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The Effect of the Preparation Method on the Content of Biologically Active Substances and Antimicrobial Activity of Extracts of Marigold Flowers (*Tagetes patula* L.)

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ABSTRACT

Introduction: The search for new sources of biologically active substances with antimicrobial properties represents a significant direction in modern biotechnology and pharmacology. A promising source of such substances is *Tagetes patula L.* (French marigold). Despite the high potential biological activity of aqueous extracts of *T. patula*, they remain insufficiently studied compared to extracts obtained using other solvents and extraction methods.

Purpose: To investigate the influence of extraction methods on the content of biologically active substances and the antimicrobial activity of aqueous extracts of *Tagetes patula L.* flowers. The study aims to determine the relationship between the extraction method and the content of biologically active substances, as well as the antibacterial properties of the aqueous extracts to create antimicrobial agents based on them. The research objectives included determining the total content of phenolic compounds and flavonoids in aqueous extracts of French marigolds obtained by various extraction methods, and testing the extracts for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria isolated from clinical material.

Materials and Methods: The objects of the study were aqueous extracts of *Tagetes patula L.* flowers with a hydromodule of 1:10, obtained by boiling, infusing with stirring, microwave, and ultrasonic extraction. The total content of phenolic compounds and flavonoids was determined using a BMG Labtech plate reader. The antibacterial effect of all obtained plant extracts was assessed using the standard disk diffusion method.

Results: The extraction method significantly influences the content of biologically active substances and the antibacterial activity of aqueous extracts of French marigolds. The highest phenolic compound content was observed in extracts obtained by microwave extraction (0.34 mg/cm^3), while the maximum flavonoid concentration was achieved by boiling for 300 seconds (0.98 mg/cm^3). The aqueous extracts demonstrated antibacterial activity against both gram-positive and gram-negative bacteria *S. aureus* and *E. coli*, with an increasing trend in the inhibition zone diameter proportional to the extraction duration.

Conclusion: The study examined the impact of various extraction methods on the total content of phenolic compounds and flavonoids in aqueous extracts of French marigold flowers. The antibacterial activity of the extracts against gram-positive and gram-negative bacteria was confirmed. The obtained data indicate the potential for further research into the composition and properties of aqueous extracts of French marigolds for the development of antimicrobial agents for use in medicine, veterinary science, and agriculture.

Keywords: French marigold; aqueous extracts; biological activity; antimicrobial agents; Tagetes patula L.; aqueous extracts; biological activity

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Влияние способа получения экстрактов цветков бархатцев распростертых (*Tagetes patula* L.) на содержание биологически активных веществ и антимикробную активность

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АННОТАЦИЯ

Введение: Поиск новых источников биологически активных веществ с антимикробными свойствами представляет собой важное направление в современной биотехнологии и фармакологии. Перспективным источником таких веществ является *Tagetes patula L.* (бархатцы распростертые). Водные экстракты *T. patula*, обладая значительным потенциалом биологической активности, остаются недостаточно изученными по сравнению с экстрактами, полученными с использованием других экстрагентов и методов экстракции.

Цель: Исследование влияния методов экстракции на содержание биологически активных веществ и антимикробную активность водных экстрактов цветков *Tagetes patula L.* Определение зависимости между способом экстракции и содержанием биологически активных веществ, а также антибактериальными свойствами водных экстрактов для создания на их основе антимикробных препаратов. В рамках исследования выполнены задачи по определению суммарного содержания фенольных соединений и флавоноидов в водных экстрактах бархатцев распростертых, полученных различными методами экстракции, и тестированию экстрактов на антибактериальную активность по отношению к бактериям *Staphylococcus aureus* и *Escherichia coli*, выделенным из клинического материала.

Материалы и методы: Объектами исследования выступали водные экстракты цветков *Tagetes patula L.* с гидромодулем 1:10, полученные кипячением, настаиванием с перемешиванием, микроволновой и ультразвуковой экстракцией. Суммарное содержание фенольных соединений и флавоноидов определялось на планшетном ридере BMG Labtech. Антибактериальный эффект всех полученных экстрактов оценивался стандартным диско-диффузионным методом.

Результаты: Метод экстракции значительно влияет на содержание биологически активных веществ и антибактериальную активность водных экстрактов бархатцев распростертых. Наибольшее содержание фенольных соединений наблюдалось в экстрактах, полученных микроволновой экстракцией (0,34 мг/см^3), а максимальная концентрация флавоноидов была достигнута при кипячении в течение 300 секунд (0,98 мг/см^3). Водные экстракты продемонстрировали антибактериальную активность против грамположительных и грамотрицательных бактерий *S. aureus* и *E. coli*, причем зона задержки роста культуры увеличивалась с продолжительностью экстракции.

Выводы: Изучено влияние различных методов экстракции на содержание фенольных соединений и флавоноидов в водных экстрактах цветков бархатцев распростертых. Доказана антибактериальная активность экстрактов против грамположительных и грамотрицательных бактерий. Полученные данные подтверждают перспективность дальнейшего исследования водных экстрактов бархатцев распростертых, их состава и свойств для создания антимикробных препаратов, применяемых в медицине, ветеринарии и сельском хозяйстве.

Ключевые слова: бархатцы распростертые; водные экстракты; биологическая активность; противомикробные препараты

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INTRODUCTION

Traditional herbal medicines have always been a natural source of health. Overproduction and overuse of synthetic products and antibiotics damage both the environment and human health. A recent comprehensive study of water pollution by pharmaceutical residues, which involved 1,052 sampling sites along 258 rivers in 104 countries, showed signs of environmental hazard in more than a quarter of them (Wilkinson, 2022).

Biotechnologists and pharmacologists are busy looking for new natural sources of biologically active substances and methods for obtaining safe antimicrobial herbal remedies to be used in medicine, veterinary, and agriculture (Katsev, 2023).

The French marigold (*Tagetes patula* L.) has good prospects as a source of biologically active substances. This plant is popular in folk medicine and agriculture due to its numerous beneficial properties. Marigolds are known for their antibacterial, antiparasitic, antiviral, and antioxidant properties (Papaiani, 2012). In addition, their fungicidal and insecticidal properties make it possible to use them in agriculture against nematodes that damage strawberries, potatoes, etc. (Astafieva, 2020). Marigolds owe their antiseptic, bactericidal, and preservative properties to limonene, an aliphatic hydrocarbon, and a mix of such acyclic monoterpene hydrocarbons as α-ocimene, β-ocimene, bicyclic terpene pinene, etc. (Gupta, 2012).

Flavonoids are secondary metabolites of plant origin; in case of marigolds, they are responsible for the color. Flavonoids possess a variety of biological activities, e.g., marigold extracts are good antioxidants (Chervonnaya, 2015) and reduce oxidative stress (Mashkovskiy, 2019). However, very few studies focus on the antimicrobial properties that make the French marigold resistant to bacteria, fungi, and viruses.

Most qualitative and quantitative studies that feature bioactive compounds of plant origin aim at selecting the optimal extraction method. Extraction is the first stage of any medicinal plant research because the final result ultimately depends on the correct extraction. Most traditional extraction methods are time-consuming and demonstrate poor selectivity; they require expensive high-purity solvents, which evaporate in large amounts and cause a thermal decomposition of heat-labile compounds (Croteau, 2000). Ultrasonic and microwave extraction methods may overcome these limitations.

Ultrasonic extraction involves cavitation, which destroys the cell walls of plant materials and forms diffusion microcurrents. The resulting cell juice contain bioactive substances that dissolve in the extractant (Poverin, 2006). Most likely, ultrasonic extraction intensifies mass transfer and accelerates access of the solvent to plant cells. Other factors include moisture content, grinding, particle size, and solvent type (Salova, 2016). In addition, temperature, pressure, frequency, and exposure time also affect the efficiency of ultrasonic processing (Elapov, 2021). Ultrasonic treatment is now as popular as traditional methods because it reduces extraction time and requires less energy and solvent (Chemat, 2008).

Microwave extraction is another modern method for extracting soluble substances into liquid from plant materials (Markin, 2014). Microwave heating has a direct effect on polar materials. Electromagnetic energy converts into heat by means of ion conduction and dipole rotation (Jain, 2009). Microwave extraction consists of three successive steps: (1) solutes separate from the active sites of the sample matrix at high temperature and pressure; (2) solvent diffuses into the sample matrix; (3) solutes pass on from the sample matrix into the solvent (Alupului, 2012). Microwave extraction is fast, simple, selective, efficient, and environmentally friendly since it requires little organic solvent (Pan, 2003).

Water extraction proved to be a cheap and safe method for extracting bioactive substances from *T. patula* flowers. As an extractant, water is available, safe, pharmacologically indifferent, and cheap. Aqueous marigold extracts exhibit significant biological activity, which depends on the extraction technique. For example, a hot water marigold extract obtained by boiling was more effective than that obtained by cold water extraction (Astafieva, 2020)

Munhoz *et al*. (Munhoz, 2017) studied the effect of aqueous marigold extract obtained by boiling on the growth, yield, and pest resistance of tomato plants. The extract increased the shoot height and the number of branches, leaves, buds, flowers, and fruits; the experimental plants were less susceptible to diseases than the control. However, the biological activity of aqueous marigold extracts attracts less scientific attention than its hydroalcoholic extracts, which makes the aqueous extraction of bioactive substances from marigold flowers a promising research direction. In addition, few studies report the effect of various extraction methods and variables on the yield of biologically active substances and the biological activity of marigold extracts.

This study established the effect of the extraction method on the content of bioactive substances and antimicrobial activity in aqueous extracts of *Tagetes patula* L. flowers. The experiment included the total phenolic content, the total flavonoid content, the antimicrobial properties, and the antibacterial activity in relation to clinically isolated *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS AND METHODS

Objects

The research featured various aqueous extracts of *Tagetes patula* L. flowers collected in Kaliningrad during the flowering period in August 2023. The water module was 1:10.

Materials

The extracts consisted of distilled water (State Standard GOST 6709-72); sodium nitrite, ≥ 97.0 % (Sigma-Aldrich); aluminum chloride-6-water of analytical grade (State Standard GOST 3759–75); chemically pure sodium hydroxide (GOST 4328-77); ≥ 94 % rutin hydrate (Sigma-Aldrich); Folin-Ciocalteu reagent 2N (LenReaktiv); sodium carbonate of analytical grade (State Standard GOST 83–79); gallic acid (Ruskhim); Mueller-Hinton II agar (Obolensk, TR 9385-227-78095326-2015); nutrient medium 1 of GRM grade (dry agar based on fish meal hydrolysate, Obolensk, TR 9398-001-78095326-2006); nutrient medium 2 of GRM Saburo grade (Obolensk, TR 9398-002-78095326-2006); disk antibiotics (Research Center for Pharmacotherapy).

Equipment

The equipment included an ultrasonic bath (Bandelin RK 102 H, Sonorex Super) with heating (20–22 kHz, 1–70 W/cm^2); analytical scales (AND GH-252); electronic technical scales (GOSMETR VLTE-5100T); a heating laboratory hotplate (SCHOTT SLH 230); an microwave oven (OASIS MW-70MW; 2,450 MHz, 700 W); a high-speed centrifuge for microtest tubes (Eppendorf 5424); a magnetic stirrer with a hot plate (Atomic Lab Prime); a drying oven (Binder FD53); an automatic refractometer (HI96801); a microplate reader (CLARIOstar BMG Labtech); a microbiological safety box (Laminar-S-1,2 NEOTERIC); an autoclave (Melag Euroklav 23 VS).

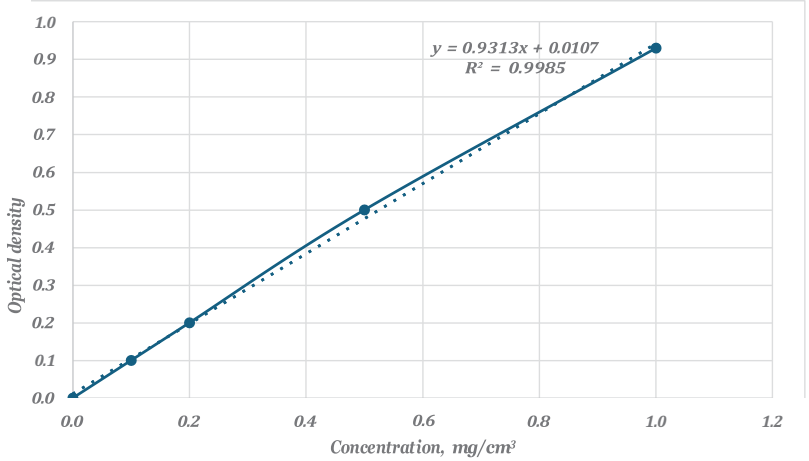
Methods

Determining the Total Phenolic Compounds in Aqueous Marigold Extracts

To define the total phenolic content, we mixed 20 µl extract, 100 µl Folin-Ciocalteu reagent (10 %), and 80 µl of 7.5 % aqueous Na_2CO_3 solution. The mix remained in the dark at room temperature for 30 min, after which the absorbance was measured at 765 nm on a BMG Labtech plate reader. To calculate the total concentration of phenolic compounds, we used a calibration curve obtained by a standard gallic acid solution (100–4,000 µg/ml). The data obtained were expressed as 1 mg gallic acid equivalent per 1 ml extract (Figure 1). The comparative analysis of the phenolic contents across the extracts involved single-factor and two-factor authentication.

Figure 1

Effect of Gallic Acid Concentration on Optical Density: Calibration Curve

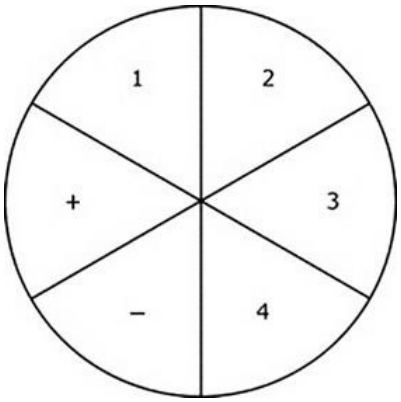


Determining the Total Flavonoid Content in Aqueous Marigold Extracts

The total flavonoid content in the extracts was determined in line with the method described in [10]. We poured 25 µl extract, 100 µl distilled water, and 7.5 µl of 5 % sodium nitrite into the wells of a 96-well plate. After 6 min, we added 7.5 µl of 10 % aluminum chloride, 100 µl of 4 % sodium hydroxide, and 10 µl distilled water to each well. The plate was kept in the dark; after 15 min, the absorbance of the solutions was measured using a BMG Labtech plate reader at 510 nm and the average absorbance value was recorded. The same procedure was repeated for the standard rutin solution to construct a calibration curve. The flavonoid content was expressed as 1 mg rutin equivalent per 1 ml extract (Figure 2). The comparative analysis of flavonoid contents across the extracts involved a single-factor and two-factor authentication.

Figure 3

Location of Discs



Notes: + — positive control; – — negative control; 1–4 — experimental aqueous extracts.

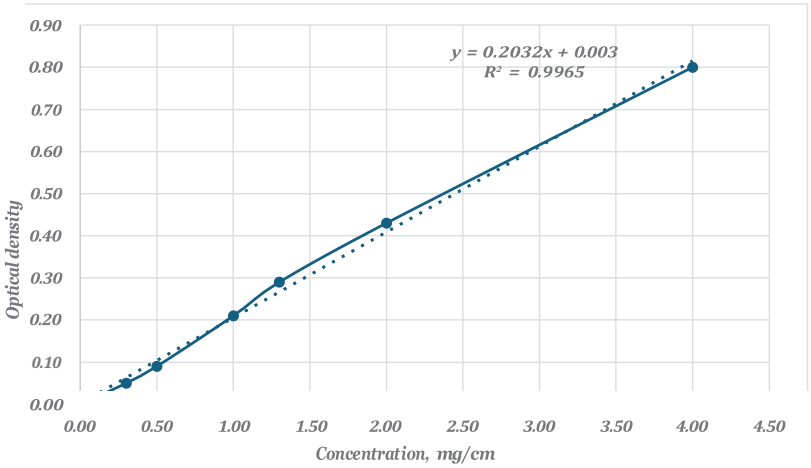
Determining the Antibacterial Activity of Marigold Extracts

To define the antibacterial effect, we appealed to the standard disk diffusion method using pure bacterial cultures of *Staphylococcus aureus* and *Escherichia coli* isolated from clinical samples. A sterile Mueller-Hinton II nutrient medium made it possible to identify the microbial resistance. The medium was pre-sterilized at 1.5 atm for 15 min. Disks with the following antibiotics served as positive control: clindamycin (2 µg) for *S. aureus*, amoxicillin/clavulanic acid (20/10 µg) for *E. coli*, and distilled water as negative control. Microorganisms were seeded under sterile conditions in a LAMSYSTEMS laminar flow hood. Bacterial suspensions were obtained by suspending the culture on a swab in saline solution; the turbidity was 0.5 on the McFarland scale.

The suspension was sown on solid media in Petri dishes using the continuous lawn technique over the entire surface of the agar until complete absorption. The paper disks were dipped in the extracts. After seeding, they went to each zone of the Petri dish with the corresponding strain, as did the discs with antibiotics and distilled water. Figure 3 shows the disk arrangement diagram. The dishes underwent thermostatic treatment at 37 °C for 16 h. At the end of incubation, we removed the dishes from the thermostat and measured the diameter of the lysis zones around the disks.

Figure 2

Effect of Rutin Voncentration on Optical Density: Calibration Curve



Research Procedure

The research consisted of several successive stages. The objective was to determine the correlation between the extraction time and the total phenolic and flavonoid contents under thermal, ultrasound, and microwave treatments. The next stage tested the antibacterial activity using the disk diffusion method in relation to clinically isolated Gram-positive and Gram-negative bacteria. To prepare the extracts, we crushed the dry raw materials with scissors down to two-millimeter pieces and sieved the resulting mass. After adding 20 cm^3 water to 2 g crushed raw materials, we covered the flasks with foil and infused the extraction material for 60 min at room temperature until swelling. After that, the extraction followed four protocols (Table 1).

Table 1

Marigold Extraction Methods

Boiling	Infusion and stirring at 70 °C, 1,000 rpm	Ultrasound extraction, 20 kHz, 30 W/cm^2	Microwave extraction, 2450 MHz, 350 W
300 s	300 s	30 s	30 s
900 s	900 s	90 s	60 s
1,800 s	1,800 s	180 s	
3,600 s	3,600 s	300 s	

After extraction, the material was filtered and centrifuged at 7,000 rpm for 3 min to remove ballast substances. To prevent biological deterioration, the aqueous extracts were refrigerated at 1 °C. The experiment took place within 24 h. Finally, the total contents of phenolic compounds and flavonoids were determined with a microplate reader.

Data Analysis

The stage of statistical processing involved IBM SPSS Statistics 22. All measurements were performed in triplicate and expressed as the mean ± standard deviation. The comparative analysis relied on single-factor and two-factor authentication using the Shapiro-Wilk test and the Tukey criterion ($p < 0.05$). A single-factor analysis of variance is possible under the following conditions: 1) a quantitative continuous data type, 2) independent samples, 3) normal distribution of total phenolics and total flavonoids; 4) equal variances for all features analyzed. After the first two conditions were met, the rest were checked separately for each extraction method. Each subsequent operation was carried out using IBM SPSS Statistics 27.

RESULTS AND DISCUSSION

This section describes the total phenolics and flavonoids in various aqueous extracts of *Tagetes patula* L. depending on the extraction time and the resulting antimicrobial properties for each extract.

Total Phenolic Content in Aqueous Marigold Extracts

The concentration of phenolic compounds in the extracts obtained by boiling, infusion, ultrasonication, and microwaving was found by calibration curve (Figure 1). Figure 4 shows the total phenolics for each extraction method and time.

The one-way ANOVA test revealed reliable differences in the average amount of phenolic compounds with different extraction times, with the exception of ultrasonic extraction ($F = 2.643$; $p \geq 0.05$) and microwave extraction ($F = 4.344$; $p \geq 0.05$). For the extraction time variable, the calculated significance levels exceeded the significance level $\alpha = 0.05$ in all gradations. Therefore, the null hypothesis that this factor had no effect was relevant. Extraction time affected the phenolic content only in the extracts obtained by boiling and infusion with stirring.

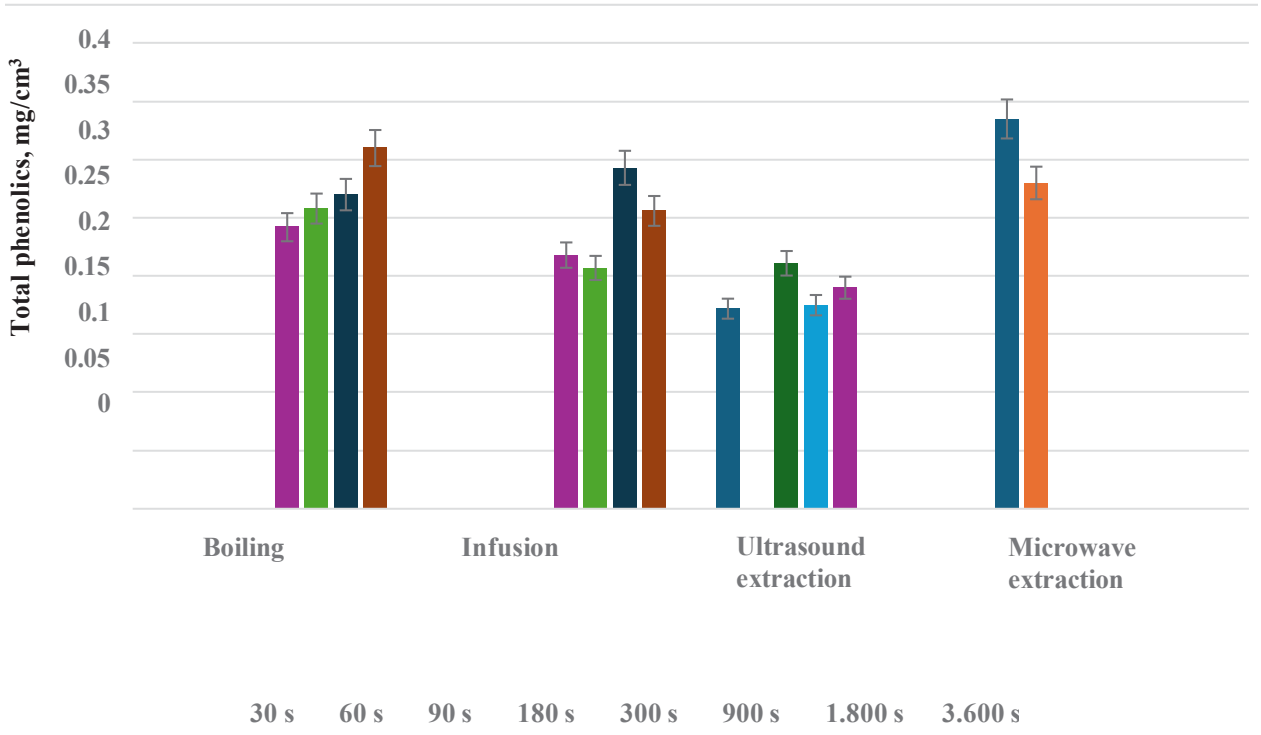
The Tukey's post hoc test showed no significant differences ($p > 0.05$) in the average total phenolic content in the samples boiled for 300 s, 900 s, and 1,800 s (0.242, 0.258, and 0.270 mg/cm^3, respectively), as well as for the boiling times of 1,800 s and 3,600 s (0.270 and 0.310 mg/cm^3, respectively).

For the extracts obtained by infusion with stirring, no significant difference was detected between 300 s and 900 s (0.218 and 0.207 mg/cm^3, respectively), 300 s and 3,600 s (0.218 and 0.256 mg/cm^3), and 1,800 s and 3,600 s (0.292 and 0.256 mg/cm^3).

A two-way ANOVA test made it possible to determine how much the production method affected the phenolic contents. The extraction method factor had a greater partial eta squared (88.3 %) than the extraction time factor (44.6 %). Thus, this factor exerted a greater effect on the total phenolics than the extraction time and their interaction.

Figure 4

Effect of Extraction Time on Total Phenolic Content in Marigold Extracts



Total Flavonoid Content in Aqueous Marigold Extracts

The concentration of flavonoids was found using the calibration curve against the rutin concentration (Figure 2). Figure 5 demonstrates the effect of extraction time on the yield of flavonoids for all extraction methods.

The one-way ANOVA test revealed reliable differences for the average total flavonoids in samples with different extraction times, with the exception of microwave extraction ($F = 0.482$; $p \geq 0.05$). The calculated significance level exceeded $\alpha = 0.05$. Therefore, the null hypothesis that the factor had no effect was relevant. The extraction time significantly affected the flavonoid content in the extracts obtained by boiling, infusion with stirring, and ultrasound extraction.

The Tukey's post hoc test showed no significant differences ($p > 0.05$) in the average total flavonoids during conventional boiling for 1,800 s and 3,600 s (0.432 and 0.266 mg/cm^3, respectively), as well as for 15 and 30 min (0.607 and 0.432 mg/ml, respectively). For the extracts obtained by infusion with stirring, no significant difference was registered in the average total flavonoids for 300 s and 3600 s (0.199 and 0.243 mg/cm^3, respectively), 1,800 s and 3,600 s (0.348 and 0.243 mg/cm^3), and 900 s and 1,800 s (0.372 and 0.348 mg/cm^3). In the ultrasonicated samples, only 30 s and 90 s differed reliably.

The two-factor ANOVA test with respect to the total flavonoid content in the extracts showed that the extraction method factor had a greater partial eta squared (87.7 %) than the extraction time factor (70.3 %). This factor exerted a greater effect on the total flavonoid content than extraction time and their interaction. Therefore, the yield of flavonoids in the extracts strongly depended on the extraction method.

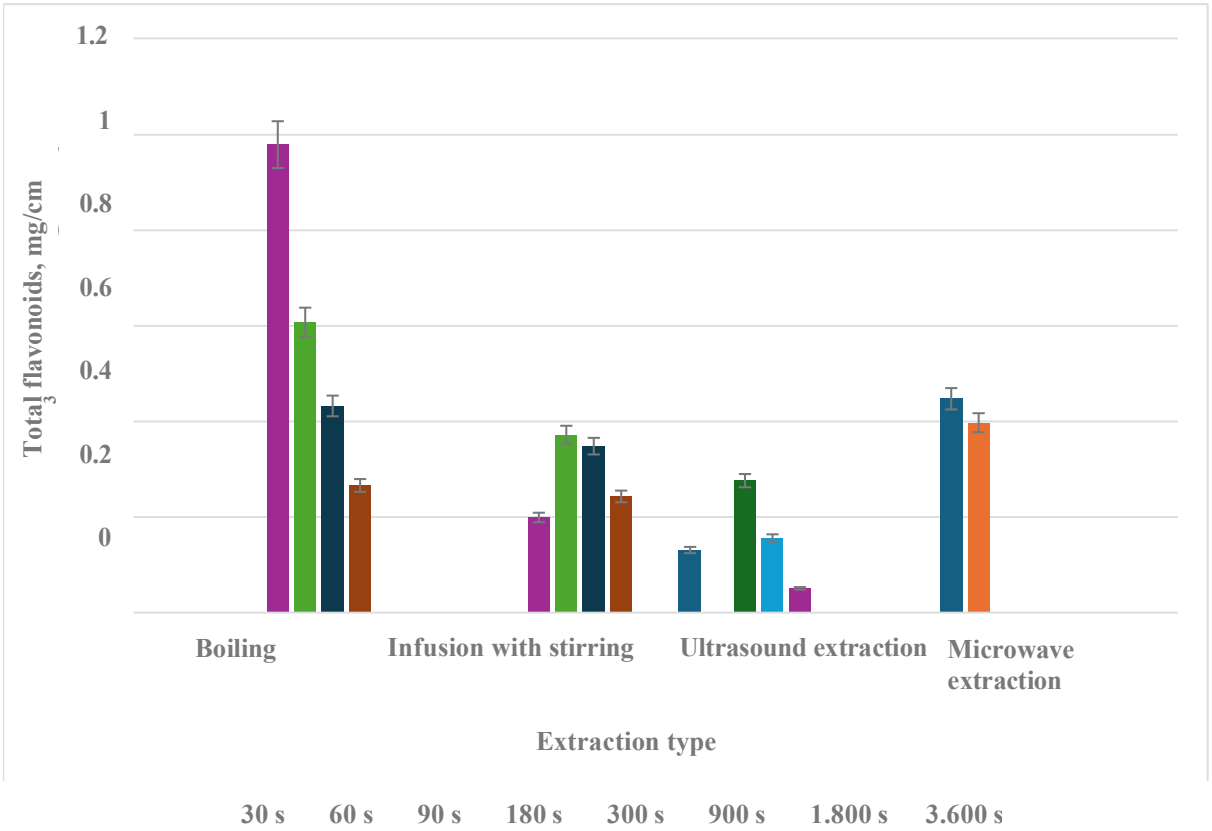
Antibacterial Activity of Aqueous Marigold Extracts

The antimicrobial properties of *T. patula* extracts were tested for extraction time effect for the extracts obtained by infusion with stirring on a magnetic stirrer.

The extracts were most effective against *E. coli* from skin, with a tendency for the lysis zone to increase with the

Figure 5

Effect of Extraction Time on Total Flavonoids in Marigold Extracts



extraction time. The results confirmed the antibacterial activity of the bioactive substances in the extracts. In the other clinical samples, the extracts acted selectively, with no pronounced dependencies detected.

The comparative statistical analysis of the empirical data revealed that the extraction method had a significant effect on the content of such biologically active substances as polyphenols and flavonoids in the aqueous extracts of *Tagetes patula* L. flowers. The largest total phenolics (0.34 mg/cm^3) belonged to the extracts obtained by microwave extraction for 30 s (350 W; 2,450 MHz). Microwave extraction proved to be an effective alternative method for extracting biologically active substances from marigold flowers: it was fast and provided a greater yield of bioactive substances.

Boiling for 300 s proved to be the optimal method for flavonoid extraction. Further boiling caused swelling, lowered the moisture content, and reduced

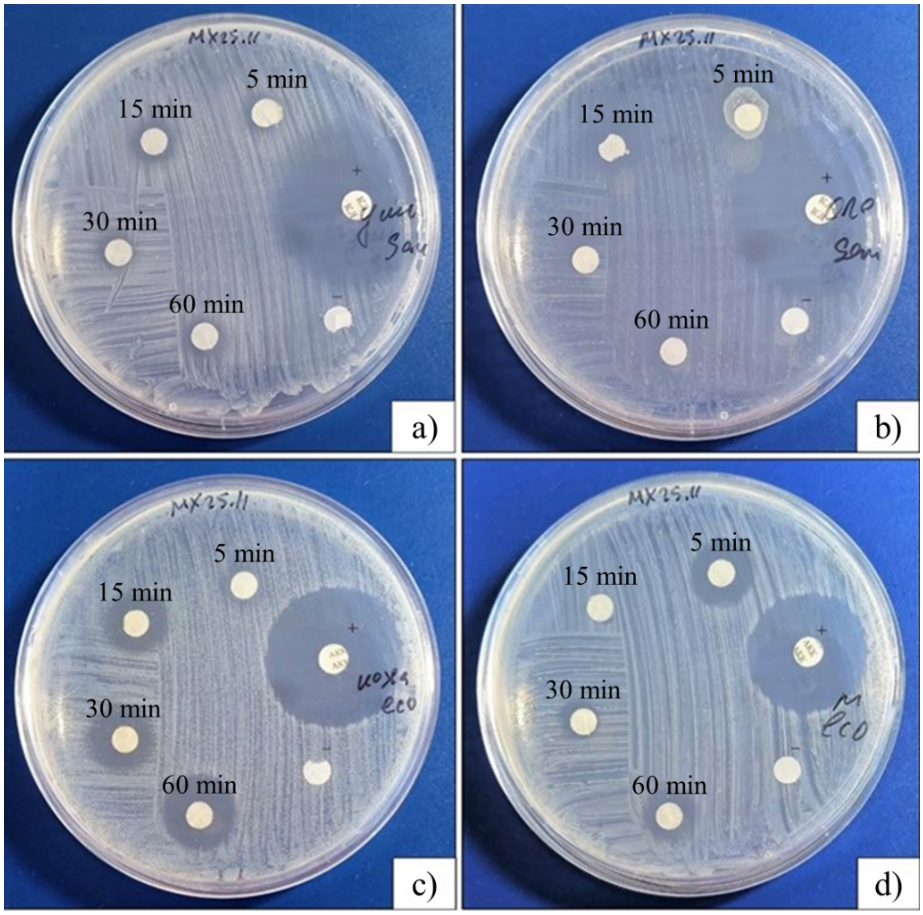
Table 2

Growth Inhibition Zones, mm

Clinical isolate	Extraction time				+	–
	5 min	15 min	30 min	60 min		
S. aureus (ears)	8.0	10.9	8.1	9.7	32.5	–
S. aureus (genitals)	14.1	12.8	–	9.8	34.8	–
E. coli (skin)	8.2	14.3	15.0	16.9	29.6	–
E. coli (urine)	14.3	–	9.2	12.4	24.2	–

Figure 6

Antibacterial Activity of Aqueous Marigold Extracts Obtained with a Magnetic Stirrer Against



Note. S. aureus from ears (a), *S. aureus* from genitals (b), *E. coli* from skin (c), and E. coli from urine (d).

the concentration of flavonoids, which indicated the insufficiency of the hydromodule in question.

The lowest yields of phenolics and flavonoids registered in the samples obtained by ultrasound extraction could be caused by low amplitude and short exposure time. However, some researchers reported ultrasound extraction as a promising method for extracting bioactive complexes from medicinal and plant materials to be applied together with aqueous extraction (Petkova, 2017; Sukhikh, 2022).

Power, frequency, temperature, and time were the variables that affected the yield of biologically active substances and their activity during ultrasonic extraction (Dzah, 2020). In addition, ultrasonic extraction is known to render aqueous extracts a certain aseptic effect that increases their shelf-life. For instance, Madhu *et al*. (Madhu, 2019) reported a parameter-dependent lower count of coliform bacteria, salmonella, yeast, and mold fungi in ultrasonic extracts.

In this regard, further studies could optimize the variables of ultrasonic aqueous extraction of biologically active substances from marigold flowers and highlight their antimicrobial activity.

Marigold flower extracts exerted a bactericidal effect on Gram-positive and Gram-negative bacteria isolated from various clinical materials. The lysis zone for *Staphylococcus aureus* was 9.7 mm for ears and 9.8 mm for genitals. The growth inhibition zone for *Escherichia coli* was 16.9 mm for skin and 12.4 mm for urine.

The extraction time correlated with the inhibition zone in the *E. coli* samples from skin. In other clinical samples, the extracts acted selectively. Probably, the extracts with different extraction time and methods had individual bioactive complexes, which targeted certain strains. This finding requires additional research in the chemical composition of aqueous marigold extracts.

Astafieva *et al*. (Astafieva, 2020) confirmed the antimicrobial properties of aqueous extracts of marigold flowers obtained by five-time one-hour boiling or infusing at room temperature for 3 days. The aqueous extracts were more aggressive to the Gram-negative culture of *E. coli* compared to the hydroalcoholic extracts. The extracts obtained by boiling had an inhibition zone of 23.0 mm; the extracts obtained by infusion demonstrated 35.0 mm of inhibition. The inhibition zones of the hydroalcoholic extracts were as low as 15.0 mm and 16.0 mm for the hot and cold methods, respectively. However, despite the initially high biological activity, the aqueous extracts gradually lost their antimicrobial properties during storage.

The antimicrobial properties of aqueous extracts of French marigold flowers could be explained by the phenolics and flavonoids in their composition. Further research in non-phenolic biologically active compounds and their effect on the antimicrobial activity of aqueous extracts seems to be a prospective direction.

CONCLUSION

This research revealed the effect of traditional and alternative aqueous extraction methods on the total content of phenolics and flavonoids in *Tagetes patula* L. flower extracts, as well as proved their antibacterial activity against Gram-positive and Gram-negative bacteria. Aqueous extracts of the French marigold demonstrated excellent chemical composition and properties for antimicrobial drugs to be used in medicine, veterinary, and agriculture. The fungicidal activity of aqueous marigold extracts could be used to develop biopreparations against pathogens and spoilage of agricultural plants.

CONTRIBUTION

Olga V. Kriger: developed the research concept; curated the date; proofread the manuscript.

Ekaterina I.Shepel: conducted the research; visualized the results; drafted the manuscript.

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