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Ultrasound-Assisted Extraction of Bioactive Compounds from *Tanacetum vulgare* L.: Antibacterial and Cytotoxic Evaluation

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Abstract

This study investigates ultrasound-assisted extraction (UAE) of bioactive compounds from *Tanacetum vulgare* L. collected in Central Kazakhstan's Akmola region, focusing on optimizing extraction parameters, analyzing chemical composition, and evaluating biological activity. The novelty lies in the first comprehensive analysis of *T. vulgare* populations under the region's extreme continental climate, known to affect metabolite accumulation. Using 70% ethanol, UAE at 20 minutes provided the highest extraction efficiency, as evidenced by a substantial recovery of phenolic compounds. High-performance liquid chromatography (HPLC) identified key bioactive components – luteolin (6.9 µg/mL), quercetin (5.0 µg/mL), apigenin (1.45 µg/mL), cynaroside (2.7 µg/mL), rutin (1.28 µg/mL), chlorogenic acid (1.1–1.14 µg/mL), and ferulic acid (2.46–2.69 µg/mL) – with extraction time significantly influencing their yield. The antibacterial assessment revealed strong inhibition against *Staphylococcus aureus*, with a 30-minute flower extract producing an inhibition zone of 34±1.1 mm, surpassing benzylpenicillin (30±1.1 mm). By contrast, weak or no activity was observed against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans*. In cytotoxicity tests using *Artemia salina*, all extracts – regardless of concentration or duration – resulted in 100% lethality, suggesting potential toxic effects. These findings underscore the impact of Kazakhstan's harsh ecological conditions on the phytochemical profile of *T. vulgare* and point to both the plant's promising pharmacological applications and the need for caution in its use.

Keywords:
Ultrasound-Assisted Extraction (UAE);
Tanacetum vulgare L.;
Phenolic Compounds;
Antibacterial Activity;
Cytotoxicity;
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1- Introduction

Medicinal plants have long been recognized as a rich source of therapeutic agents and continue to play a significant role in drug development and healthcare [1]. They produce a wide array of bioactive compounds that not only serve in the plants' own defense and survival but also provide valuable pharmacological effects for humans [2]. Given their importance, continuous research is required to document the chemical composition of medicinal flora and identify novel compounds with potential health benefits. This is particularly relevant for wild medicinal species, which may offer new phytochemicals and lead compounds for drug discovery [3].

One such plant is *Tanacetum vulgare* L. (common tansy), a perennial herbaceous species of the Asteraceae family, widely distributed throughout Eurasia and North America. Owing to its content of essential oils, phenolic compounds, and terpenes, *T. vulgare* exhibits a broad spectrum of biological activities. In traditional medicine, tansy extracts have been used for treating gastrointestinal disorders, stimulating appetite, and combating parasitic infections [4]. Additionally, its beneficial effects on the nervous system have been documented: phenolic compounds show antioxidant activity, reduce stress and anxiety levels, and make tansy a promising candidate for managing anxiety

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disorders [5]. *T. vulgare* is also widely applied in dermatology and cosmetology due to its antiseptic, analgesic, and wound-healing properties. Topical preparations based on this plant are employed as rinses, baths, compresses, and lotions for treating wounds, ulcers, pharyngitis, dermatitis, seborrhea, muscle spasms, bruises, arthritis, rheumatism, gout, and joint pain [6].

The pharmacological activity of tansy is attributed to its complex chemical composition, which includes terpenes, flavonoids, and phenolic acids. The primary therapeutic effects of this plant – choleretic, anthelmintic, antiseptic, hemostatic, spasmolytic, anti-inflammatory, and antimicrobial – are linked to compounds such as camphor, caryophyllene, borneol, cineole, and sabinene [7, 8]. The flowers of *T. vulgare* are considered valuable herbal raw material due to their high flavonoid content, which includes luteolin, apigenin, acacetin, quercetin, isorhamnetin, cynaroside, eupatilin, jaceidin, tilianin, scutellarin, and apigetrin [9]. These compounds play a key role in plant adaptation by protecting against ultraviolet radiation, pests, and pathogens. Flavonoids also exhibit strong antioxidant and anti-inflammatory activities, making them promising candidates for the development of novel phytopharmaceutical preparations [10].

At the same time, it is well established that the phytochemical profile of a medicinal plant can vary significantly depending on the ecological and climatic conditions of its habitat [11]. Plants of the same species growing in different environments often develop distinct chemotypes – populations characterized by dominant chemical constituents. Among the key factors influencing the accumulation of bioactive compounds in species such as *T. vulgare* are climate, altitude, soil composition, and environmental stressors [12]. Temperature, solar radiation, and precipitation levels directly affect the synthesis of secondary metabolites, including phenolic compounds that play a crucial role in plant defense [13]. High-altitude conditions, marked by increased ultraviolet radiation and low temperatures, stimulate the production of protective compounds, which may enhance the biological activity of plants [14]. The mineral composition of the soil, particularly nitrogen content, also affects the biosynthesis of certain metabolites, such as alkaloids [15]. Finally, abiotic stressors like drought and pollution activate plant defense mechanisms, thereby intensifying the synthesis of secondary metabolites [16].

T. vulgare is no exception. Recent studies confirm the considerable chemical diversity of *T. vulgare* and its intraspecific variability as influenced by environmental conditions [17]. It has been demonstrated that different tansy chemotypes vary in their terpene composition and may interact differently with surrounding biotic and abiotic factors, including insects and microbial communities [18, 19]. These findings support the idea that the phytochemical profile of tansy is shaped by a complex set of habitat-specific variables, including climate, soil composition, altitude, and ecological stress levels.

Central Kazakhstan presents a particularly compelling context for studying this variability. The region is characterized by a sharply continental climate, featuring extremely hot summers, cold winters, and low annual precipitation levels [20]. Such harsh environmental conditions impose significant stress on plants, which in turn can influence their metabolism. It has been hypothesized that plants growing under extreme climatic conditions may synthesize and accumulate unique secondary metabolites as adaptive responses [21]. Therefore, populations of *T. vulgare* growing in Central Kazakhstan may develop an unusual chemical profile or elevated levels of certain bioactive compounds, making them worthy of detailed phytochemical investigation. Analyzing *T. vulgare* collected in this region may reveal novel or enhanced components, contributing to the broader understanding of the species' chemical diversity.

Another critical factor influencing the composition of plant extracts is the extraction method used to isolate phytochemicals. Various techniques – such as maceration, Soxhlet extraction, and steam distillation—can produce extracts with differing qualitative and quantitative profiles of chemical constituents [22]. In recent years, ultrasound-assisted extraction (UAE) has emerged as a promising method for isolating bioactive compounds from plant material. UAE employs high-frequency sound waves to generate cavitation in the solvent, effectively disrupting plant cell walls and enhancing the release of intracellular contents [23]. This technique offers several advantages over conventional extraction methods, typically yielding a higher concentration of target compounds in a shorter time and at lower temperatures, thereby preserving thermolabile constituents. Moreover, UAE often requires less solvent and energy, aligning with the principles of green chemistry [24-27]. Consequently, it is considered a highly efficient method for extracting valuable phytochemicals. By applying ultrasound-assisted extraction, it is possible to obtain a broader spectrum of compounds from tansy and to maximize the yield of bioactive substances compared with traditional methods.

The objective of this study is to conduct a comprehensive analysis of the chemical composition and biological activity of ultrasound extracts of *Tanacetum vulgare* L. from Central Kazakhstan, determining the optimal extraction conditions for maximizing the yield of bioactive compounds and evaluating their antibacterial and cytotoxic properties.

2- Materials and Methods

2-1- Materials

2-1-1- Plant Material

The aerial parts of *T. vulgare* were collected from a population in the Akmola region, along the banks of the Ishim River in the Republic of Kazakhstan, in July 2020, during the full flowering stage. The botanical identification was

confirmed by a faculty member of the Department of Biomedicine at Karaganda Medical University [28, 29]. The plant material was dried in a dark, well-ventilated area and then ground to a particle size of 2–3 mm using an electric mill, passing through a 5 mm sieve. The raw material was not separated into flowers and leaves but combined into a single batch, forming a light green powder with a distinct, strong aroma.

2-1-1- Solvents and Reagents

For the experimental studies, chemical reagents and solvents of analytical grade (*p.a.*), highly pure (*puriss.*), and special purity (*puriss. spec.*) were used.

Hexane (C_6H_{14}) (Mr 86.2). 1042600. [110-54-3]. A colorless, highly flammable liquid, practically insoluble in water.

Ethyl acetate ($C_4H_8O_2$) (Mr 88.1). 1035300. [141-78-6]. A transparent, colorless liquid, soluble in water, miscible with 96% ethanol [30]. Used as a solvent.

96% Ethanol (C_2H_5OH) (Mr 46.07). 102500. [64-17-5]. A colorless, volatile, and highly flammable liquid. Hygroscopic, miscible with water and dichloromethane. Burns with a blue flame without soot. Ethanol (96%) was selected as a solvent due to its efficiency in extracting phenolic compounds and its compatibility with ultrasound-assisted extraction (UAE) [30].

Purified water (H_2O) (Mr 18.02). 1095500. [7732-18-5]. Water intended for the preparation of pharmaceutical formulations, except for sterile and pyrogen-free preparations, unless otherwise specified.

Metallic zinc (Zn) (Mr 65.38). 1049500. [7439-95-4]. A silvery-white wire or gray powder [31].

Acetic acid ($C_2H_4O_2$) (Mr 60.1). 1000300. [64-19-7]. A colorless liquid or white, shiny crystals resembling fern leaves. Easily miscible or soluble in water, 96% ethanol, glycerol (85%), and most fats and essential oils [31].

Hydrochloric acid (HCl) (Mr 36.46). 1043500. [7647-01-0]. A transparent, colorless, fuming liquid, miscible with water [31].

2-2- Methods

2-2-1- Ultrasound extraction

Ultrasound extracts of *T. vulgare* were obtained using a two-step extraction method from air-dried plant material (leaves, stems, and flower heads) with 70% ethanol, without prior maceration. The extraction was performed sequentially, using a plant material-to-solvent ratio of 1:20, in an ultrasound bath at a frequency of 20 kHz at room temperature (20–22°C) for 15, 20, and 30 minutes. After ultrasound treatment, the liquid extracts were filtered, and the solvent was evaporated at room temperature. The dried plant material was then re-extracted with 70% ethanol using the same ultrasound extraction (UE) procedure for 15, 20, and 30 minutes.

To enhance the extraction process from *T. vulgare* leaves and flowers, an ultrasound bath "Grad 40-35" was used at a frequency of 20 kHz, varying the number of extraction cycles.

At the initial stage, the ground plant material was soaked in hexane to extract the terpenoid fraction. The dried plant material was subsequently re-extracted using 70% ethanol.

The prepared extraction containers were placed in an ultrasound chamber, where they underwent ultrasound treatment at a frequency of 22 kHz for 15, 30, and 45 minutes. The extracts were then filtered and concentrated to dryness using a rotary evaporator [32, 33] (Figure 1).

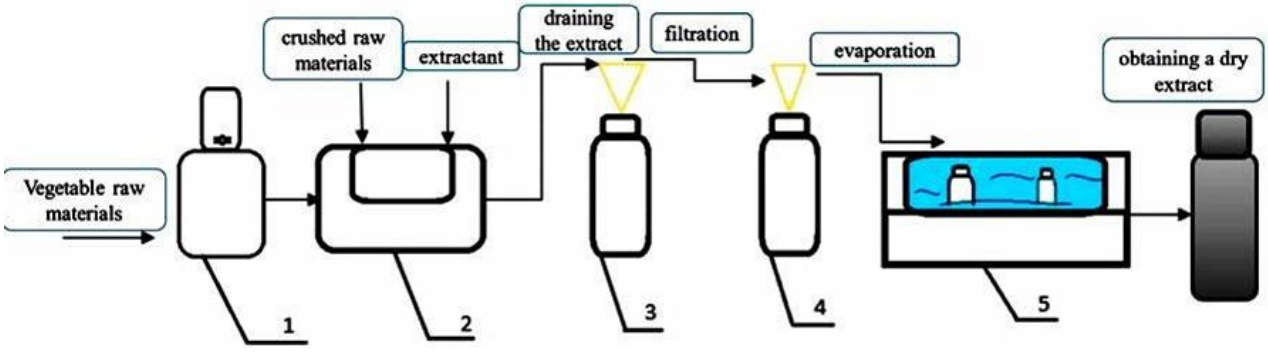

Figure 1. Apparatus scheme for obtaining dry ultrasound extracts of *T. vulgare*. (1 – Electric mill; 2 – Ultrasound extractor; 3 – Bottle with product; 4 – Bottle after additional filtration; 5 – Rotary evaporator)

After Ultrasound processing, the liquid extracts were filtered and left to dry at room temperature until a dry extract was obtained. As a result of the extraction, 10 samples of dark green color with a pleasant herbal aroma were obtained (Figure 2).

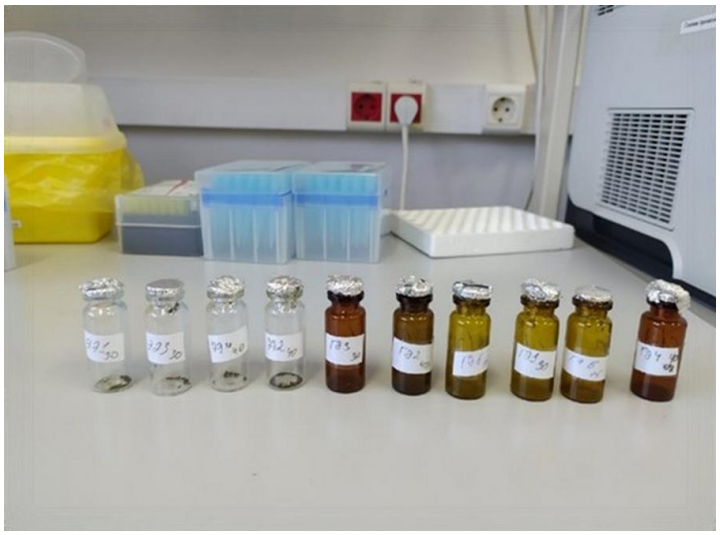

Figure 2. Dry extracts of *T. vulgare* obtained by the Ultrasound method at 15, 30, and 45 minutes

2-2-2- High-Performance Liquid Chromatography (HPLC)

The composition of polyphenolic components in the extracts was analyzed using high-performance liquid chromatography (HPLC) combined with ultraviolet detection and tandem mass spectrometry with electrospray ionization (ESI-MS/MS) in real-time mode [34]. The experiment utilized the following reagents: acetonitrile (ACN) for HPLC (≥99.9%, Sigma-Aldrich, France), formic acid (99–100%, AnalaRNORMAPUR®, VWR Chemicals, France), and purified water processed using the Milli-Q system (Millipore, France).

A set of 20 different phenolic compounds was used as standards, including caffeic, gallic, and chlorogenic acids, as well as luteolin, apigenin, kaempferol, and others (Sigma-Aldrich, USA).

All analyses were performed on an Agilent 1260 Infinity HPLC system (Agilent Technologies, USA), equipped with a four-channel pump (model G1311C 1260 Pump VL), an autosampler (G1329B 1260 ALS), a column thermostat (G1316A 1260 TCC), a variable-wavelength detector (G1314C 1260 VWD VL+), and a quadrupole mass spectrometer (G6130A Quadrupole LC-MS/MS). The ChemStation software package under Windows NT was used for data processing.

Separation was carried out on a reversed-phase Zorbax Eclipse Plus C18 column (150 × 4.6 mm, 3.5 µm sorbent, Agilent Technologies, USA). The gradient system included phases A and B, both containing 2.5% formic acid. In phase A, water was used as the solvent, whereas in phase B, acetonitrile served as the solvent. The sequential changes in eluent composition occurred over 40 minutes as follows: Start (0 min) – 3% B; 7 min – 20% B; 7.10 min – 30% B; 27 min – 40% B; 35 min – 50% B; 35.10 min – 20% B; 40 min – 3% B.

The flow rate was maintained at 0.4 mL/min, and the column was kept at 30 °C. Before injection, all Ultrasound extracts and standard samples were prepared in an acetonitrile: water (1:1, v/v) mixture at a volume of 20 µL. After passing through the column, the flow proceeded through a UV detector (wavelengths 280 nm and 360 nm) before entering the mass spectrometer with an electrospray ionization source.

The optimal measurement parameters in negative ESI mode were set as follows: capillary temperature 350 °C, nitrogen gas flow 8 L/min, and atomizer pressure 45 psi. Registration was conducted in multiple reaction monitoring (MRM) mode, tracking specific mass transitions at predefined retention times.

To confirm the structure and identify compounds, retention times were compared with known standards, and data obtained via the Agilent G6130A LC-MS/MS system were analyzed. Quantitative and qualitative analysis of phenolic compounds was performed using the external standard method, wherein the corresponding extracts, dissolved in a formic acid: water (1:1) mixture, were loaded into 1 mL vials.

2-2-3- Study of Antibacterial Activity

The antibacterial activity of the obtained samples was evaluated using the disk diffusion method and serial dilution method. Sterile disks impregnated with a predetermined dose of the sample were placed on the surface of agar plates inoculated with test microorganisms. The antibacterial properties of the sample solutions (10, 100, and 250 mg/mL) were determined using the agar diffusion method with wells (8 mm in diameter). A volume of 0.1 mL of the test solutions

was introduced into each well. Petri dishes were pre-incubated in a refrigerator for two hours to allow diffusion of the tested substance. The presence of antibacterial activity was assessed based on the formation of inhibition zones, where microbial growth was absent, after 24-hour incubation [35].

Benzylpenicillin sodium salt was used as a reference standard for antibacterial activity, while nystatin was used as the standard for antifungal activity. The concentrations of the tested compounds were set at 1 µg for antibacterial activity and 1 µg for antifungal activity. The reference compounds were tested at a concentration of 1 mg/mL. The antimicrobial activity of the samples was assessed by measuring the diameters of the inhibition zones around the test wells (in mm). The activity was categorized as follows: inhibition zones smaller than 10 mm or complete microbial growth were considered as no activity, zones of 10–15 mm indicated weak activity, 15–20 mm represented moderate activity, and zones larger than 20 mm were classified as strong activity. Each sample was tested in three parallel experiments.

Dilutions were prepared at a ratio of 1 mg of the substance per 1 mL of solvent. The sensitivity of bacteria to the tested samples was determined using the disk diffusion method. Four bacterial strains were used in the experiment: *Staphylococcus aureus* 6538, *Bacillus subtilis* 6633, *Escherichia coli* BL/Pet32/VPI, and *Candida albicans* ATCC 885-653.

The tested cultures were inoculated using the lawn-spreading method on the following selective media: nutrient agar (NA), Endo agar, standard nutrient agar, and Sabouraud agar. Petri dishes were then incubated for 24 hours at 37°C, except for the Sabouraud agar plates, which were incubated at 28°C.

For *S. aureus*, a suspension containing 10^5 colony-forming units (CFU) of the 24-hour culture was applied to a Petri dish with NA. Similarly, for *B. subtilis*, a suspension containing 10^5 CFU of the test strain was applied to a Petri dish with meat-peptone agar (MPA). The test sample, ethanol, and sterile physiological saline (control) were added to the wells in 20 µL volumes. After 24 hours of incubation at 37°C, the inhibition zones were measured. The absence of an inhibition zone was considered indicative of no antimicrobial activity.

2-2-4- Study of the Cytotoxic Activity of Tanacetum vulgare L. Extracts

To assess the cytotoxic activity, brine shrimp (*Artemia salina*) were used. The methodology was based on comparing the mortality rate of *A. salina* (Leach) in the experimental samples with that in the control water, which did not contain toxic substances. The criterion for acute lethal toxicity was considered to be a mortality rate of 50% or more of *A. salina* (Leach) in the experimental sample compared to the control.

Dilutions were prepared at a ratio of 0.25 g of the test substance per 1 mL of solvent. Each sample was tested in three parallel experiments. The experiment was conducted at a temperature of 20 ± 2°C under natural light conditions. The salinity of the artificial seawater used as a control was maintained at 8.0–8.5 (pH). During the bioassay, *A. salina* larvae were two days old, with a stocking density of 6–12 individuals per test tube. The bioassay conditions included pH, temperature (within +23 ± 2°C), oxygen content, and salinity. The cytotoxic activity was evaluated using the *A. salina* survival assay. The incubator was filled with artificial seawater, and *A. salina* cysts were added. Over two days, with gentle aeration, the brine shrimp hatched from the cysts [36]. As reference substances, 70% ethanol and the commercial herbal preparation "Troychatka Evalar" (manufactured by Evalar, Russia) were used. "Troychatka Evalar" is a standardized phytopreparation composed of extracts from *Artemisia absinthium* (wormwood), and *Syzygium aromaticum* (clove), commonly used for its antiparasitic and digestive health properties. The tested samples were evaluated at concentrations of 0.25 g/mL, 0.2 g/mL, and 0.1 g/mL. The exposure duration for the test organisms was 48 hours (Table 1).

Table 1. Experimental design for assessing the cytotoxic activity of *Tanacetum vulgare* extracts using *Artemia salina*

Sample ID	Extract Dilution	Initial Number of *Artemia salina* in Test Water	Expected Number of Dead *Artemia salina* After 48 Hours
XV-I	0.25 g/mL	6	To be determined
XV-II	0.2 g/mL	6	To be determined
XV-III	0.1 g/mL	6	To be determined
XX-I	0.25 g/mL	6	To be determined
XX-II	0.2 g/mL	6	To be determined
XX-III	0.1 g/mL	6	To be determined
XXX-I	0.25 g/mL	6	To be determined
XXX-II	0.2 g/mL	6	To be determined
XXX-III	0.1 g/mL	6	To be determined
Control (water with NaCl for required salinity)	Control	6	Expected survival
Control (70% ethanol)	Control	6	To be determined
Control (Troychatka Evalar)	0.25 mg/mL	6	To be determined

2-3-Statistical Methods

All experiments were conducted in triplicate (n=3), and the results are presented as mean ± standard deviation (SD). Only descriptive statistical methods were applied to summarize and interpret the data. Data processing and graphical representations were carried out using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA).

3- Results

3-1- Ultrasound Extraction of Tanacetum vulgare L. Plant Material

To evaluate the efficiency of ultrasound-assisted extraction (UAE) for bioactive compounds from *Tanacetum vulgare*, two solvents were utilized: nonpolar hexane and 70% ethanol. The selection of these solvents was based on their differing polarities, allowing for the assessment of the extraction efficiency of both lipophilic and hydrophilic compounds.

At the first stage of the experiment, hexane extraction was performed at three different time intervals: 15, 30, and 45 minutes. The results demonstrated that the highest yield of extractive substances was observed at 30 minutes, amounting to 0.6% for leaves and 0.5% for flowers. Increasing the extraction time to 45 minutes led to a decrease in yield by 1.2-fold for leaves and 22% for flowers. This decline may be attributed to the partial degradation of thermolabile compounds under prolonged ultrasound exposure (Table 2).

Table 2. Yield of *T. vulgare* extracts at different ultrasound extraction durations

Sample	Time of extraction	Yield
HE1 (leaves)	45 min	0.25±0.1%
HE2 (flowers)		0.41±0.2%
HE3 (leaves)	30 min	0.6±0.2%
HE4 (flowers)		0.5±0.4%
HE5 (leaves)	15 min	0.35±0.15%
HE6 (flowers)		0.28±0.3%
EE1 ((leaves)	30 min	9.54±0.13%
EE2 (flowers)		10.0±0.2%
EE3 (leaves)	45 min	13.6±0.21%
EE4 (flowers)		9.7±0.1%

HE – USE with hexane; EE – USE with ethanol.

As the second solvent for ultrasound extraction, 70% ethanol was selected. The analysis of data obtained using 70% ethanol in ultrasound-assisted extraction (UAE) revealed the following patterns: at 15 minutes, no significant yield of extractive substances was observed. The maximum content of extractives was achieved at 45 minutes of extraction. Compared to the yield at 30 minutes, extending the extraction time to 45 minutes led to a substantial increase (by 45%) in extract yield from leaves. However, the yield from flowers remained unaffected by extraction time, staying within the statistical margin of error (Table 2).

At the next stage of the study, a modified experiment was conducted using mixed plant material, without separating leaves and flowers. 70% ethanol was again used as the extraction solvent. Based on the results of the first experiment, new time intervals of 15, 20, and 30 minutes were tested. The experimental results demonstrated that ultrasound extraction with 70% ethanol provided the highest yield of dry matter and bioactive compounds after 20 minutes of extraction (Table 3).

Table 3. Yield of extractive substances (%) from *Tanacetum vulgare* L. herb at different ultrasound extraction durations

Sample number	Liquid extract mass (grams)	Dry extract mass (grams)	Extractive yield (%)
15 minutes USE sample 1	3.5399	0.3757	10.6
15 minutes USE sample 2	1.8843	0.3763	19.9
15 minutes USE sample 3	3.0432	0.368	10.09
20 minutes USE sample 1	2.3511	0.3292	14
20 minutes USE sample 2	1.7811	0.6934	35.8
20 minutes USE sample 3	2.2594	0.2474	10.94
30 minutes USE sample 1	5.8217	0.1064	1.8
30 minutes USE sample 2	3.9933	0.1792	4.5
30 minutes USE sample 3	3.2983	0.3049	9.2

The 15-minute extraction yielded a dry residue mass comparable to that of the 20-minute extraction but with a 60% reduction in active compounds. In contrast, the 30-minute extraction resulted in a decreased yield of both dry residue and active compounds. Specifically, the concentration of active compounds in the 30-minute extraction was 2.5 times lower than in the 15-minute UAE and 4 times lower than in the 20-minute UAE (Table 3).

3-2- Analysis of the Phenolic Composition of Ethanol-Based Ultrasound Extracts of T. Vulgare Using High-Performance Liquid Chromatography (HPLC)

At the third stage of the study, the phenolic composition of ethanol-based ultrasound extracts of *Tanacetum vulgare* was analyzed using high-performance liquid chromatography (HPLC). All samples exhibited a similar qualitative composition; however, the concentrations of individual phenolic acids and flavonoids varied. The most significant components of the extracts were apigenin, luteolin, quercetin, cynaroside, and rutin. Additionally, all samples contained chlorogenic acid (1.1–1.14 µg/mL) and ferulic acid (2.46–2.69 µg/mL). Among the predominant phenolic compounds, flavonoids were of particular value: luteolin (6.9–4.3 µg/mL) and quercetin (5.0–3.2 µg/mL) (Figure 3).

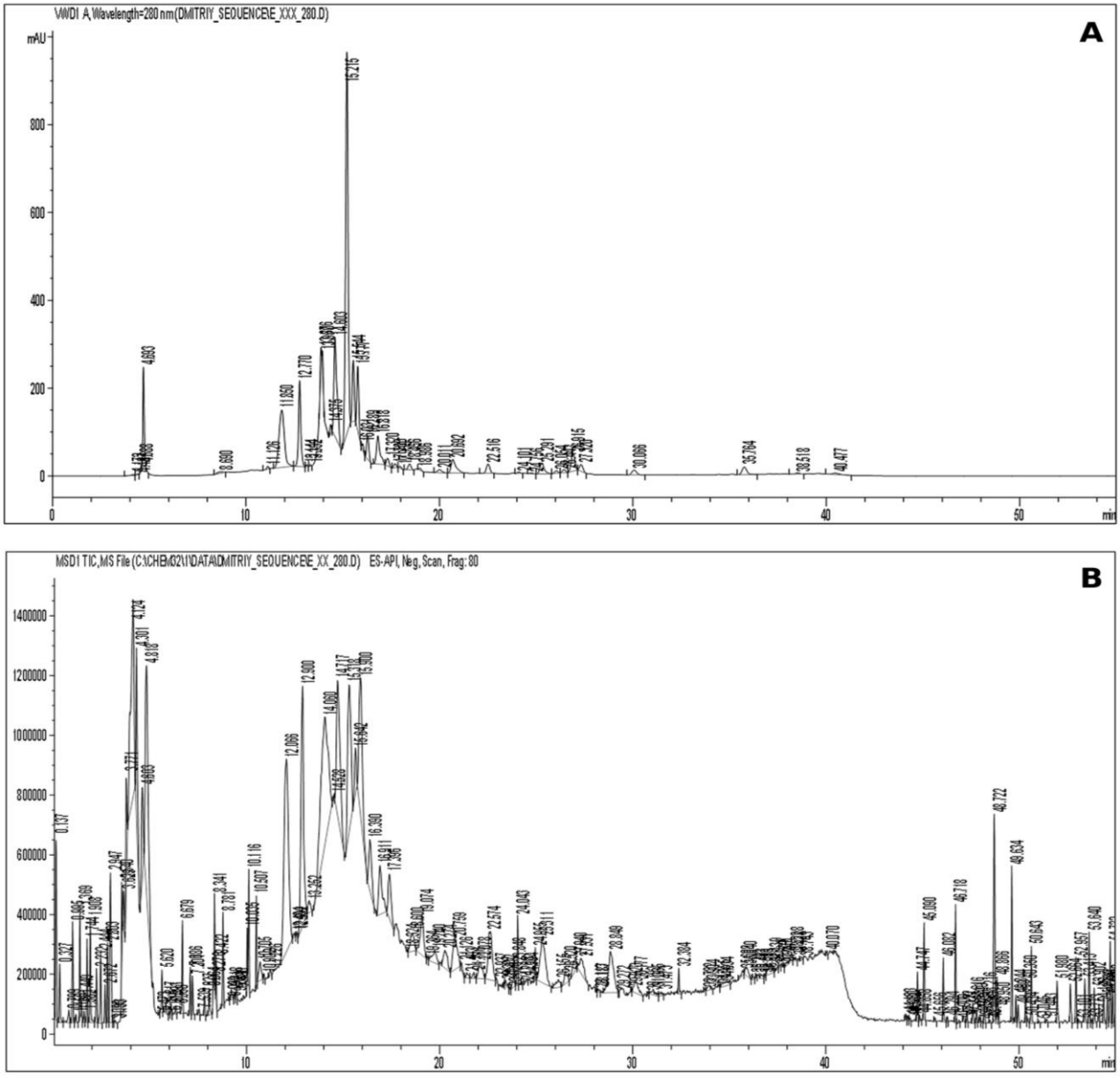

Figure 3. Chromatographic profile of phenolic compounds in Ultrasound extracts of *Tanacetum vulgare* L. (A. Chromatographic profile of extracts obtained at 15 minutes, showing the highest content of luteolin (6.9 µg/mL) and quercetin (5.0 µg/mL); 20 minutes – an increase in the concentration of cynaroside (2.7 µg/mL) and apigenin (1.45 µg/mL); B. Chromatographic profile of extracts obtained at 20 minutes, revealing a high content of chlorogenic acid (1.1–1.14 µg/mL) and ferulic acid (2.46–2.69 µg/mL). The x-axis (horizontal axis) represents retention time (minutes), indicating the time required for each compound to pass through the chromatographic column. The y-axis (vertical axis) represents absorbance (mAU – milli-absorbance units), which correlates with the concentration of detected compounds.

Notably, the 15-minute extraction yielded the highest content of luteolin (6.9 µg/mL) and quercetin (5.0 µg/mL), whereas increasing the extraction time to 20 minutes led to an increase in the concentration of cynaroside (2.7 µg/mL) and apigenin (1.45 µg/mL). Further prolongation of the extraction time to 30 minutes resulted in a higher rutin content (1.28 µg/mL). However, at 45 minutes of extraction, a decrease in the overall content of phenolic compounds was observed, indicating potential degradation of thermolabile components due to prolonged Ultrasound treatment (Figure 4).

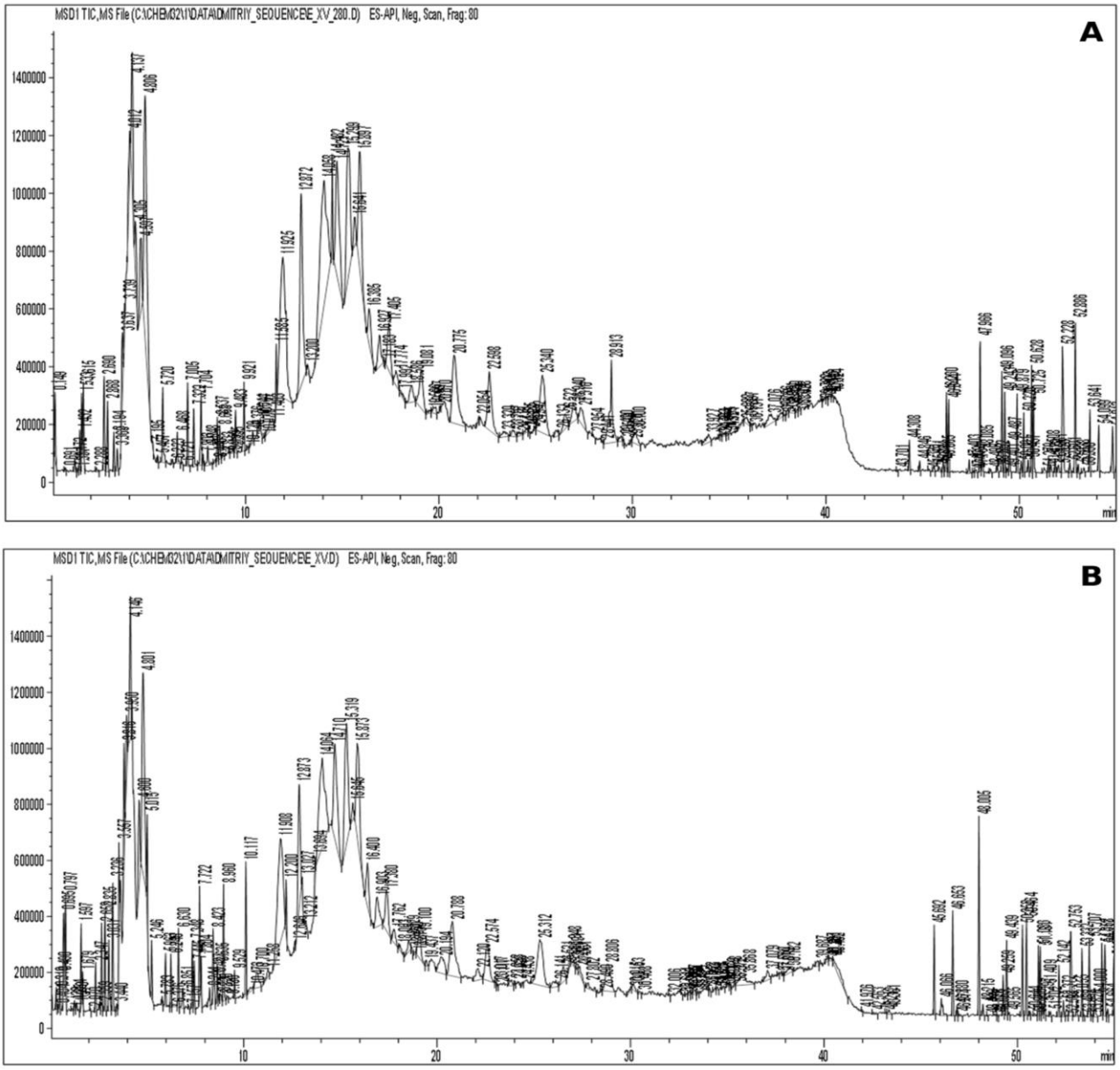

Figure 4. Dynamics of changes in phenolic compounds in Ultrasound extracts of *Tanacetum vulgare* L. with increasing extraction time. (A. 30 minutes – an increase in rutin content (1.28 µg/mL), accompanied by a decrease in luteolin and quercetin levels; B. 45 minutes – a decline in the overall content of phenolic compounds, indicating possible degradation of thermolabile components due to prolonged Ultrasound treatment). The x-axis (horizontal axis) represents retention time (minutes), indicating the time required for each compound to pass through the chromatographic column. The y-axis (vertical axis) represents absorbance (mAU – milli-absorbance units), which correlates with the concentration of detected compounds.

Thus, the results of the study confirm that Ultrasound extraction with 70% ethanol is most effective at 20 minutes of processing. The obtained data allow for the identification of patterns in the accumulation of phenolic compounds depending on the extraction time, which has practical significance for the standardization of methods for obtaining *T. vulgare* extracts.

3-3-Study of the Antibacterial Activity of Tanacetum vulgare L. Extracts

This study also assessed the antibacterial activity of Ultrasound ethanolic extracts of *Tanacetum vulgare* L., revealing their selective action against various microorganisms.

The ethanolic Ultrasound extract of *T. vulgare* flowers, obtained after 30 minutes, demonstrated the most pronounced bactericidal effect against *S. aureus* (34±1.1 mm), exceeding the reference value—benzylpenicillin (30±1.1 mm). Against *B. subtilis*, the flower extract (30 min) showed weak activity (11±1.1 mm), while against *E. coli* and *Candida albicans*, the activity was almost absent (6±1.1 mm). The flower extract (30 min) exhibited moderate activity against *Ps. aeruginosa* (19±1.1 mm) (Table 4).

Table 4. Mean values of inhibition zone diameters for test strains (mm)

Sample Code	*Staphylococcus aureus* 6538 (mm)	*Bacillus subtilis* 6633 (mm)	*Escherichia coli* BL/Pet32/VPI (mm)	*Pseudomonas aeruginosa* ATCC 9027 (mm)	*Candida albicans* ATCC 885-653 (mm)
Ultrasound ethanolic extract of *T. vulgare* leaves (30 min)	15±1.1	9±1.1	16±1.1	6±1.1	6±1.2
Ultrasound ethanolic extract of *T. vulgare* leaves (45 min)	22±1.1	10±1.2	15±1.1	6±1.2	6±1.1
Ultrasound ethanolic extract of *T. vulgare* flowers (30 min)	34±1.1	11±1.1	6±1.1	19±1.1	6±1.1
Ultrasound ethanolic extract of *T. vulgare* flowers (45 min)	6±1.2	10±1.2	6±1.2	18±1.2	6±1.2
Ultrasound ethanolic extract of *T. vulgare* leaves and flowers (15 min)	15±1.1	11±1.1	8±1.1	-	-
Ultrasound ethanolic extract of *T. vulgare* leaves and flowers (20 min)	12±1.1	10±1.1	17±1.1	-	-
Ultrasound ethanolic extract of *T. vulgare* leaves and flowers (30 min)	15±1.2	11±1.2	12±1.2	-	-
Benzylpenicillin sodium salt	30±1.1	30±1.2	13±1.2	30±1.1	-
Nystatin	-	-	-	-	18±1

Note: "-" indicates no inhibition zone. Growth inhibition diameters <10 mm or full bacterial growth in the Petri dish were considered as no antimicrobial activity, 10–15 mm as weak activity, 15–20 mm as moderate activity, and >20 mm as strong activity.

The extracts from leaves (30 and 45 min) demonstrated moderate and high activity, respectively, against *Staphylococcus aureus* (15±1.1 and 22±1.1 mm) and moderate activity against *Escherichia coli* (16±1.1 and 15±1.1 mm), but were less effective against *Bacillus subtilis* (9±1.1 and 10±1.2 mm). The extracts obtained from the mixed plant material (leaves and flowers) exhibited generally weak to moderate activity against *S. aureus* and *E. coli*, while no inhibitory effect was observed against *Pseudomonas aeruginosa* and *Candida albicans*.

3-4- Cytotoxic Activity of Tanacetum vulgare L. Extracts

All ethanol-based ultrasonic extracts of *T. vulgare* exhibited strong cytotoxic effects at all tested concentrations and exposure durations. The results indicate that all ethanol ultrasonic extracts of *T. vulgare* L. (XV, XX, XXX), regardless of the applied concentration (0.25, 0.2, or 0.1 mg/mL) and the duration of ultrasonic extraction (15, 20, or 30 min), resulted in 100% mortality of the tested larvae (Table 5).

Table 5. Cytotoxic activity of tested *Tanacetum vulgare* L. extracts

Sample Code	Extract Concentration (mg/mL)	Number of Larvae in Control	Number of Larvae in Sample	Survival Rate in Control (%)	Mortality Rate (%)
XV-I	0.25	12	0	100	100
XV-II	0.2	12	0	100	100
XV-III	0.1	12	0	100	100
XX-I	0.25	12	0	100	100
XX-II	0.2	12	0	100	100
XX-III	0.1	12	0	100	100
XXX-I	0.25	12	0	100	100
XXX-II	0.2	12	0	100	100
XXX-III	0.1	12	0	100	100
Control (Trojchatka Evalar)	0.25 mg/mL	12	0	100	100
Control (70% Ethanol)	Control	12	0	100	100
Control (NaCl-enriched water)	Control	12	12	100	0

XV – ethanol ultrasonic extract of *T. vulgare* obtained within 15 min; XX – ethanol ultrasonic extract of *T. vulgare* obtained within 20 min; XXX – ethanol ultrasonic extract of *T. vulgare* obtained within 30 min.

Similarly, 100% mortality was recorded in control samples containing Trojchatka Evalar (0.25 mg/mL) and 70% ethanol, whereas full survival (0% mortality) was observed in the control sample containing NaCl-enriched water. These findings indicate a pronounced cytotoxic activity of the tested extracts, which remains unaffected even with a decrease in concentration and variations in ultrasonic extraction time.

4- Discussion

In recent years, considerable attention has been paid to studying the biologically active compounds found in wild flora. The value of wild-growing plants is attributed to their high adaptability to diverse environmental conditions, which is reflected in their unique chemical composition [37]. The accumulation of biologically active substances in plants directly depends on factors such as soil composition, climatic conditions during growth, and specific developmental phases. Understanding the composition of plant raw materials is crucial, as it allows for evaluating their potential applications in medicine, pharmacy, and cosmetics [38]. Despite numerous scientific investigations into the chemical composition of *Tanacetum vulgare*, it is important to recognize that the extract composition may vary depending on geographical origin and extraction method.

It has been shown that ultrasound accelerates and enhances the disruption of plant cell membranes, intensifying the extraction process and increasing the concentration of target metabolites in solution [39]. However, an excessive increase in ultrasound treatment duration does not always lead to a higher yield of extracts and can cause the degradation of thermolabile phenolic compounds [40], as well as the formation of free radicals that damage certain flavonoid structures. A similar effect was noted in more recent reviews on ultrasonic extraction (UAE), which reported that prolonged processing time or high ultrasound power reduces the yield of some polyphenols (e.g., catechins) due to oxidative or thermal degradation [41]. Therefore, when planning extraction, it is necessary to balance the sufficient disruption of cellular structures with the minimal structural loss of sensitive compounds.

On the other hand, the choice of solvent critically affects both the selectivity and yield of the extracted fractions. Using hexane (a nonpolar solvent) primarily isolates terpenoids and other lipophilic components, whereas 70% ethanol (a mixture of water and ethanol) extracts polar metabolites, including phenolic acids and flavonoids [42]. Several studies have shown that hydroethanolic solutions (50–70% ethanol) provide a significant yield of phenolic compounds from various parts of *T. vulgare* [43]. In our study, using hexane resulted in the highest yield of extractable substances from leaves and flowers at 30 minutes of ultrasonic extraction (0.6%), but extending the processing time to 45 minutes led to a decrease in yield, likely due to the partial degradation of thermolabile compounds or solvent saturation.

By contrast, with 70% ethanol extraction, the maximum content of extractable substances (up to 13.6% in certain fractions) was observed at 45 minutes of ultrasonic treatment when leaves and flowers were considered separately. However, in experiments with a mixture of raw materials (leaves + flowers), the highest total mass of the dry extract and the most balanced phenolic content were already recorded at 20 minutes (35.8%). Notably, a 30-minute extraction under the same conditions sometimes led to a decrease in total phenol yield, which agrees with observations of possible flavonoid degradation under prolonged ultrasonic exposure [44].

Our results are consistent with reports that *T. vulgare* contains a rich spectrum of phytochemicals, including terpenoids, essential oils, and phenolic components [45]. In particular, tansy essential oils are characterized by high levels of mono- and sesquiterpenes (camphor, sabinene, borneol, 1,8-cineole, etc.) [36, 37], which justifies the use of nonpolar solvents (hexane, CO_2, etc.) to isolate volatile compounds. At the same time, many studies indicate a high content of flavonoids (luteolin, quercetin, apigenin, rutin, etc.) and phenolic acids (chlorogenic, caffeic, dicaffeoylquinic) in ethanol extracts of *T. vulgare* [46]. As our experiments showed, the highest concentrations of luteolin (6.9 µg/mL) and quercetin (5.0 µg/mL) were achieved with a 15-minute ultrasonic extraction using 70% ethanol, while 20 minutes was required to maximize the extraction of compounds such as cinaroside (2.7 µg/mL) and apigenin (1.45 µg/mL). Interestingly, with a 30-minute extraction, the rutin content (1.28 µg/mL) increased, whereas the overall amount of several free flavonoids decreased. These dynamics can be explained by differences in solubility, binding strength with cellular matrices, and flavonoid stability under ultrasound.

The geographical origin of tansy samples also influences their composition, as shown by our findings. We determined that the total phenolic content in tansy extracts from arid zones can surpass values reported for more humid regions. The literature indicates significant variability in the phytochemical composition of *T. vulgare* depending on the growing location. For instance, Šukele et al. (2023) compared tansy samples collected from various regions of Latvia and reported a range of total phenolic content from about 40 to 140 mg GAE/g, with such differences being difficult to explain by geography alone. According to their data, the concentration of phenolic substances strongly depends on habitat type and growth conditions [47].

In our case, tansy from arid steppe habitats may accumulate more phenolic compounds as an adaptive response to stress conditions, which aligns with the general trend of increased polyphenol synthesis under plant abiotic stress. For comparison, a study examining the chemical properties of *T. vulgare* in different natural habitats found that the highest concentration of flavonoids (0.52%) was present in samples collected from ruderal and reclaimed sites, while the maximum content of phenolic acids (2.42%) was recorded in samples from ruderal areas. The authors attributed the observed differences in *T. vulgare* chemical composition to ecological and genetic factors [4]. An analysis of *T. vulgare*

from two different locations in western Romania using ultra-high-performance liquid chromatography (UHPLC) identified ascorbic acid, riboflavin, pyrocatechol, rutin, quercetin, and kaempferol [40]. Moreover, volatile compounds, phytochemical profiles, and antioxidant activities of several medicinal plants from Bulgaria (including *T. vulgare*) have been studied by GC-MS, showing total phenolic content ranging from 0.55 to 47.39 mg GAE/g dry weight and flavonoid content from 0.45 to 17.27 mg CE/g dry weight. Our data fall toward the higher end of this range, possibly due to the more extreme conditions (high insolation, drought) in the collection region.

Overall, our phytochemical analysis confirms that *T. vulgare*, regardless of region of growth, is a rich source of phenolic compounds (flavonoids, caffeoylquinic acids, tannins), reinforcing the conclusion that this species contains high levels of valuable biologically active substances.

All the aforementioned flavonoids and acids are known for their antioxidant, anti-inflammatory, and antimicrobial properties [48]. Our evaluation of antimicrobial activity confirmed the selective effects of *T. vulgare* extracts against various Gram-positive and Gram-negative strains. In particular, the ultrasonic ethanol extract of flowers (30 minutes) was most active against *Staphylococcus aureus*, surpassing benzylpenicillin in effectiveness [49]. Similarly, the literature describes high sensitivity of *S. aureus* to tansy essential oil, including strains from the ESKAPE group [10]. Meanwhile, activity against *Bacillus subtilis* in our experiments was considerably lower, and activity against *E. coli* and *Candida albicans* was practically negligible. This specificity may stem from differences in the cell wall structure of Gram-negative bacteria and fungi, as well as the differing modes of action of phenolic compounds and terpenoids. Several authors note that phenolic-rich fractions of *T. vulgare* typically show stronger inhibition of Gram-positive pathogens, whereas essential oils or lipophilic components exhibit certain fungicidal effects—though often less pronounced against *Candida* yeasts [46].

In addition to antimicrobial potential, we found that ultrasonic extracts of *T. vulgare* exhibit high cytotoxic activity. In our experiments, all ethanol extracts (15, 20, and 30 minutes) caused 100% lethality of *Artemia salina* larvae at all tested concentrations. Similar data on the pronounced cytotoxic effects of tansy – against both cancer and normal cell cultures – have also been reported by other researchers [50]. It is suggested that sesquiterpene lactones (e.g., artecanin, tanacetin), along with high concentrations of phenolic metabolites, may exert proapoptotic effects, especially at elevated doses. Indeed, methanol and ethanol extracts of *T. vulgare* rich in phenolics have shown a potent antiproliferative effect against HeLa and other tumor cell lines; however, these compounds appear to have low selectivity and can also suppress the viability of normal cells [51]. This underscores the necessity for strict control of the concentration and duration of use of such phytoextracts when developing herbal preparations.

In summary, our findings support the conclusion that ultrasonic extraction with a moderately polar solvent (70% ethanol) efficiently extracts phenolic components from *T. vulgare*, whereas hexane extraction enriches the extract with lipophilic terpenes. The composition of the extracts directly influences their biological activity: phenolic compounds primarily determine antioxidant and anti-inflammatory properties, whereas terpenoid fractions (essential oils) impart strong antimicrobial effects, particularly against Gram-positive microorganisms. The high cytotoxicity of both types of extracts suggests that sesquiterpene lactones and certain flavonoids may be the main agents responsible for their potent cytotoxic mechanism of action. The duration of ultrasonic treatment must be optimized: overly short extraction times do not ensure complete extraction of bound glycosidic forms, whereas prolonged ultrasound exposure may lead to degradation or oxidation of thermolabile compounds [52].

Thus, our research underscores the importance of a comprehensive approach in selecting both ultrasonic extraction parameters and solvent types. It also demonstrates that *T. vulgare* from Central Kazakhstan possesses a phytochemical profile similar to that described in other regions [53], potentially enhanced by harsh climatic conditions. The data obtained on the selective extraction of diverse groups of metabolites and the high biological activity of ethanol-based ultrasonic extracts (especially against *S. aureus*) provide a basis for further studies aimed at developing new phytopreparations – with careful consideration of safety and dosage due to the high cytotoxicity observed.

5- Conclusion

This study demonstrates the efficiency of ultrasound-assisted extraction (UAE) for obtaining bioactive compounds from *Tanacetum vulgare* L. and highlights the crucial role of extraction parameters in determining yield and biological activity. Our findings reveal that using 70% ethanol under optimized ultrasonic conditions can substantially increase the extraction of phenolic compounds, including key flavonoids (luteolin, quercetin, apigenin, cynaroside, rutin) and phenolic acids (chlorogenic and ferulic acids). Notably, moderate processing times (15–20 minutes) produced the highest yields of target metabolites, while prolonged ultrasound exposure led to partial degradation of thermolabile components.

In terms of biological properties, the ethanolic extracts showed pronounced antibacterial activity against *Staphylococcus aureus*, at times exceeding the efficacy of benzylpenicillin, whereas the impact on *Escherichia coli*, *Bacillus subtilis*, and *Candida albicans* was weak or negligible. These data underscore the selective antimicrobial potential of *T. vulgare* extracts and suggest that the unique ecological conditions of Central Kazakhstan may enhance their flavonoid content.

However, all tested extracts displayed 100% lethality in the *Artemia salina* assay, indicating potent cytotoxic effects that necessitate caution in potential therapeutic applications. The concurrent high antibacterial efficacy and cytotoxicity likely reflect the complex interplay of phenolic acids, flavonoids, and sesquiterpene lactones found in tansy. Taken together, these findings support the continued exploration of UAE protocols for standardizing *T. vulgare* extracts while emphasizing the importance of balancing the extraction efficiency of bioactive compounds with their safety profile.

Future studies should focus on refining extraction parameters, elucidating underlying modes of action, and establishing standardized dosages to harness the pharmacological value of this plant while mitigating its potential toxicity.

6- Declarations

6-1- Author Contributions

Conceptualization, Y.B. and Y.P.; methodology, D.S.; software, D.S.; validation, D.S., S.A., and Y.P.; formal analysis, D.S.; investigation, D.S. and S.A.; resources, Y.B.; data curation, Y.P.; writing—original draft preparation, Y.P.; writing—review and editing, Y.P.; visualization, D.S.; supervision, Y.B.; project administration, Y.B. All authors have read and agreed to the published version of the manuscript.

6-2- Data Availability Statement

The data presented in this study are available on request from the corresponding author.

6-3- Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

6-4- Institutional Review Board Statement

Not applicable.

6-5- Informed Consent Statement

Not applicable.

6-6- Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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