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# Investigating the chemical properties and antibacterial activity of *Tanacetum balsamita* L. essential oil: a study *in vitro*

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**ABSTRACT**

This study aimed to investigate the chemical composition, antioxidant properties, and antimicrobial activity of *Tanacetum balsamita* L. essential oil. The essential oil was extracted using the water distillation method, and then the total phenolic content (using the Folin-Ciocalteu method), total flavonoid content (using the aluminium chloride colorimetric method), antioxidant activity (using DPPH and ABTS free radical scavenging methods), and antimicrobial activity (using disk diffusion agar, well diffusion agar, minimum inhibitory concentration, and minimum bactericidal concentration methods) were evaluated. The total phenolic and flavonoid contents of the essential oil were found to be 29.50 mg gallic acid equivalent per gram and 14.90 mg quercetin equivalent per gram, respectively. The essential oil exhibited significant antioxidant activity, with the ability to scavenge 50.40% and 53.55% of free radicals DPPH and ABTS, respectively. According to the results of disc diffusion agar method, *Bacillus subtilis* and *Salmonella typhi* were the most sensitive and resistant bacterial strains, respectively, with zone of inhibition diameters of 16 mm and 10.50 mm. The zone of inhibition diameters for these bacteria in the well diffusion agar method were obtained as 16.30 mm and 10.80 mm, respectively. The minimum inhibitory concentration and minimum bactericidal concentration for *B. subtilis* were determined as 4 mg/mL and 32 mg/mL, respectively, and for *S. typhi*, they were 64 mg/mL and greater than 512 mg/mL, respectively. According to the results, *T. balsamita* essential oil can be used as a natural antioxidant and antimicrobial agent in food products.



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## 1.Introduction

Foodborne diseases are a significant global concern in public health, primarily caused by microbial contamination. Microbial contamination is the main cause of foodborne diseases and a decrease in food quality. To prevent microbial contamination, artificial food preservatives are commonly used. However, the use of these artificial preservatives poses risks to human health and can result in adverse effects such as headaches, nausea, fatigue, cognitive disorders, seizures, cancer, and loss of appetite. Additionally, there is growing concern about the emergence of drug-resistant microorganisms [1-4]. Food industries also use artificial food preservatives to prevent food oxidation during packaging. However, due to the undesirable effects of artificial compounds on health and the environment, as well as the increasing problem of drug-resistant strains, the food industry is now focusing on using natural antioxidant and antimicrobial compounds extracted from plants as preservatives. Natural food preservatives are safe, environmentally friendly, and cost-effective, unlike artificial compounds, and they have a wide range of applications. Among various natural products, essential oils have the most application for food preservation [5-13].

Essential oils are aromatic and volatile liquids extracted from different parts of plants as secondary metabolites. Secondary metabolites play important roles in the environmental and biological defense of plants as they often possess antimicrobial and antioxidant properties. Essential oils have been widely used for centuries in the pharmaceutical, agricultural, personal care, and cosmetic industries, as well as added to foods as spices or herbal ingredients. They are extracted from plant materials such as flowers, roots, peels, seeds, fruit peels, and wood [14].

*Tanacetum balsamita* L., also known as Costmary, is famous for its traditional use as a flavoring agent in Mediterranean, Balkan, and South American countries. This species is scattered in Southeastern Europe and Southwest Asia but is also widely present worldwide. It is cultivated in Iran, Turkey, Romania, Germany, Italy, Spain, and England and has traditional aromatic uses in Europe and Asia. The fresh and dried leaves have a strong lemon-mint flavor and a sweet astringent taste. The dried leaves have a long history of use as a flavoring agent in soups, meats, sausages, cakes, and for preparing invigorating tea. The leaves of *T. balsamita* have been used as a liver protector, invigorating agent, sedative, pain reliever, and astringent [15]. The essential oil and extract of *T. balsamita* have also been reported to possess analgesic, anti-inflammatory, antimicrobial, and antioxidant activities [16-18].

Considering the widespread use of *T. balsamita* in Iranian and other traditional medicines for pain and inflammation relief, and the high content of essential oil present in the aerial parts of the plant, this study aimed to investigate the phenolic compounds, antioxidant activity, and antimicrobial properties of the essential oil of this medicinal plant.

## 2. Materials and methods

### *2.1. Materials*

The chemical materials used in this study were obtained from reputable companies, Merck (Germany) and Sigma-Aldrich (USA).

### *2.2. Essential oil extraction*

After obtaining the plant and confirming its scientific name, essential oil extraction was performed using a Clevenger apparatus and water distillation method for 3 h. The obtained essential oil was dehydrated using sodium sulphate and stored at 4 °C in a sterilized dark glass until microbiological and chemical tests were conducted [19].

### *2.3. Total phenolic content*

The Folin-Ciocalteu method was used to measure the total phenolic content of the essential oil. For this purpose, 1.0 mL of the oil or gallic acid solution (0-0.5 mg/mL) was



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mixed with 1.0 mL of 10% Folin-Ciocalteu reagent and then 3.0 mL of 10% $Na_2CO_3$ was added. The mixture was incubated at room temperature for 2 h and the absorbance was recorded at 756 nm. The total phenolic content was expressed as milligrams of gallic acid per gram of essential oil [1].

### *2.4. Total flavonoid content*

The aluminium chloride colorimetric method was used to evaluate the total flavonoid content of the essential oil. In this method, 1.0 mL of the oil or quercetin solution (0-0.5 mg/mL) was added to 3.0 mL of 5% $NaNO_2$ solution. Then, the obtained solution was mixed for 5 min and 3.0 mL of 10% $AlCl_3$ (w/v) was added. After adding 2 mL of 1 M NaOH, absorption at a wavelength of 510 nm was measured, and the total flavonoid content was reported as milligrams of quercetin per gram of extract [1].

### *2.5. Antioxidant activity*

#### *2.5.1. DPPH radical scavenging methods*

In this assay, 37.5 µL of the essential oil or methanol (control sample) were mixed with 2 mL of methanolic DPPH solution (0.0001 M). The samples were kept in darkness for 20 min, and then their absorption at a wavelength of 517 nm against methanol was measured. The following equation was used to calculate the DPPH radical scavenging activity of the extract:

$$\text{Antioxidant activity (\%)} = [(Ab - As)/Ab] \times 100$$

In this equation, Ab represents the absorption of the control and As represents the absorption of the sample.

#### *2.5.2. ABTS radical scavenging methods*

The method described by Noshad et al. [21], with slight modifications, was used to determine the radical scavenging activity of the extract using the ABTS assay. In summary, equal volumes of a 7 mM ABTS solution and a 2.5 mM K2S2O8 solution were mixed together and kept in darkness at 25 °C for 16 h. The resulting cationic radical ABTS solution was then combined with methanol to reach an absorbance of 0.70 at 734 nm. Subsequently, the extract (0.1 mL) was mixed with the radical ABTS solution (3.9 mL), and the resulting solution was kept at room temperature for 6 minutes. The absorption of the solution at 734 nm relative to the control sample was measured. Finally, the antioxidant activity of the extract was calculated according to the following formula.

$$\text{Antioxidant activity (\%)} = [(Ab - As)/Ab] \times 100$$

In this equation, Ab represents the absorption of the control and As represents the absorption of the sample.

### *2.6. Antimicrobial activity*

Disc diffusion agar, well diffusion agar, minimum inhibitory concentration, and minimum bactericidal concentration were used to evaluate the antimicrobial effect of the essential oil against *Bacillus subtilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Enterobacter aerogenes*, and *Salmonella typhi* bacteria.

#### *2.6.1. Disc diffusion agar*

Blank disks were soaked in 20 µL of essential oil and kept at room temperature for 15 min. Then, these blank disks were placed on Mueller-Hinton agar plates contaminated with bacterial strains. After incubation at 37 °C for 24 h, the zone of inhibition around the disks was measured and reported as the antibacterial potential of the essential oil [22].

#### *2.6.2. Well diffusion agar*

For this purpose, 20 µL of the essential oil were added to 6 mm diameter wells previously created on the surface of Mueller-Hinton agar plates and contaminated with bacterial strains. Petri dishes were kept at 37 °C for 24 h, and then the zone of inhibition around the wells was



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measured and reported as the antibacterial effect of the essential oil [22].

### *2.6.3. Minimum inhibitory concentration and minimum bactericidal concentration*

The method presented by Tabatabaee Yazdi et al. [19] was used with necessary modifications to determine the minimum inhibitory concentration and killing concentration of the essential oil. First, a stock solution with a concentration of 512 mg/mL was prepared, and then sequential dilutions of the stock solution were made (512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 mg/mL). Next, 100 µL of each concentration and 10 µL of the standard microbial suspension were added to each well of a 96-well microplate. The plates were incubated at 37 °C. After 24 h, 20 µL of 5% triphenyl tetrazolium chloride indicator was added to the wells. If the essential oil prevented microbial growth, no reddish or pink color formation occurred. The first concentration at which no color change was observed was determined as the minimum inhibitory concentration of the essential oil.

In the test for determining the minimum bactericidal concentration of the essential oil, 10 µL were taken from the wells of the 96-well microplate where no color change was observed. These samples were transferred under sterile conditions and cultured on Mueller-Hinton agar plates. The plates were incubated at 37 °C, and after 24 h, the microbial growth on the plates was examined. The first concentration of the essential oil that prevented colony formation was reported as the minimum bactericidal concentration [19].

### *2.7. Statistical analysis*

The experiments were repeated three times. The data were analyzed using Minitab software (version 16), and the Tukey-test was used to determine the difference between the means of the data with a confidence level of 95%.

## 3. Results and discussion

### *3.1. Total phenolic and flavonoid content*

The results of the total phenolic and flavonoid content of *T. balsamita* essential oil are presented in Figure 1. According to the results, the essential oil contained 29.50 mg gallic acid per gram of total phenols and 14.90 mg per gram of total flavonoids.

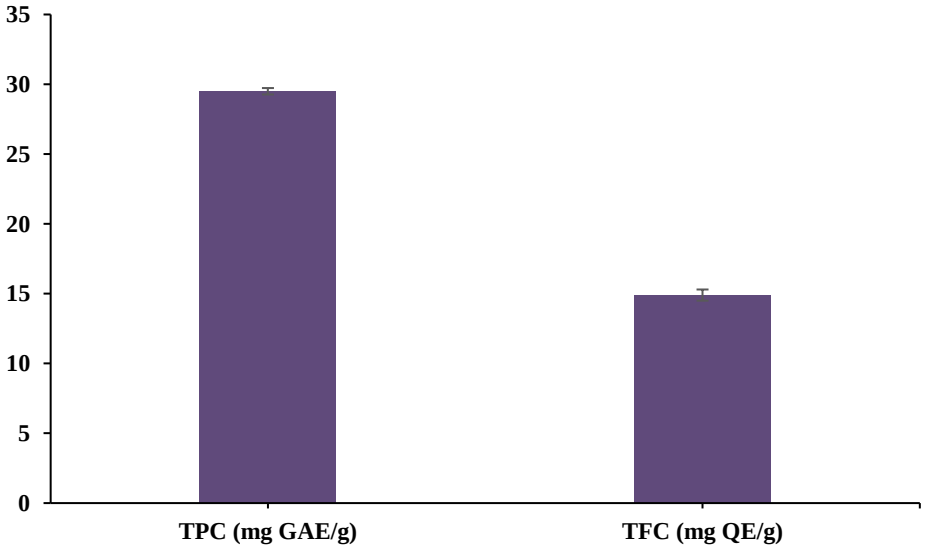


**Figure 1**. Total phenol content (TPC) and total flavonoid content (TFC) of the essential oil. GAE = Gallic acid equivalent; QE = quercetin equivalent.



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The total phenolic content in the extract of leaves, roots, and flowers of *T. balsamita* were reported to be 82.30, 41.43, and 75.59 mg gallic acid per gram, respectively. The total flavonoid content was reported to be 97.18, 74.3, and 2.41 mg quercetin per gram, respectively [23]. Bączek et al. [24] investigated the phenolic and flavonoid compounds in the essential oil and extract of *T. balsamita*. Among the phenolic compounds, 5 acids, including 3,4-dihydroxy-cinnamic (caffeic), 4-hydroxy-3-methoxycinnamic (ferulic), 3-caffeoylquinic (chlorogenic), 3,4-dihydroxycinnamoyl-3-(3,4-dihydroxyphenyl) lactic (rosmarinic) and dicaffeoyltartaric (cichoric) acids, were identified. Regarding flavonoids, 4 compounds, quercetin, apigenin 7-O-glucoside (cosmosiin), luteolin 7-O-glucoside and luteolin 3′-methyl ether (chrysoeriol), were identified in the essential oil and extract of *T. balsamita* [24]. In the study by Gecibesler et al. [25], the total phenolic content and total flavonoid content of *Tanacetum cilicicum* extract were reported to be 53.99-268.02 mg gallic acid per gram and 76.26-41.86 mg quercetin per gram, respectively. The differences in the results of this study compared to other researchers indicate that the composition and quality of the extract and essential oil derived from plant sources are strongly influenced by the age and diversity of the plant, geographical conditions, drying methods, and extraction methods [20, 26].

### *3.2. Antioxidant activity*

Antioxidants delay or inhibit oxidation, thereby protecting food and plants from oxidative damage and increasing their shelf life and quality. Therefore, their consumption can be beneficial in the treatment of oxidative damage-related diseases such as cardiovascular diseases, inflammations, diabetes, and cancer [27]. The results of the antioxidant activity of *T. balsamita* essential oil are presented in Figure 2. In this regard, the antioxidant effects of the essential oil against free radicals DPPH and ABTS were found to be 40.50% and 55.53%, respectively.

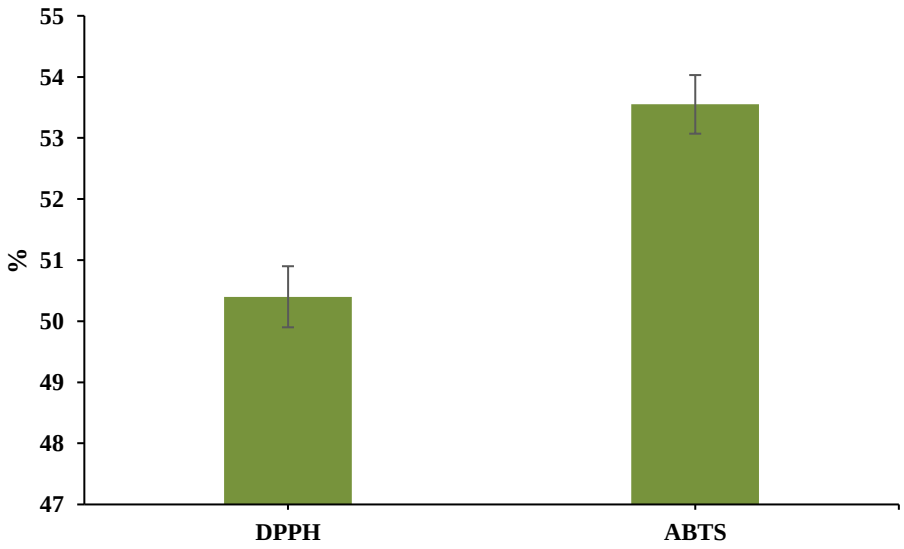


**Figure 2**. Antioxidant effect of the essential oil based on DPPH and ABTS radical scavenging methods.

The antioxidant activity of different parts of *T. balsamita* plant extract based on DPPH and ABTS free radical scavenging methods has been investigated by Gevrenova et al. [23]. The results showed that the antioxidant activity of leaf, root, and flower extracts based on DPPH inhibition method were 87.43, 87.44, and 54.84 equivalents of Trolox per gram, respectively,



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and these values for the ABTS radical scavenging assay were 64.65, 52.91, and 35.96 equivalents of Trolox per gram, respectively [23]. The antioxidant activity of *Tanacetum cilicicum* extract was also reported based on its reducing power, DPPH free radical scavenging, superoxide anion scavenging, chelating activity, and thiocyanate free radical scavenging methods [25]. It seems that the antioxidant potential of *T. balsamita* essential oil can be mainly attributed to the presence of phenolic acids. Phenolic acid derivatives, especially rosmarinic acid, are known for their high antioxidant activity [28]. However, it has been reported that the antioxidant activity of *T. balsamita* essential oil and extract may not only be associated with phenolic acids but also with flavonoids [24]. Flavonoids with hydroxyl groups in their chemical structure are highly effective in scavenging free radicals, especially when these hydroxyl groups are substituted on the B ring. Phenolic acids with multiple hydroxyl groups also exhibit strong antioxidant activity. It is hypothesized that phenolic and flavonoid compounds may act as reducing agents and react with free radicals to convert them into more stable products, thus terminating the free radical chain reaction [25].

### *3.3. Antimicrobial activity*

The antibacterial activity of *T. balsamita* essential oil against certain pathogenic Gram-positive bacteria (*B. subtilis*, *S. pyogenes*, and *S. aureus*) and Gram-negative bacteria (*Sh. dysenteriae*, *E. aerogenes*, and *S. typhi*) was investigated using disc diffusion agar, well diffusion agar, minimum inhibitory concentration, and minimum bactericidal concentration methods, and the results are reported in Figures 3 and 4 and Table 1.

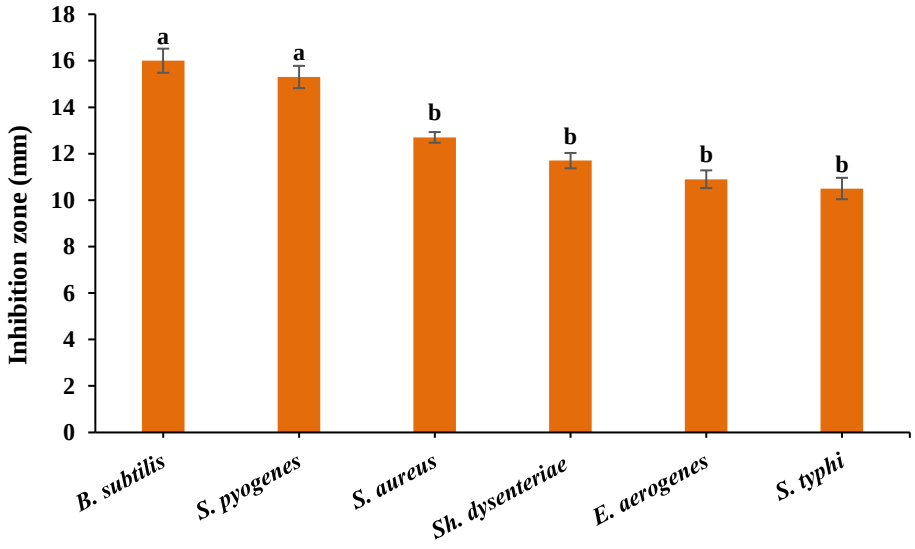


**Figure 3**. Antibacterial effect of the essential oil based on disc diffusion agar method.

The essential oil exhibited a strong antibacterial effect against all tested microorganisms, and its antimicrobial effect was dependent on the type of bacteria ($p < 0.05$). The highest (16 mm) and lowest (10.50 mm) diameters of growth inhibition zones were observed for *B. subtilis* and *S. typhi*, respectively ($p < 0.05$) (Figure 3). These values were 16.30 mm and 10.80 mm, respectively, in the well diffusion agar method (Figure 4). Additionally, the antibacterial activity or diameter of growth inhibition zone was higher in the well diffusion agar method compared to the disc diffusion agar method. In fact, bacterial species are in direct contact with the essential oil in the well diffusion agar method, but the rate of diffusion of the antimicrobial agent from the disk surfaces to the medium determines its inhibitory effect in the disc diffusion agar test [8, 29, 30].





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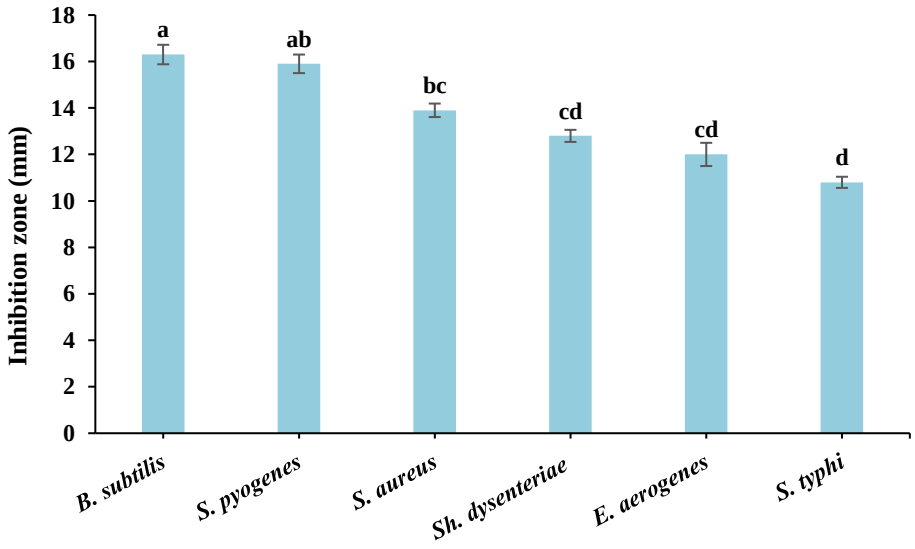


**Figure 4**. Antibacterial effect of the essential oil based on well diffusion agar method.

The minimum inhibitory concentration and the minimum bactericidal concentration of *T. balsamita* essential oil against the above bacteria are also reported in Table 1. *B. subtilis* and *S. typhi* were the most sensitive and resistant strains, respectively, with minimum inhibitory concentrations of 4 and 64 mg/mL. Furthermore, the minimum bactericidal concentration for the above bacteria was 32 and greater than 512 mg/mL, respectively.

**Table 1**. Antibacterial effect of the essential oil based on minimum inhibitory concentration and minimum bactericidal concentration methods.

| Bacterial type | Minimum inhibitory concentration (mg/ml) | Minimum bactericidal concentration (mg/ml) |
|---|---|---|
| *B. subtilis* | 4 | 32 |
| *S. pyogenes* | 8 | 128 |
| *S. aureus* | 8 | 256 |
| *Sh. dysenteriae* | 32 | 512 |
| *E. aerogenes* | 32 | > 512 |
| *S. typhi* | 64 | > 512 |

As clearly observed, Gram-positive bacteria were more sensitive to lower concentrations of the essential oil compared to Gram-negative bacteria, which is mainly due to the presence of a unique mucopeptide layer in their cell membrane that makes them more susceptible to antimicrobial agents. On the other hand, the cell membrane of Gram-negative bacteria contains a complex lipopolysaccharide and phospholipid layer, which slows down the diffusion of





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antimicrobial compounds into the cell [20]. Bączek et al. [24] demonstrated that *T. balsamita* essential oil is capable of inhibiting the growth and eliminating Gram-positive (*Bacillus cereus*, *B. subtilis*, *S. aureus*, and *Listeria monocytogenes*) and Gram-negative bacteria (*E. aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Shigella sonnei*, and *Yersinia enterocolitica*). Similar results have been reported by Yousefzadi et al. [31]. Furthermore, it has been reported that the diameter of the growth inhibition zone for *B. subtilis*, *S. aureus*, *E. coli*, and *S. typhi* in the presence of *T. balsamita* essential oil is 13, 17, 15, and 13 mm, respectively [17]. The observed antibacterial activity in *T. balsamita* essential oil can be attributed to the presence of phenolic compounds and flavonoids. These compounds are capable of inhibiting nucleic acid synthesis, disrupting the function of the cytoplasmic membrane, and interfering with the energy metabolism of bacterial cells [24].

## 4. Conclusion

This study provides an in-depth investigation of the phenolic compounds, antioxidant activity, and antimicrobial effect of *T. balsamita* essential oil. The results showed that the oil contains phenolic and flavonoid compounds, which contribute to its significant antioxidant activity. The antimicrobial effect of the oil was dependent on the type of bacteria, with Bacillus subtilis and Salmonella typhi being the most sensitive and resistant strains, respectively. Therefore, *T. balsamita* essential oil can be a suitable choice for the production of new antimicrobial and antioxidant drugs with fewer side effects compared to chemical drugs. However, identifying the effective compounds associated with the biological activity of this oil and investigating its biological effects under in vivo conditions are recommended for future studies.

## 5. Acknowledgement

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# بررسی ویژگی های شیمیایی و فعالیت ضدباکتریایی اسانس شاهسپران: مطالعه در شرایط برون تنی

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**چکیده**

این مطالعه با هدف بررسی ترکیبات شیمیایی، اثر آنتی‌اکسیدانی و فعالیت ضدمیکروبی اسانس شاهسپران انجام شد. اسانس شاهسپران با کمک روش تقطیر با آب استخراج شد و سپس محتوای فنول کل (روش فولین سیوکالتو)، فلاونوئید کل (روش رنگ سنجی آلومینیوم کلرید)، اثر آنتی‌اکسیدانی (با استفاده از روش‌های مهار رادیکال آزاد DPPH و ABTS) و ضدمیکروبی (مطابق روش‌های دیسک دیفیوژن آگار، چاهک آگار، حداقل غلظت مهارکنندگی و حداقل غلظت کشندگی) اسانس ارزیابی گردید. محتوای فنول و فلاونوئید کل اسانس به ترتیب برابر با ۲۹/۵۰ میلی‌گرم گالیک اسید و ۱۴/۹۰ میلی‌گرم کوئرستین در گرم بود. فعالیت آنتی‌اکسیدانی اسانس قابل‌توجه بود و به ترتیب قادر به مهار ۵۰/۴۰ درصد و ۵۳/۵۵ درصد رادیکال‌های آزاد DPPH و ABTS بود. مطابق نتایج دیسک دیفیوژن آگار، *باسیلوس سوبتیلیس* و *سالمونلا تیفی* به ترتیب با قطر هاله عدم رشد ۱۶ و ۱۰/۵۰ میلی‌متر حساس‌ترین و مقاوم‌ترین سویه‌های باکتریایی نسبت به اسانس بودند. قطر هاله عدم رشد برای این باکتری‌ها در روش چاهک آگار به ترتیب ۱۶/۳۰ و ۱۰/۸۰ میلی‌متر بدست آمد. نتایج حداقل غلظت مهارکنندگی رشد و حداقل غلظت کشندگی برای باکتری *باسیلوس سوبتیلیس* به ترتیب ۴ و ۳۲ میلی‌گرم در میلی‌لیتر و برای باکتری *سالمونلا تیفی* به ترتیب ۶۴ و بزرگتر از ۵۱۲ میلی‌گرم در میلی‌لیتر بدست آمد. مطابق نتایج، اسانس شاهسپران را می‌توان بعنوان عامل آنتی‌اکسیدان و ضد میکروب طبیعی در مواد غذایی استفاده نمود.



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