inhibitory concentration (MIC) [13].

III. RESULTS AND DISCUSSION

The Phytochemical screening carried out on the German chamomile (Aqueous Extract) allowed us to identify the existing biomolecules in this plant in order to obtain an overall evaluation of the types of phenols existing in this plant [2]. Phytochemical Analysis were conducted for aqueous extract of Chamomile Flowers. The aqueous extract showed positive results for Alkaloids, Tanins, Coumarins, Phenolic compounds, Glycosides, Flavanoids and Proteins & Amino acids while negative results for Saponins, Anthraquinones, Triterpinoids and Carbohydrates as shown in Table I.

Table I. Phytochemical Analysis

Sr. No.	Phytochemicals	Results
1.	Alkaloids	Positive
2.	Saponins	Negative
3.	Tanins	Positive
4.	Anthraquinones	Negative
5.	Triterpinoids	Negative
6.	Coumarins	Positive
7.	Phenolic compounds	Positive
8.	Glycosides	Positive
9.	Flavanoids	Positive
10.	Proteins & Amino acids	Positive
11.	Carbohydrates	Negative

Antibacterial Activity

For different Concentrations, Aqueous extract of chamomile flowers showed antibacterial activity against different bacterial strains (*Salmonella*, *Micrococcus luteus*, *Staphylococcus saprophyticus*, *Yersinia kritensenii*, *Staphylococcus aureus* and *Serratia rubidaea*). M. chamomilla had a high inhibitory activity with a high value (20mm and 17mm) for 200mg/3ml concentration against *Salmonella typhii* and *Staphylococcus saprophyticus* respectively and had low inhibitory activity (7mm) and no zone of inhibition for 25mg/3ml against *Staphylococcus saprophyticus* and others respectively. The Zone of Inhibition were shown in Table II given below.

Table II. Inhibition zone (mm) for different bacterial strains

Sr.No		Concentrations(mg/3ml)			
		200	100	50	25
		Zone of Inhibition(mm)			
1.	<i>Salmonella</i>	20	14	10	-
2.	<i>Micrococcus luteus</i>	12	13	9	-
3.	<i>Staphylococcus saprophyticus</i>	17	7	12	7
4.	<i>Yersinia Kritensenii</i>	15	9	-	-
5.	<i>Staphylococcus aureus</i>	12	11	8	-
6.	<i>Serratia rubidaea</i>	15	13	10	-

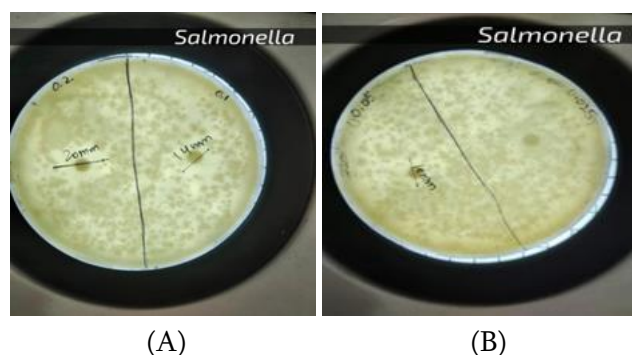


Fig.1 Antibacterial activity of chamomile extract against *Salmonella*

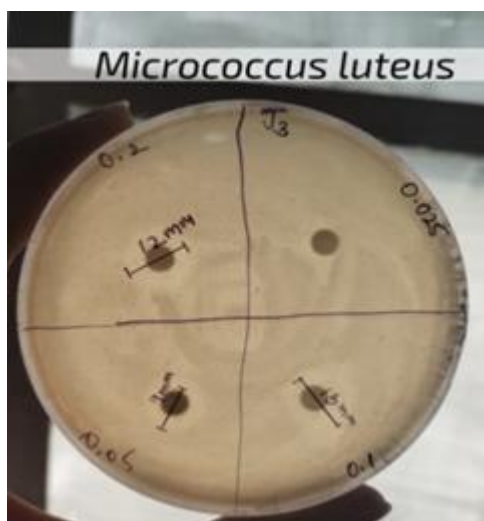


Fig 2. Antibacterial activity of Chamomile extract against *micrococcus luteus*

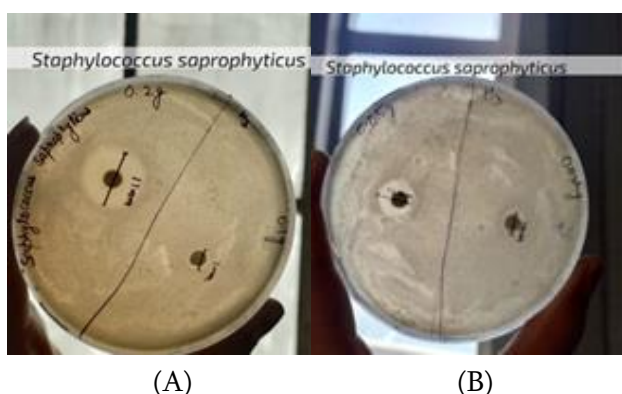


Fig.3 (A) and (B) showing Antibacterial activity of Chamomile extract against *Staphylococcus saprophyticus*

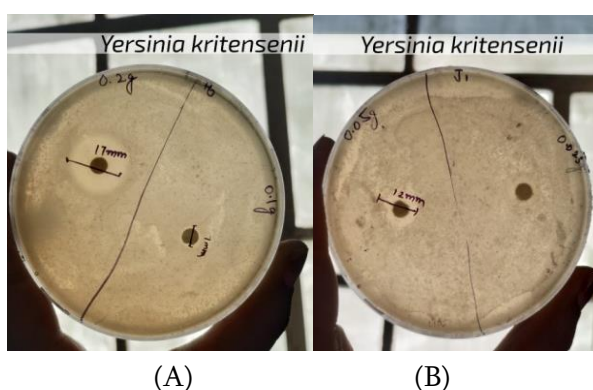


Fig. 4 Antibacterial activity of Chamomile extract against *Yersinia kritensenii*

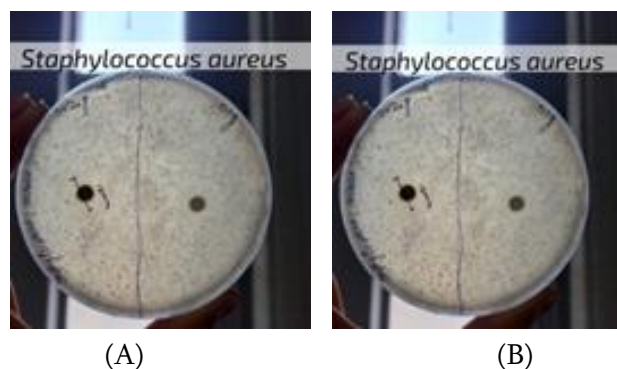


Fig.5 (A) and (B) Antibacterial activity of Chamomile extract against *Staphylococcus aureus*



Fig.6 Antibacterial activity of Chamomile extract against *Serratia rubidaea*

Antibiotic Susceptibility Test

Aqueous extract of chamomile flowers showed antibacterial activity resulting in the zone of Inhibition of about 20mm,13mm,9mm (for 200mg/3ml,100mg/3ml,50mg/3ml respectively) around the discs, also doxycycline(Antibiotic) show zone of Inhibition of 20mm against Salmonella and zone of Inhibition of about 22mm,17mm,21mm (for 200mg/3ml,100mg/ml,50mg/ml respectively) against *Staphylococcus saprophyticus* with antibiotic - doxycycline showing zone of inhibition of about 30mm (as shown in Fig.7).

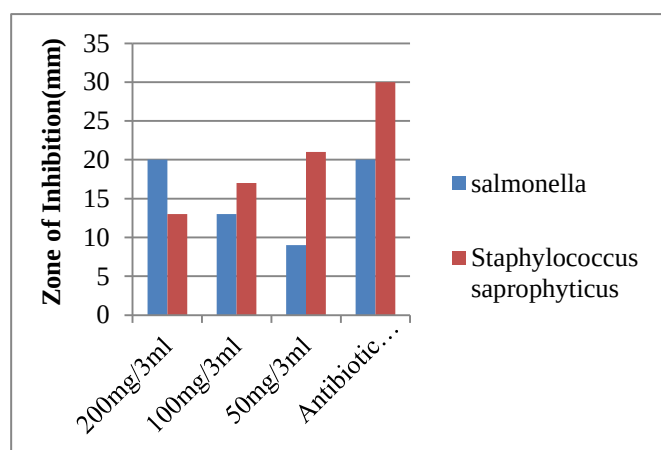


Fig.7 Zone of Inhibition (mm) of Aqueous extract of Chamomile flowers against *Salmonella* and *Staphylococcus saprophyticus* by using AST.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) for different concentrations (45mg, 40mg, 35mg, 30mg) against different bacterial strains (as mentioned in AST) was done for observing minimal inhibition. It has been observed that MIC of Aqueous extract of chamomile for investigated bacteria including *Salmonella*, *Micrococcus luteus*, *Staphylococcus saprophyticus*, *Yersinia kristensenii*, *Staphylococcus aureus* and *Serratia rubidaea* were 35µg/ml, 35µg/ml, 30µg/ml, 30µg/ml, 30µg/ml and 30µg/ml respectively.

IV. CONCLUSION

According to an antimicrobial assessment of chamomile (*Matricaria chamomilla*) flowers, chamomile has significant antibacterial qualities against a variety of bacterial strains. Additionally, it showed that there were components that were medicinally active. According to this study of the plant extract, chamomile contains coumarins, flavonoids, glycosides, phenols, proteins, amino acids, and tannins, all of which may contribute to its antibacterial properties (Table I). The plant extract under investigation had no Carbohydrates, Triterpenoids, Saponins and Anthraquinones. It is known that these bioactive substances have

antibacterial properties and work through several mechanisms. Additionally, it may be inferred that, at varying concentrations, the chamomile extract can prevent the growth of the diverse microorganisms that were identified in this study. While the effects on *Micrococcus luteus* were generally less noticeable, chamomile shown considerable activity against *Salmonella* and *Staphylococcus saprophyticus*. However, depending on the extract concentration and preparation techniques, measurable inhibition still occurred. This study backs up the historic usage of chamomile in herbal medicine and raises the possibility that it might be used as a natural antibacterial agent that is further developed for therapeutic uses or as an adjuvant to standard antibiotics.

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VI. Authors Contributions

Experimentation was done by Neelam. The manuscript preparation was done by Neelam and Gurinder. The proof reading was done by Dr. Puneet and lab facility was provided by Harpreet Kaur.

VII. Conflicts of Interests

There was no conflict of Interest.

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