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Studying the chemical properties of chamomile and thyme and their effectiveness against the growth of microorganisms isolated from cheeses and chicken meat

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Abstract

This study aimed to evaluate the chemical properties and biological activity of alcoholic extracts of thyme and chamomile by examining their antibacterial effects against certain bacterial species isolated from chicken meat and soft cheese, namely *Escherichia coli* and Klebsiella spp. The study also included an assessment of the antioxidant activity of both extracts, in addition to the qualitative identification of their active compounds. The results showed that the alcoholic extract of thyme possessed high inhibitory activity, which increased with increasing extract concentration. While the alcoholic extract of chamomile exhibited lower inhibitory activity compared to thyme, it was still effective against some bacterial isolates. Regarding antioxidant activity, the results indicated that both extracts demonstrated a significant ability to inhibit free radicals, suggesting their potential use as natural antioxidants, with chamomile extract showing relative superiority at certain concentrations.

I. Introduction

Plants are among the most important natural sources of active compounds used in therapeutic and pharmaceutical fields. They contain a wide range of chemical substances with varying effects, from beneficial to toxic. Humans have relied on these plants for thousands of years to treat numerous diseases due to their therapeutic properties, particularly their antibacterial activity. It is worth noting that some compounds may exhibit significant therapeutic effects when used in safe and specific doses [1]. Chamomile is used as an antibacterial agent [2]. Chamomile has been used for centuries as a medicinal herb, traditionally to treat digestive disorders and various skin problems. Modern studies have also shown its effectiveness in wound care, demonstrating its ability to promote healing and improve recovery speed[3]. Historically, chamomile has been used as a medicinal and aromatic plant in Europe, the United States, Japan, and other countries. Due to its numerous medicinal properties, chamomile extract has been incorporated into herbal products in recent years, including therapeutic ointments and lotions [4]. As for thyme, it contains a group of phytochemical compounds, terpenes, flavonols, and carvacrol, which possess antioxidant, antibacterial, and antifungal properties [5]. Thyme is a plant of great medicinal importance, as it can treat many diseases, supported by its rich chemical composition and diverse therapeutic properties [6]. Thyme is a plant rich in bioactive compounds and a wide range of medicinal properties. Studies have highlighted the therapeutic effects of thyme, demonstrating its antioxidant and antimicrobial activities [7]. The aim of the research was to study the chemical properties of chamomile and thyme and their effectiveness in inhibiting bacteria isolated from cheese and chicken meat in order to benefit from their active compounds in the field of food preservation.

II. Materials and Methods

Isolation of Microorganisms

Twenty-five samples of cheese and chicken meat were collected and cultured on MacConkey agar and Nutrient agar. The plates were then incubated aerobically at 37°C for 24 hours. To obtain pure isolates, the culture process was repeated several times. Subsequently, different bacterial colonies were selected based on

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variations in color, size, shape, and texture, and subjected to microscopic and biochemical examinations to identify the isolated bacterial species.

Preparation of bacterial suspension

Test tubes, each containing 5 mL of physiological saline solution, were inoculated with 150 µL of bacterial cultures grown on a solid medium. After inoculation, the contents were thoroughly mixed to ensure a homogeneous distribution of bacterial cells. The resulting bacterial suspension was compared to the McFarland standard to determine the required density, resulting in a final concentration of 10^8 cells per ml. This step enables the preparation of a homogeneous bacterial suspension for use in various tests, thereby contributing to the accuracy of results regarding the biological activity of plant extracts against bacterial growth.

Preparation of Thyme and Chamomile Plant Extract

Place 200 ml of 70% alcohol in a clean glass flask and add 20 g of the plant powder. Place the solution in a magnetic stirrer for 8 hours. Filter the solution using Whatman No. 1 filter paper and centrifuge for 10 minutes at 3000 rpm. Concentrate the filtrate using a rotary evaporator and then place it in an electric freezer at 40°C. Finally, store it in opaque glass bottles at 4°C. Place in an electric freezer at 40°C, then store in opaque glass bottles at 4°C.

Qualitative Identification of Active Compounds

Qualitative chemical analysis was performed on the alcoholic extract of thyme and chamomile to confirm the presence of secondary active compounds.

Alkaloids Detection

Alkaloids were detected using the Marcus reagent, as described in [8].

Flavonoids Detection

Flavonoids were detected based on [9].

Phenols Detection

Phenols were detected as described in [9].

Tannins Detection

Tannins were detected using the method described in [10].

Saponins Detection

The extract of the plant sample was shaken, and the appearance and persistence of foam indicate the presence of saponins [10].

Testing the inhibitory effect of the extract

The agar diffusion method was followed, using etching based on [11]

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Antioxidant Activity Testing

The free radical scavenging activity of DPPH was estimated using the method described by [12]. This method measures the ability of antioxidant compounds to inhibit free radicals through their interaction with 1,1-diphenyl-2-picrylhydrazyl (DPPH). This is achieved by monitoring the change in absorbance at a specific wavelength using a spectrophotometer. A decrease in absorbance indicates an increase in the free radical scavenging activity of the compounds. This method aids in evaluating the antioxidant capacity of the studied sample, thereby contributing to the determination of its importance in various medical and industrial applications.

III. Results and Discussion

. Isolation and Identification

Preliminary identification of bacteria isolated from chicken meat and cheese using MacConkey agar showed bacterial growth. Ten isolates were selected from 25 samples to study the biological effectiveness of the alcoholic extract of chamomile and thyme. (2) Klebsiella spp. were isolated from chicken meat and (2) from cheese. (3) *E. coli* were isolated from meat and (3) from cheese.

The inhibitory effect of the alcoholic extract of thyme and chamomile

Table 1 presents the effect of the alcoholic extract of thyme on the growth of *E. coli*. At lower concentrations (1% and 2%), only a weak inhibitory effect was observed, with inhibition zones ranging from 9 to 15 mm. In contrast, higher concentrations (3% and 4%) exhibited a marked increase in inhibitory activity, reaching the highest values, particularly for isolate **E24**, which showed an inhibition zone of 23 mm at 4% concentration compared to 15 mm at 3%.

Table 1: Effect of alcoholic extract of thyme on E.coli isolates isolated from cheese and chicken meat

S/N	Bacteria	concentrations			
		1%	2%	3%	4%
1	E 1	9	10	10	9
2	E 16	10	9	9	13
3	E 17	10	11	11	19
4	E 18	10	12	12	18
5	E 23	11	12	12	20
6	E 24	11	10	15	23

Table 2 illustrates the effect of the alcoholic extract of thyme on **Klebsiella spp.** Isolates. At a concentration of 1%, the extract exhibited a weak inhibitory effect, with similar inhibition zones observed across the different isolates. Increasing the concentration to 2% resulted in a greater inhibitory effect compared to 1%. The activity of the extract further increased at 3%, with isolate **K1** showing a significant inhibition zone of 19 mm. At the highest tested concentration (4%), the inhibitory effect reached its maximum, with isolate **K18** exhibiting an inhibition zone of 22 mm and isolate **K16** showing 21 mm, indicating that higher concentrations of the extract have a pronounced effect on bacterial growth.

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Table 2: Effect of alcoholic extract of thyme on Klebsiella spp isolates isolated from cheese and chicken meat

S/N	Bacteria	concentrations			
		1%	2%	3%	4%
1	K1	15	16	19	19
2	K 4	10	10	9	20
3	K 16	11	9	9	21
4	K 18	9	11	11	22

Table 3 shows that isolate E1 was clearly affected by the extract, exhibiting inhibition zones at concentrations of 1%, 2%, 3%, and 4%, with the diameter of the inhibition zone increasing with increasing concentration, indicating a clear response to the extract. Isolates E16 and E23 were also affected at the same concentrations, but the inhibition zone was larger at 4%. Isolates E17 and E18 were unaffected by the extract at all concentrations, indicating resistance to the extract's effects. It is noted that the inhibitory effect increases with increasing extract concentration, with 4% being more effective than the other concentrations. This finding is consistent with that reported in [13]

Table 3 Effect of chamomile alcoholic extract on E.coli isolates isolated from cheese and chicken meat

S/N	Bacteria	concentrations			
		1%	2%	3%	4%
1	E1	9	10	11	14
2	E 16	9	9	11	16
3	E 17	0	0	0	0
4	E 18	0	0	0	0
5	E 23	9	10	15	15
6	E 24	9	10	11	12

Table 4 shows that the alcoholic extract of chamomile has a clear effect on the growth of Klebsiella spp., as growth inhibition increases with increasing concentration of the extract. The highest rate of inhibition diameter was reached at a concentration of 4%, while the lowest rate of inhibition diameter was at a concentration of 1% and 2%. It is noted that there is a convergence in the inhibition rate between isolates K1 and K4 and isolates K16 and K18.

Table 4 Effect of chamomile alcoholic extract on Klebsiella spp isolated from cheese and chicken meat

S/N	Bacteria	concentrations			
		1%	2%	3%	4%
1	K1	10	11	12	16
2	K 4	0	11	14	15
3	K 16	11	11	11	19
4	K 18	9	10	9	19

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The results indicate that increasing the extract concentration leads to higher rates of inhibition zone diameters. [14] demonstrated that increasing the extract concentration has a direct effect on increasing the diameter of the inhibition zone. This increase is attributed to the direct relationship between the extract concentration and the amount of secondary metabolites, which in turn contribute to enhancing the inhibitory effect. This result is consistent with what [15] found when using other plant extracts, as their study showed that increasing the concentration of extracts obtained from leaves and roots leads to higher rates of inhibition.

Qualitative detection of active compounds

Table 5 shows the results of the qualitative analysis of the active compounds. The results showed that the extract contained all the active compounds except for the saponins. [16] indicated that alcohol is a polar solvent characterized by its high ability to extract chemical compounds, especially since most of the active compounds found in plants are polar in nature. [17] also stated that phenolic compounds, saponins, tannins, and alkaloids possess microbial growth inhibitory activity. These compounds adhere to the bacterial cell wall, leading to the disruption of enzyme activity, protein denaturation, and impaired function of the bacterial cell membrane.

Table 5. Qualitative detection of active compounds

S/N	Content	Alcoholic extract of chamomile	Alcoholic extract of thyme
1	Tannins	+	+
2	Phenols	+	+
3	Alkaloids	+	+
4	Flavonoids	+	+
5	saponins	-	-

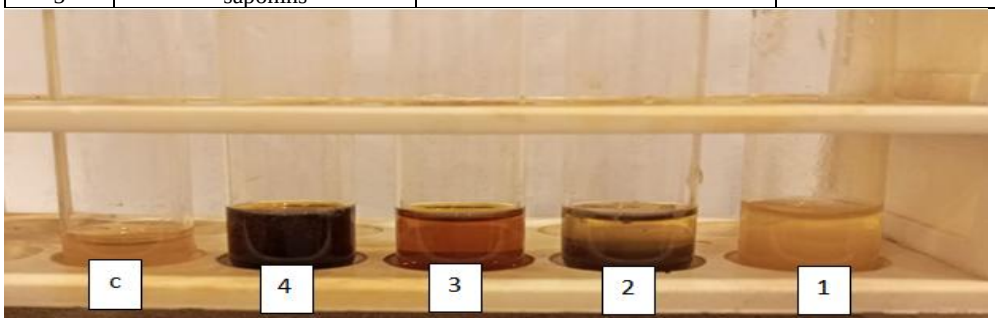

Figure 1. Qualitative detection of the active compounds of thyme

3-4. Antioxidant activity

Figure 2 shows that the alcoholic extract of thyme possesses potent antioxidant activity, as evidenced by the decreasing absorption values and increasing free radical inhibition rate with increasing extract concentration. The control sample exhibited the highest absorption value, indicating a lack of antioxidant activity, while a gradual decrease in absorption was observed with the use of higher concentrations of the sample, reflecting its effectiveness in inhibiting free radicals.

The results also showed a decrease in the absorbance value from 0.3073 at a concentration of 0.12 mg/ml to 0.1149 at a concentration of 1 mg/ml. This is attributed to the ability of the active compounds in thyme to react with free radicals, which leads to a reduction in the intensity of the color resulting from the reaction, which is a direct indicator of antioxidant activity.

On the other hand, the percentage of free radical inhibition (scavenging) clearly increased as the concentration rose. It reached 73.28% at the lowest concentration and gradually climbed to 90.01% at the highest. These results indicate a direct relationship between the concentration of the alcoholic extract of

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thyme and its antioxidant activity. This demonstrates the efficacy of its active compounds, particularly phenolic or flavonoid compounds, in inhibiting free radicals. Thyme is a plant rich in antioxidants, as its various types contain polyphenolic and flavonoid compounds that contribute to showing remarkable antioxidant activity[18][19]

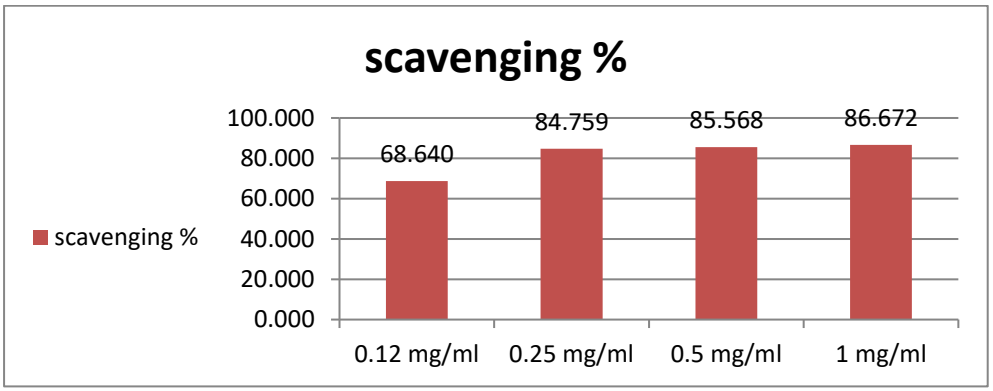

Figure 2. Antioxidant activity of thyme

When comparing the antioxidant activity of chamomile, as shown in Figure 3, with the antioxidant activity of thyme, it becomes clear that both samples showed antioxidant activity, but the activity of chamomile is relatively less than the activity of thyme at all concentrations, and this is shown through the absorbance values and free radical inhibition ratios.

The alcoholic extract of chamomile exhibited higher absorption values compared to thyme at the same concentrations, indicating a relatively lower capacity to inhibit free radicals. At a concentration of 1 mg/ml, the absorption value of chamomile was 0.1533, while it was lower for thyme. This difference suggests a lower antioxidant capacity in chamomile compared to thyme. Furthermore, the results showed lower free radical inhibition rates for chamomile compared to thyme, with the highest inhibition rate being 86.67% for chamomile and 90.01% for thyme at the highest concentration. This lower rate indicates that the active compounds in chamomile are less effective at scavenging free radicals or are present in lower concentrations than those in thyme. [20] It was mentioned that chamomile extracts have a high ability to inhibit oxidation, which reflects its high efficiency in removing free radicals and acting as a hydrogen donor. [21] indicated that chamomile extract contributes to reducing the accumulation of oxidative agents such as hydrogen peroxide and free iron, thus confirming its effective role as a natural antioxidant.

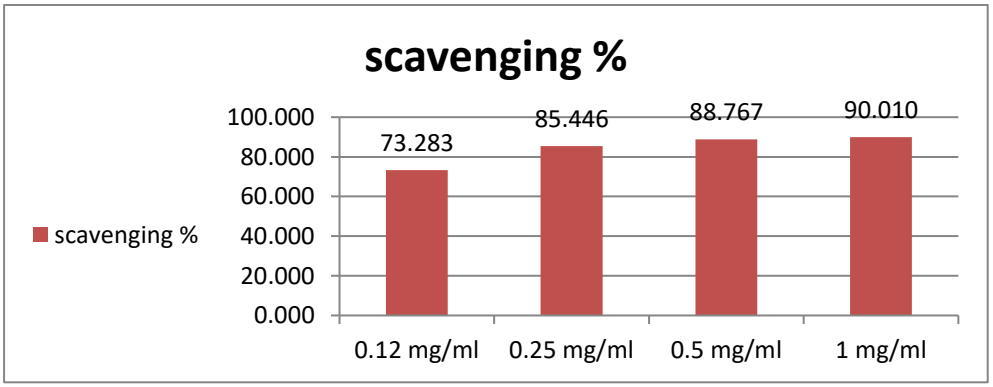

Figure 3. Antioxidant activity of chamomile

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