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BIOACTIVE COMPOUNDS FROM *Taraxacum officinale* EXTRACTS OBTAINED BY OPTIMIZED ULTRASOUND-ASSISTED EXTRACTION

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ABSTRACT. In this study, the extraction of plant species *Taraxacum officinale* (F. H. Wigg) was optimized providing the maximum extraction of phenolic compounds using Response Surface Methodology (RSM). The optimum extraction parameters were as follows: extraction temperature of 60°C, extraction time of 30 min and 1:32.7 g/mL solid-liquid ratio for ethanol extract, and 60°C, 30 min, 1:14.2 g/mL ratio for acetone extract. The optimized extracts exhibited different biological activities. Ethanol extract showed better DPPH radicals scavenging activity and reducing power compared to acetone extract. The extracts had low antibacterial activity. In addition, the extracts had no effect on the healthy MRC-5 cell line, while acetone extract reduced the viability of HCT-116 carcinoma cells. This paper provides a basis for further research on the optimization of dandelion extraction under different conditions and testing the bioactivity of this medicinal plant.

Keywords: antibacterial activity, antioxidant activity, cytotoxic activity, RSM, *Taraxacum officinale*, ultrasound-assisted extraction.

INTRODUCTION

Secondary metabolites of plants are a group of bioactive compounds. They are synthesized in secondary metabolism in specialized plant cell types. There are different ways of classifying secondary metabolites such as based on their chemical structure, composition, the pathway of synthesis, and their solubility in different solvents. However, they can be divided into three basic groups: terpenoids, phenols, and nitrogen-containing compounds. It is assumed that their synthesis is increased due to stress factors because they have a role in protecting plants from various negative influences. Due to their bioactivity, plants are widely used in medicine, food, and pharmaceutical industries. Nowadays, the emphasis is placed on the study of plants' secondary metabolites as potential agents in the struggle against various problems of the modern age, such as diseases, antimicrobial resistance, food preservation, etc. So far, many studies have been published in which the antimicrobial, anti-inflammatory,

antioxidant, and antitumor effects of various plant extracts have been confirmed (CHINOU, 2008; JIMENEZ-GARCIA *et al.*, 2013; PRINSLOO and NOGEMANE 2018; JAIN *et al.*, 2019).

Taraxacum officinale (F. H. Wigg), known as dandelion, is a member of the family Asteraceae. It is a perennial plant that grows in temperate regions. Young leaves are used as food because they are rich in minerals, vitamins, fiber, and essential fatty acids (DI NAPOLI and ZUCCHETTI, 2021). In traditional medicine, dandelion is used to treat various diseases due to its healing properties and the presence of secondary metabolites such as flavonoids and phenolic acids (MIŁEK *et al.*, 2019). Recently, dandelion root polysaccharides exhibited protective effects against acetaminophen- and H_2O_2-induced oxidative damage in hepatic cells (CAI *et al.*, 2019).

Extraction is one of the key steps in the isolation and utilization of bioactive compounds from various plant materials. Ultrasound-assisted extraction except that it has better extraction efficiency compared to conventional solid-liquid extraction methods, it is also suitable for use due to higher extract yield, shorter extraction time, energy savings, and less use of solvents (FANG *et al*., 2014; TROJANOWSKA *et al.*, 2019). Ultrasound-assisted extraction is based on the action of ultrasonic waves that propagate through the solvent during the extraction of active components and cause negative solvent pressure. As a result, cavitation bubbles form, grow and implode. During the implosion, there is an increase in temperature and pressure at the molecular level, which damages the plant cell wall and releases out plant intracellular material (FU *et al*., 2020).

In order to obtain extracts with the highest possible content of phenolic compounds, optimization of ultrasound-assisted extraction was used in this research. The extraction of *T. officinale* was optimized using Response Surface Methodology (RSM) which represents a statistical and mathematical method that is currently widely used in the optimization of various processes (AYDAR, 2018). As far as we know, no paper has been published about the optimization of the extraction of phenolic compounds from *T. officinale*. Hence, the aim of this study was to provide optimum extraction parameters for phenolic compounds isolation from *T. officinale*. Furthermore, we aimed to perform the *in vitro* evaluation of antioxidant, antibacterial and cytotoxic activity as well as phytochemical analyses of optimized *T. officinale* extracts.

MATERIALS AND METHODS

Plant material

Plant material was collected in Malo Laole (44°19′30″ N, 21°27′51″ E), a village in Eastern Serbia, during April 2020. The taxonomic identification of the plant species *T. officinale* was confirmed at the Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Serbia. Afterwards, the aerial parts of the plant were dried in a shadow, then grounded, and used for extraction in the form of powder.

Experimental design

The extraction parameters were optimized using an experimental design by Response Surface Methodology (RSM) (KHURI and MUKHOPADHYAY, 2010). A Central Composite Face-Centered (CCFC) design with three factors and three levels consisting of 20 randomized runs with six central points was selected. The experimental design was without blocks. The independent variables were extraction temperature (25 – 60°C, A), extraction time (10 – 30 min, B) and plant material to solvent ratio (1:10 – 1:40 g/mL, C). The levels of independent variables were selected based on preliminary experimental results. Table 1. shows the high

(+1), medium (0), and low (-1) levels of the independent variables. The dependent variables (responses) were total phenolic content (TPC) and total flavonoid content (TFC).

Table 1. Independent variables and their coded and actual values.

		Coded levels		
Independent variables	**Units**	**-1**	**0**	**+1**
Extraction temperature (A)	°C	25	42.5	60
Extraction time (B)	min	10	20	30
Solid/solvent ratio (C)	g/mL	10	25	40

A second-order polynomial equation was used to develop an empirical model which correlated the responses to the independent variables. The general form of second-order polynomial equitation (1) is:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad (1)$$

where Y is the dependent variable; A, B, C represent the independent variables (temperature, time, and solid/solvent ratio), while β_0 is the model intercept coefficient; β_i, β_{ii}, β_{ij} represent linear, quadratic, and regression coefficients of interaction, respectively (AYYILDIZ *et al*., 2018).

After determination of the extraction parameters, based on the CCFC design, samples were prepared for extraction using two solvents (ethanol and acetone) separately. An amount of 1 g of the plant material was mixed with an appropriate quantity of solvent in prepared glass bottles which were later placed in an ultrasonic bath where extraction was performed under parameters that were set according to the experimental design. Ultrasound-assisted extraction of plant material was performed using an ultrasonic bath (Vabsonic, Niš, Serbia) with an ultrasonic frequency of 40 kHz. After extraction, the samples were centrifuged at 3000 rpm for 5 min, after which the obtained supernatants (extracts) were used for TPC and TFC analysis.

The TPC and TFC were determined using methods described by WOOTTON-BEARD *et al.* (2011) and QUETTIER-DELEU *et al.* (2000) respectively. The TPC is expressed as a microgram of gallic acid equivalents (GAE) per gram of dry plant weight (μgGAE/g DW). The TFC is expressed as a microgram of rutin equivalents (RUE) per gram of dry plant weight (μgRUE/g DW). All experiments were performed in triplicate and mean values were calculated.

Statistical analysis

The statistical software Design Expert (Trial version 11.0, STAT-EASE Inc., Minneapolis, USA) was used to analyze the experimental data and to find the 3D response surfaces of the response models. The significance of the independent parameters and their interactions were estimated by the ANOVA. The adequacy of the developed models was tested by performing coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}), and predicted coefficient of determination (R^2_{pre}), while the F-test and *p*-value were used to check the significance of the regression coefficient. A p-value of less than 0.05 was statistically significant. Finally, the desirability function method was applied to generate optimum extraction conditions.

Preparation of plant extracts under optimum extraction parameters

Ultrasound-assisted extraction was performed according to the optimum extraction conditions obtained by experimental design. An amount of 10 g of dry plant material was

extracted by ethanol and acetone, separately. After extraction, the filtrate from each solvent was evaporated using a rotary evaporator (IKA, Germany) at 40°C. The dry extracts were stored at -20°C until use.

Phytochemical analysis of plant extracts

Determination of total phenolic content, total flavonoid content and total proanthocyanidin content

The total phenolic content of the extracts was quantified according to the Folin-Ciocalteu's method as described by WOOTTON-BEARD *et al.* (2011). The concentration of the tested extracts was 1 mg/mL. Gallic acid (Sigma Aldrich, St. Louis, USA) was used as the standard and the total phenolic content was expressed as milligram of gallic acid equivalents (GAE) per gram of extract (mg GAE/g of extract).

The total flavonoid content of the extracts was determined using the aluminium chloride method as described by QUETTIER-DELEU *et al.* (2000). The concentration of the tested extracts was 1 mg/mL. Rutin (Sigma Aldrich, St. Louis, USA) was used as the standard and the concentrations of flavonoids were expressed as milligram of rutin equivalents (RUE) per gram of extract (mg of RUE/g of extract).

The total proanthocyanidin content was measured by the butanol-HCl method with ferric ammonium sulfate as a catalyst as described by PORTER *et al.* (1986). The concentration of the tested extracts was 1 mg/mL. Cyanidin chloride (Sigma Aldrich, St. Louis, USA) was used as the standard and the total proanthocyanidin content was expressed as milligrams of cyanidin chloride equivalents (CChE) per gram of extract (mg CChE/g of extract).

Determination of antioxidant activity

DPPH radicals scavenging capacity assay

The ability of *T. officinale* extracts to scavenge DPPH free radicals was assessed using the method described by TAKAO *et al.* (1994). The tested concentrations of plant extracts were from 62.5 µg/mL to 1000 µg/mL. Diluted solutions of extract (2 mL each) were mixed with 2 mL of DPPH methanolic solution (40 µg/mL). The absorbance of the mixture was measured at 517 nm. Ascorbic acid (Sigma Aldrich, St. Louis, USA) was used as a reference compound. The percentage of DPPH radical scavenging activity was calculated using the given formula (2):

$$\% = \frac{\left(A_{control} - A_{sample}\right)}{A_{control}} \times 100 \qquad (2)$$

where $A_{control}$ is the absorbance of DPPH solution and A_{sample} is the absorbance of tested extract. All experiments were performed in triplicate, while results are expressed as mean values ± standard deviation.

Reducing power assay

The reducing power of ethanol and acetone extracts of *T. officinale* was tested at concentrations from 15.625 µg/mL to 1000 µg/mL by the method of OYAIZU (1986). The absorbance of the reaction mixture was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the reference compound. All experiments were performed in triplicate, while results are expressed as mean values ± standard deviation.

Determination of antibacterial activity

Antibacterial activity was tested against 29 strains of 10 species of bacteria. The list of tested bacterial strains is given in Table 7. The bacterial strains were kept in glycerol stock at -80°C. For experiments, the bacterial strains were grown on Nutrient agar (Torlak, Belgrade, Serbia) at 37°C for 18 hours.

Microdilution method

Antibacterial activity was tested by determining the minimum inhibitory concentration (MIC) using the microdilution method with resazurin (SARKER *et al.*, 2007). Twofold serial dilutions of the plant extracts were made in sterile 96-well microtiter plates containing 0.1 mL of Mueller-Hinton broth (HiMedia Laboratories, Mumbai, India) per well. The tested concentration range was from 0.312 mg/mL to 40 mg/mL. The microtiter plates were inoculated with the bacterial suspensions to give a final concentration of 5×10^5 colony forming units (CFU)/mL. The inoculated microtiter plates were incubated at 37°C for 24 h. The growth of the bacteria was monitored by adding resazurin, the indicator of microbial growth. Resazurin is a blue nonfluorescent dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells. MIC was defined as the lowest concentration of tested plant extracts that prevented resazurin color change from blue to pink. Stock solutions of crude extracts were obtained by dissolving in 10% dimethylsulfoxide (DMSO) (Sigma-Aldrich, St. Louis, USA), which was used as the control. Each test included growth control and sterility control. The antibiotic tetracycline (Sigma-Aldrich Co., St. Louis, USA) was used as the positive control.

Determination of cytotoxic activity - MTT assay

The MRC-5 (healthy human lung fibroblast cell line) and HCT-116 (colorectal carcinoma cell line) cells were cultivated in the Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, USA) supplemented with 10% fetal bovine serum, and antibiotics (100 IU/mL penicillin and 100 μg/mL streptomycin, Invitrogen, USA) and cultured in a humidified atmosphere of 5% CO_2 at 37°C. All experiments were done with cells at 70 to 80% confluence. The MTT assay was used for the determination of cell viability after 24 and 72 h treatment with *T. officinale* extracts *(*concentrations 1, 10, 50, 100, 250, and 1000 μg/mL). The plant extracts were dissolved in 100% DMSO and diluted in a DMEM medium for cell treatment. The final treatment concentration of DMSO was <0.5% (HOSTANSKA *et al*., 2007). The absorbance was measured at 550 nm using an Elisa reader (RT-2100C, China). Cell viability (%) was calculated as a ratio of the absorbance of the treated group divided by the absorbance of the control group, multiplied by 100. IC_{50} values (a value that represents the concentration of a drug that inhibits 50% of cell growth *in vitro*) were calculated from the dose curves by the software tool *CalcuSyn*.

RESULTS AND DISCUSSION

Optimization of ultrasound-assisted extraction

After reviewing the literature data, it was decided to examine the optimization of ultrasound-assisted extraction in this study. Because in the comparisons of two types of extraction, the conventional method - maceration and ultrasound-assisted extraction, it was observed that ultrasonic extraction shows greater efficiency in the yield of secondary

metabolites (MAJD *et al.*, 2014; JOVANOVIĆ *et al.*, 2017; SAVIC *et al.*, 2019; DZAH *et al.*, 2020).

The TPC and TFC responses of each run of the experimental design are presented in Table 2. From the presented results it can be concluded that the lowest amount of TPC/ethanol (940.69 μgGAE/gDW), TPC/acetone (548.63 μgGAE/gDW), and TFC/ethanol (2146.39 μgRUE/gDW) was obtained under the following extraction conditions 25°C, 10 min, and 1:10 g/mL (Run 20), while the lowest amount of TFC/acetone (1679.72 μgRUE/gDW) was obtained at 25°C, 10 min, and 1:40 g/mL (Run 16). The highest amount of TPC/ethanol (3518.04 μgGAE/gDW) and TFC/acetone (3523.33 μgRUE/gDW) was obtained at 60°C, 30 min, and 1:40 g/mL (Run 12), TPC/acetone (2161.57 μgGAE/gDW) at 60°C, 30 min, and 1:10 g/mL (Run 18), and TFC/ethanol (3774.48 μgRUE/gDW) at 42.5°C, 30 min, and 1:25 g/mL (Run 4).

Table 2. The CCFC experimental design and response values for the optimization of extraction conditions.

	Independent variables			Responses			
				Ethanol		Acetone	
Run	**A**	**B**	**C**	**TFC**	**TPC**	**TFC**	**TPC**
1	42.5	20	25	3234.90	2100.98	3199.31	766.67
2	25	30	10	2634.58	1459.61	3157.15	1106.96
3	60	10	40	3174.31	3087.84	2967.67	1842.35
4	42.5	30	25	**3774.48**	2344.85	3445.83	1157.11
5	42.5	10	25	3495.31	2457.35	2870.66	869.61
6	42.5	20	25	3557.81	2254.66	3348.09	709.07
7	25	20	25	2954.51	1632.60	2782.64	624.90
8	60	10	10	2848.13	2752.84	2948.13	1522.65
9	42.5	20	25	3302.60	2213.73	3392.01	866.67
10	25	30	40	2850.00	1678.82	2455.14	925.10
11	42.5	20	25	3543.92	2334.07	3365.10	661.03
12	60	30	40	3565.00	**3518.04**	**3523.33**	2084.31
13	60	20	25	3247.92	3179.17	3355.56	1679.66
14	42.5	20	10	2677.99	1986.47	3157.15	745.39
15	42.5	20	40	3218.33	2491.76	2774.72	829.02
16	25	10	40	2389.86	1559.22	1679.72	771.77
17	42.5	20	25	3414.58	2414.22	3392.01	822.55
18	60	30	10	3157.15	3029.31	3261.67	**2161.57**
19	42.5	20	25	3310.42	2608.82	3484.03	990.20
20	25	10	10	2146.39	940.69	2600.90	548.63

A – Extraction temperature (°C); B – Extraction time (min); C – Solid/solvent ratio (g/mL); TFC – Total flavonoid content (μgRUE/gDW); TPC – Total phenolic content (μgGAE/gDW). The highest TFC and TPC values are given in bold.

Non-significant variables with a *p*-value higher than 0.05 were eliminated in order to achieve a simple and realistic model. So, the results of ANOVA for the reduced models are summarized in Table 3. High F-values with very low *p*-values demonstrate that generated model is statistically significant. Based on ANOVA, it is concluded that all four models were statistically significant. The F-values indicated that the models were significant and that there is a 0.01% chance that F-values may cause noise. The *p*-values for all four models were less than 0.05 indicating that the model terms were significant. The non-significant lack of fit test

showed that the models properly fit the experimental data, thus further validating the models. According to the *p* and F-values, certain independent variables significantly influenced the extraction of the mentioned secondary metabolites (Table 3).

Based on the fitting statistics from Table 4, it could be noticed that for all four responses the difference between the predicted R^2 and the adjusted R^2 is less than 0.2, which is acceptable. For TPC of ethanol extract and TFC of acetone extract, R^2 values were 0.9537 which indicated that only 4.63% of the total variations were not explained by the model. For TFC of ethanol extract, R^2 values were 0.9413 which indicated that only 5.87% of the total variations were not explained by the model, while R^2 for TPC of acetone extract was 0.9821 which indicated that only 1.79% of the total variations were not explained by the model. The reproducibility of the model is observed based on the coefficient of variation, C.V.%, whose value should be less than 10%. Based on the presented results, it can be noticed that the C.V.% values for all four responses are acceptable. The value of adequate precision is preferably greater than 4 and it is a measure of the signal-to-noise ratio. In this case, the values of adequate precision between the responses ranged from 22.23 to 34.66, which indicates the importance of the models for the extraction process.

Table 3. Analysis of variance (ANOVA) results for the experiments.

	F-value				**p-value**			
	Ethanol		**Acetone**		**Ethanol**		**Acetone**	
Sources	**TF**	**TPC**	**TFC**	**TPC**	**TFC**	**TPC**	**TFC**	**TPC**
Model	34.7	109.82	44.68	118.86	< 0.0001	< 0.0001	< 0.0001	< 0.0001
A	58.8	302.19	84.47	437.98	< 0.0001	< 0.0001	< 0.0001	< 0.0001
B	24.0	6.67	56.95	54.84	0.0003	0.0200	<0.0001	< 0.0001
C	19.4	20.61	21.98	2.09	0.0007	0.0003	0.0004	0.1716
AB								
AC			33.50				< 0.0001	
BC				12.47				0.0037
A^2	17.2		8.75	62.66	0.0011		0.0111	< 0.0001
B^2	8.80			23.23	0.0109			0.0003
C^2	38.3		20.66		< 0.0001		0.0006	
Lack of	0.76	0.6072	1.91	0.1255	0.6496	0.7724	0.2466	0.9943

Table 4. Fit statistics.

	Ethanol		**Acetone**	
	TFC	**TPC**	**TFC**	**TPC**
Std. Dev	124.39	150.92	116.32	80.28
Mean	3124.91	2302.25	3058.04	1084.26
R^2	0.9413	0.9537	0.9537	0.9821
Adjusted R^2	0.9142	0.9450	0.9324	0.9738
Predicted R^2	0.8513	0.9310	0.8826	0.9690
Adeq Precision	22.23	34.66	26.04	34.51
C.V. %	3.98	6.56	3.80	7.40

The models that best fit the responses were quadratic for TFC/ethanol, TFC/ethanol, and TPC/acetone, only the linear model fit best to the TPC/ethanol response. Based on that, three quadratic and one linear equation were set up. By analyzing the equations (Table 5) and

3D response surfaces (Figs. 1 and 2), it was possible to conclude which experimental parameters have a positive effect on the extraction.

Table 5. Model and coded equations.

Dependent factor	Model	Coded equation
TFC/ethanol	quadratic	TFC = 3401.39 + 301.72 A + 192.72 B + 173.33 C - 311.19 A^2 + 222.49 B^2 - 464.25 C^2
TPC/ethanol	linear	TPC = 2302.25 + 829.63 A + 123.27 B + 216.68 C
TFC/acetone	quadratic	TFC = 3302.00 + 338.08 A + 277.60 B - 172.44 C + 238.05 AC - 192.38 A^2 - 295.54 C^2
TPC/acetone	quadratic	TPC = 798.46 + 531.32 A + 188.00 B + 36.73 C - 100.25 BC + 355.26 A^2 + 216.33 B^2

According to the presented data and 3D representation of the response surface, it can be noticed that in all four analyzed models, the greatest effect on the extraction had temperature and time of extraction, while the solid-liquid ratio had less impact. With increasing extraction temperature and extraction time, the yield of tested secondary metabolites also increased.

From the linear equation TPC/ethanol, it can be seen that all three extraction conditions had a positive effect on TPC yield. All three factors individually (A, B, C) had a positive effect on TPC/acetone extraction, and quadratic factor of temperature (A^2) and quadratic factor of time (B^2), while the interaction between extraction time and solid/solvent ratio (BC) had a negative effect. Individual factors also had a positive effect on the extraction of TFC/ethanol and the quadratic factor of time (B^2), while the quadratic factor of temperature (A^2) and the quadratic factor of solid/solvent ratio (C^2) showed a negative effect on the extraction. TFC/acetone extraction was positively affected by individual temperature (A) and time (B) factors, while the solid/solvent ratio (C) showed a negative effect on extraction. The interaction between temperature and solid/solvent ratio (AC) had a positive effect on TFC/acetone extraction, while the quadratic factor of temperature (A^2) and the quadratic factor of solid/solvent ratio (C^2) had a negative effect.

Using the desirability function in the Design Expert Software program, the optimum extraction conditions were determined by combining both responses for each solvent separately. In order to obtain optimized extraction conditions, the independent variables were set to be in range, while the responses were set to maximum. The desirability values for the independent variables and the corresponding responses were close to 1, so all responses individually, as well as the combined response effect, showed significant acceptability (Fig. 3). The conditions for obtaining the maximum concentration of flavonoids and phenols in the ethanol extract were as follows: 60°C, 30 min, and 1:32.7 g/mL ratio. While the following optimum extraction conditions were required to obtain the maximum concentration of the mentioned compounds in acetone extract: 60°C, 30 min, 1:14.2 g/mL ratio

In the research published by SUN *et al.* (2014) the ultrasound-assisted extraction of flavonoids was optimized under different research conditions than the ones described in this study, and it was shown that the optimal parameters for extraction were ethanol concentration of 39.6, liquid/solid ratio of 59.5, and extraction time of 43.8 min.

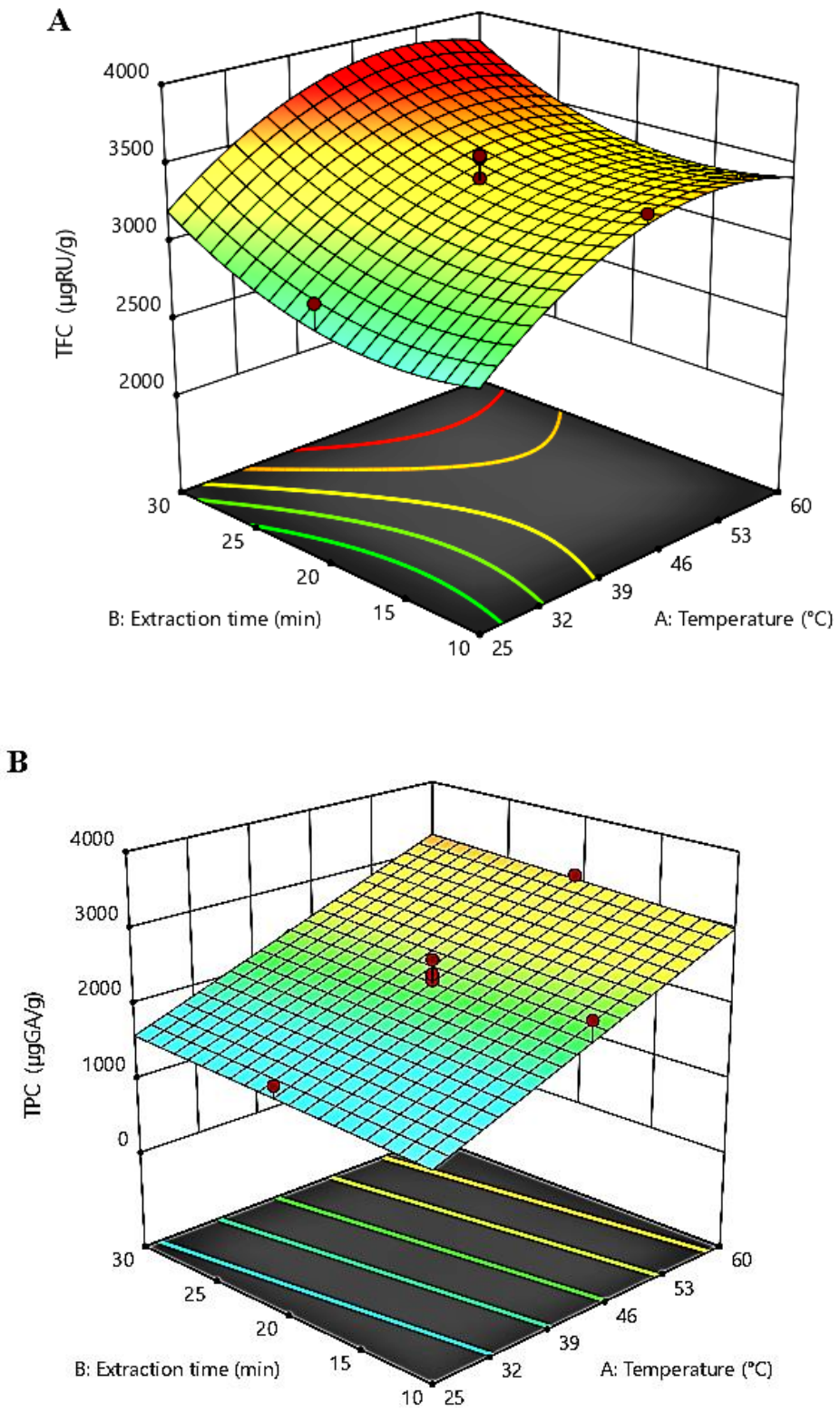

Figure 1. Response surface plots of significant model terms for ethanol extract: A – TFC (total flavonoid content) and B – TPC (total phenolic content), and effect of extraction temperature and extraction time.

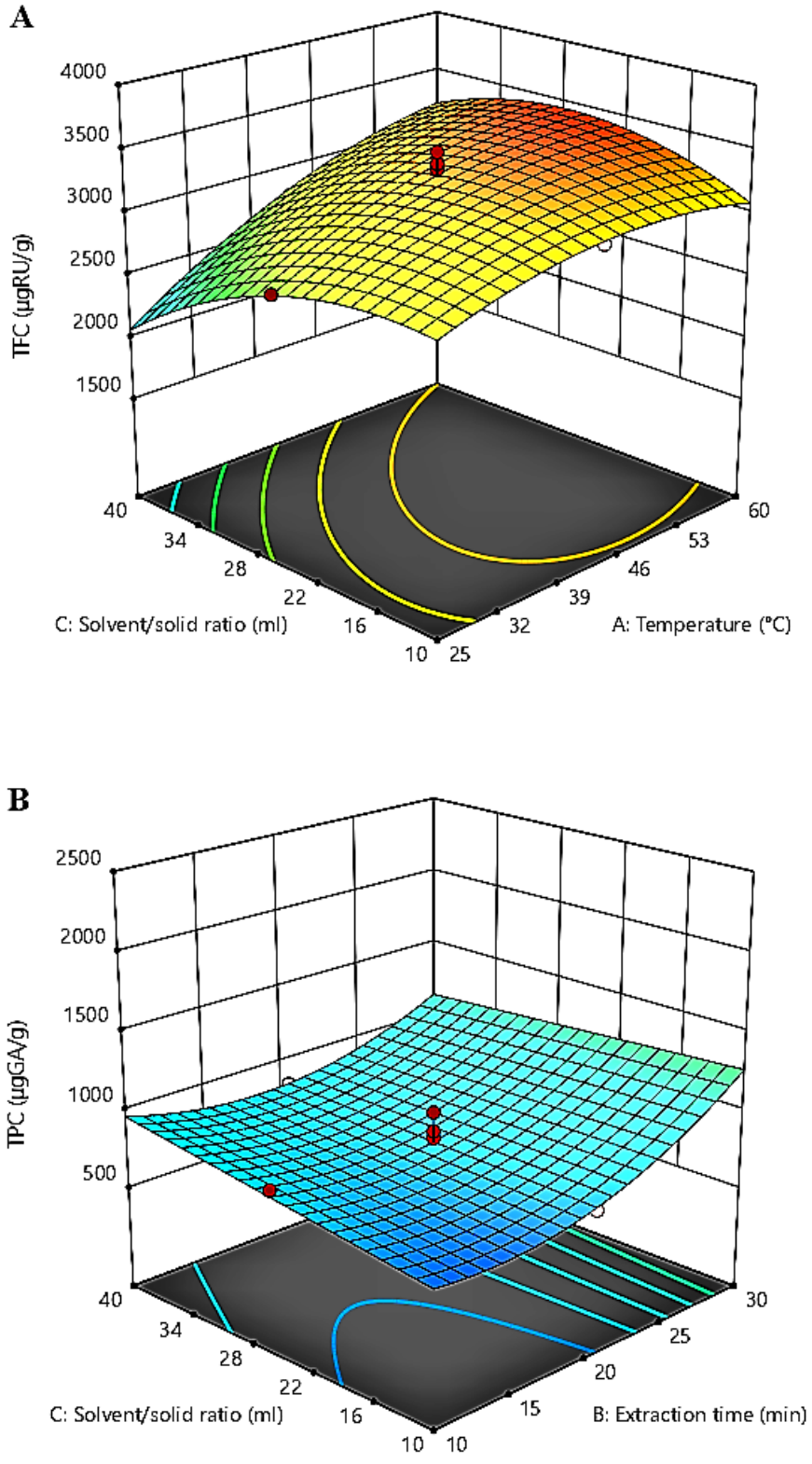

Figure 2. Response surface plots of significant model terms for acetone extract:
A – TFC (total flavonoid content) and effect of extraction temperature and solid/solvent ratio,
and B – TPC (total phenolic content) and effect of extraction time and solid/solvent ratio.

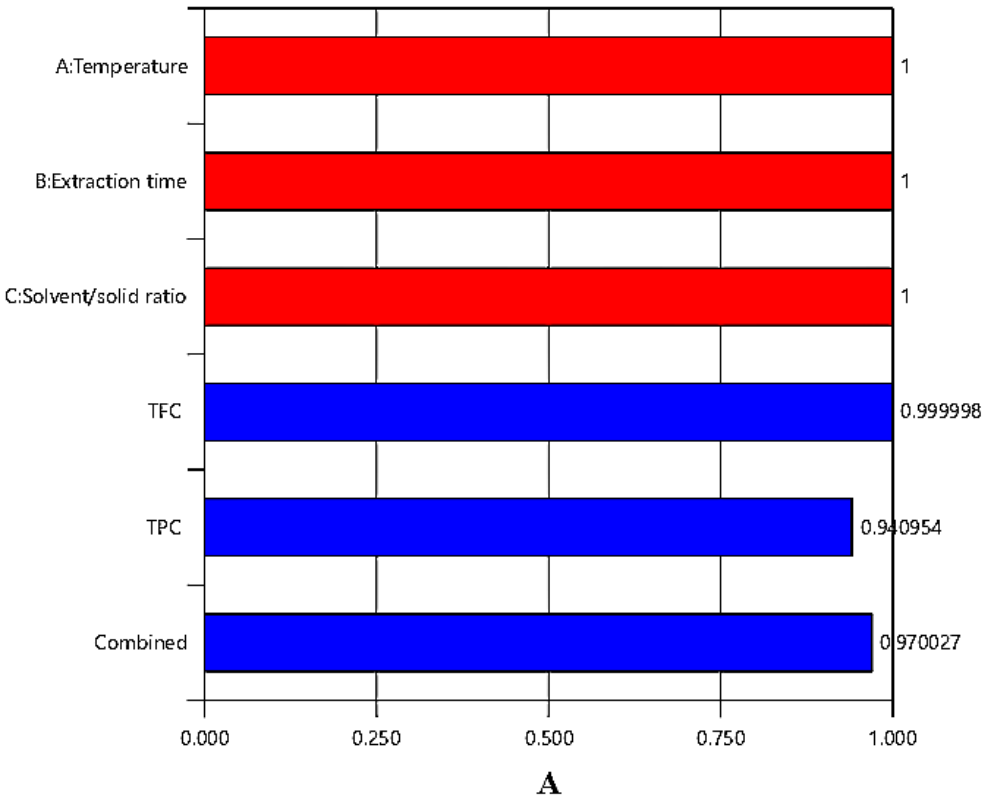

Figure 3. Desirability graphs for the independent variables and the corresponding responses individually and the combined response effect for A – optimization extraction of ethanol extract and B – optimization extraction of acetone extract.

Phytochemical analysis

The concentration of total flavonoids, total phenols, and total proanthocyanidins in the optimized extracts were measured. Analyzing the data shown in Table 6, it can be noticed that in both tested extracts the concentration of flavonoids was the highest among studied groups of phenolic compounds. The acetone extract had almost two times higher concentration of flavonoids than the ethanol extract. The concentration of flavonoids in acetone extract was 117.04 ± 11.0 mgRUE/g, while the concentration of flavonoids in ethanol extract was 49.40 ± 0.5 mgRUE/g. The concentration of total phenols in both extracts was approximately similar. Ethanol extract had a slightly higher concentration of total phenols, 31.29 ± 0.7 mgGAE/g, then acetone extract, 21.73 ± 0.3 mgGAE/g. The content of proanthocyanidins in both extracts was the lowest compared to other tested secondary metabolites. The amount of proanthocyanidins in acetone extract was 7.00 ± 0.5 mgCChE/g and was two times higher than the amount in ethanol extract, 3.45 ± 0.4 mgCChE/g.

Table 6. Total phenolic (TPC), total flavonoid (TFC), and total proanthocyanidin (TPAC) content in extracts of *T. officinale.*

Type of extract	TFC (mgRUE/g)	TPC (mgGAE/g)	TPAC (mgCChE/g)
Ethanol	49.40 ± 0.5	31.29 ± 0.7	3.45 ± 0.4
Acetone	117.04 ± 11.0	21.73 ± 0.3	7.00 ± 0.5

The values are mean ± standard deviations.

The amount of total phenols in *T. officinale* ethyl acetate extract measured in the study by GHAIMA *et al.* (2013) was 10.2 mg GAE/gDW. In the study by ROSHANAK *et al*. (2021), in the aqueous leaf extract of *T. officinale*, the concentration of total phenolic content was 22.82 ± 97 mg/gDW, while the concentration of total flavonoid content was 116.89 ± 2 mg/gDW.

In the study by EPURE *et al*. (2022), an ethanol extract of dandelion was prepared. Plant material was collected on the territory of Romania, during the flowering phase. The extract was prepared by the maceration method and the aerial parts of the plant were used. The amounts of TPC (13.15 ± 0.81 mgGAE/gDW) and TFC (6.87 ± 0.34 mgRE/gDW) obtained by this extraction method were shown to be much lower than the values in this study.

Another study optimized the extraction processes of phenolic compounds from the plant of the genus *Taraxacum* (*T. assemanii*) and also showed that a better yield of tested secondary metabolites was given by ultrasound-assisted extraction compared to the conventional method. However, this study further demonstrated that the combination of ultrasound pretreatment and maceration extraction produced the highest possible yield of secondary metabolites (YAZICI, 2021).

Antioxidant activity

The results of DPPH radicals scavenging capacity of ethanol and acetone extract of *T. officinale* and the positive control, ascorbic acid, are shown in Fig. 4.

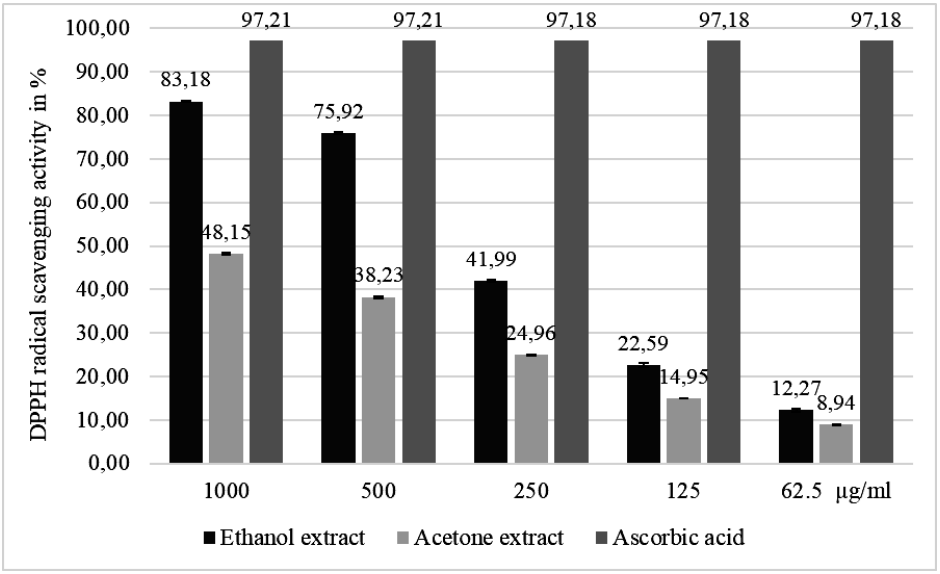

Figure 4. DPPH radicals scavenging activity of ethanol and acetone extracts of *T. officinale.*

Ethanol extract has better antioxidant activity than acetone extract. Compared with the positive control, ethanol extract has significant antioxidant activity at concentrations of 1000 µg/mL and 500 µg/mL. As the concentration of the tested extracts decreased, so did their antioxidant activity. In relation to the concentration, the DPPH radicals scavenging activity of ethanol extract ranged from 83.2% to 6.1%, while for the acetone extract it was from 48.2% to 4.9%. The research conducted by PĂDUREŢ *et al.* (2016) showed that DPPH radicals scavenging activity of aerial parts of *T. officinale* was 69%.

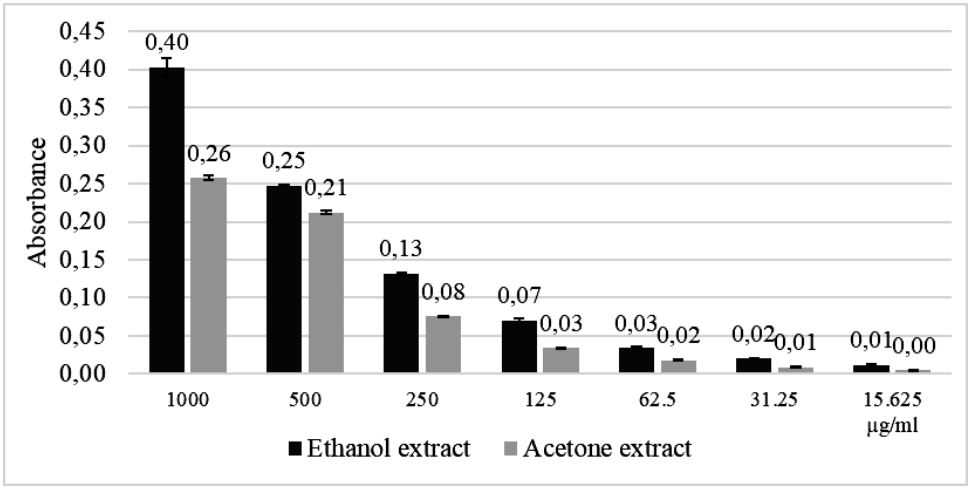

Figure 5. Reducing power of ethanol and acetone extracts of *T. officinale.*

The reducing power of plant extracts is an indicator of the potential antioxidant activity due to their ability to transfer electrons. Results of reducing power of ethanol and acetone extract of *T. officinale* are shown in Fig. 5. Ethanol extract showed higher reducing power compared to acetone extract. Within both extracts, the reducing power of the extracts increased with increasing tested concentration. The absorbance values for the ethanol extract ranged from 0.012 to 0.403, while the acetone extract absorbance values ranged from 0.005 to 0.258. For comparison, the absorbance value for the reference compound, ascorbic acid, was 2.943.

Antibacterial activity

The effect of *T. officinale* extracts against 29 bacterial strains of food and human origin, expressed as MIC values, are shown in Table 7. The 10% DMSO had not any inhibitory effect on tested bacterial strains. The extracts exhibited no activity against tested bacterial strains at tested concentrations. Though *Bacillus cereus* showed sensitivity to both extracts, the growth of this bacterial strain was inhibited at 40 mg/mL. In addition, the growth of *Acinetobacter* sp. was inhibited by acetone extract at the same concentration of 40 mg/mL.

Table 7. Antibacterial activity (MIC values) of
ethanol (E) and acetone (A) extracts of *T. officinale* and an antibiotic tetracycline (T).

Bacterial strains	E (mg/mL)	A (mg/mL)	T (μg/mL)
***Escherichia coli* ATCC 259222**	>40	>40	4
***E. coli* LM14**	>40	>40	>128
***E. coli* LM15**	>40	>40	4
***E. coli* LM16**	>40	>40	64
***E. coli* LM3**	>40	>40	4
***Pseudomonas aeruginosa* ATCC 10145**	>40	>40	32
***P. aeruginosa* LM2**	>40	>40	32
***P. aeruginosa* LM3**	>40	>40	16
***P. aeruginosa* LM7**	>40	>40	32
***Proteus mirabilis* ATCC 12453**	>40	>40	64
***P. mirabilis* LM1**	>40	>40	16
***Proteus sp.* LM2**	>40	>40	64
***Proteus* sp. LM3**	>40	>40	128
***P. mirabilis* LM4**	>40	>40	128
Salmonella enterica	>40	>40	4
***Salmonella* Typhimurium**	>40	>40	4
***Klebsiella oxytoca* LM1**	>40	>40	4
***Klebsiella* sp. LM2**	>40	>40	4
***Klebsiella* sp. LM4**	>40	>40	4
***Acinetobacter* sp. LM1**	>40	40	32
***Providencia* sp. LM1**	>40	>40	32
***Staphylococcus aureus* ATCC 25923**	>40	>40	2
***S. aureus.* LM1**	>40	>40	32
***S. aureus* LM10**	>40	>40	0.25
***S. aureus* LM11**	>40	>40	0.25
***S. aureus* LM12**	>40	>40	0.25
***Enterococcus* sp. LM4**	>40	>40	64
***Enterococcus* sp. LM5**	>40	>40	32
Bacillus cereus	40	40	0.5

The effect of *T. officinale* ethanol extract was examined against *E. coli* strains, the extract had an effect at a concentration of 25 mg/mL against *E. coli* ATCC 25922 and at a concentration of 12.5 mg/mL against the pathogenic strain of *E. coli* (NAZIR *et al.*, 2021), indicating a better antibacterial effect compared to the extract tested in this paper. On the other side, in the study by ROSHANAK *et al.* (2021) a much higher inhibitory concentration was observed. The growth of *Salmonella typhimurium* and *Bacillus subtilis* was inhibited at

256 mg/mL. ARIF *et al.* (2020) reported that methanol extract of *T. officinale* showed weak antibacterial activity.

Cytotoxic activity

The results of cytotoxic activity of *T. officinale* ethanol and acetone extracts on HCT-116 and MRC-5 cells, after 24h and 72h of treatment are presented in Fig. 6. The extracts had no effect on MRC-5 cell viability, while acetone extract of *T. officinale* reduced viability in HCT-116 cells only at the highest concentrations (250 and 1000 µg/mL). Based on IC_{50} values, acetone extract at a concentration of approximately 500 µg/mL caused a 50% reduction in HCT-116 cell viability (Table 8). The present study indicated a slight cell-selective response to the acetone extract of *T. officinale*, whereat HCT-116 cells were more sensitive to treatments compared to MRC-5 cells.

Table 8. IC_{50} values (µg/mL) of ethanol and acetone extracts of *T. officinale*.

	HCT-116		MRC-5	
Type of extract	**24 h**	**72 h**	**24 h**	**72 h**
Ethanol extract	> 1000	> 1000	> 1000	> 1000
Acetone extract	499.13	588.38	> 1000	> 1000

Based on KOO *et al*. (2004) research data, *T. officinale* showed significant cytotoxic activity via secretion of TNF-a and IL-1a in HepG2 cells. Compared to HepG2 cells, HCT-116 cells are more aggressive and show a different response and sensitivity to the same treatment. LEE and LEE (2008) showed that ethanol extract of *T. officinale* leaves had no cytotoxic effect on Calu-6, HCT-116, and SNU-601 cell lines as well. After 48 h of treatment, their result was over 800 µg/mL, which is correlated with our results.

CONCLUSION

RSM was successfully used to optimize ultrasound-assisted extraction parameters for plant species *T. officinale* based on the highest total phenolic and flavonoid content. The following optimum extraction conditions were obtained: extraction temperature of 60°C, extraction time of 30 min, and plant material-solvent ratio of 1:32.7 g/mL for ethanol extract, while extraction temperature of 60 °C, extraction time of 30 min, and plant material-solvent ratio of 1:14.2 g/mL for acetone extract. Flavonoids were present in the highest concentration in acetone extract. Based on the presented data compared with the results of other studies, it can be concluded that ultrasonic extraction gives a higher yield of extracted secondary metabolites, for a shorter period of extraction time, with lower solvent usage and represents one technique of green extraction. Ethanol extract showed better DPPH radicals scavenging activity and reducing power compared to acetone extract. The extracts had a weak antibacterial effect, only two bacterial strains *B. cereus* and *Acinetobacter* sp., were sensitive. In addition, the extracts had no effect on the healthy MRC-5 human lung fibroblast cell line, while acetone extract reduced the viability of the HCT-116 colorectal carcinoma cell line at a concentration of approximately 500 µg/mL. The extraction of secondary metabolites is greatly influenced by various factors, such as extraction time, temperature, type of solvent, etc. This paper provides a basis for further research on the optimization of dandelion extraction under different conditions, in which an extract that shows better bioactivity would potentially be obtained.

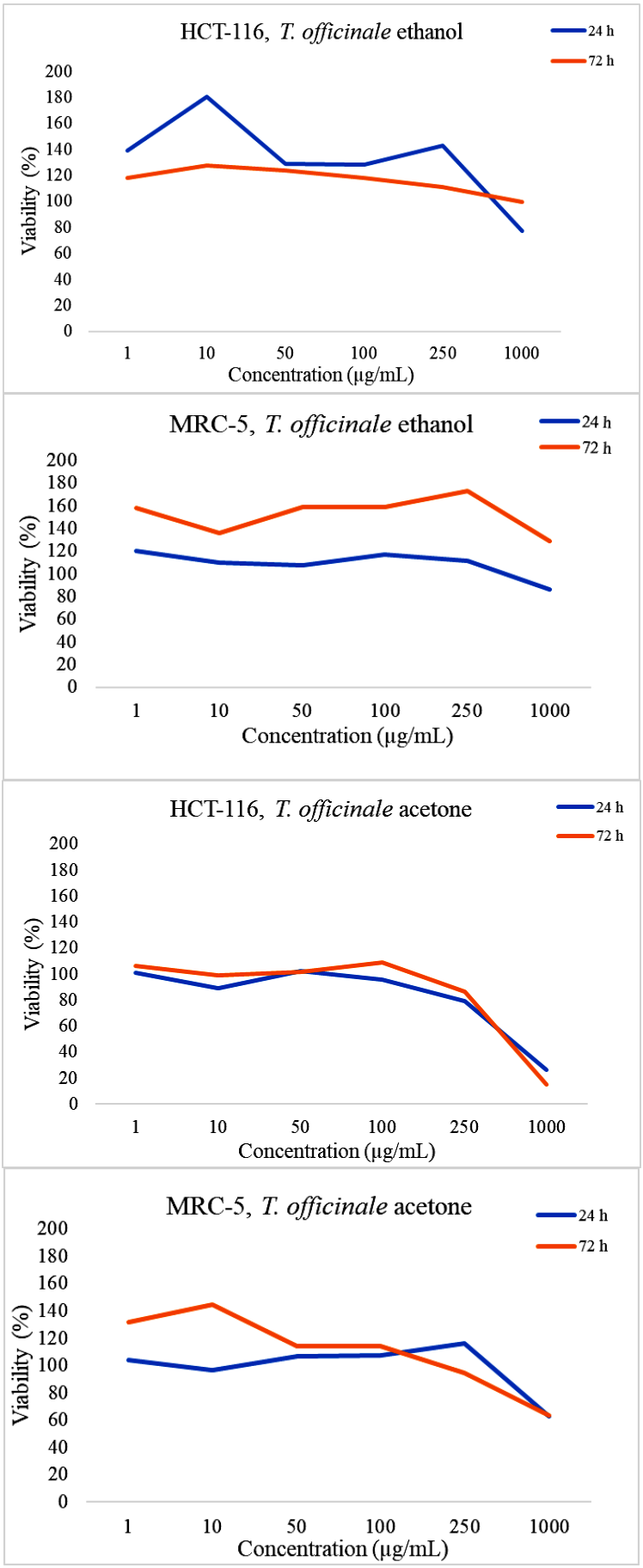

Figure 6. Cell viability (%) after treatment with ethanol and acetone extract of *T. officinale*.

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