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UTILIZATION OF RESIDUES OF AJWAIN, SUMMER SAVORY AND OREGANO ESSENTIAL OILS DISTILLATION

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water distillation, water residue, solid residue, phenolic and flavonoid compounds, antioxidant and antibacterial

ABSTRACT

Water distillation (WD) is a traditional technique for extracting essential oils (EOs). This technique yields significant amounts of water residue (WR) and solid residue (SR) as by-products. The goal of this study was to examine the effectiveness of using WR and SR by-products as antioxidant and antimicrobial agents. The chemical profile of EOs extracted from some aromatic plants rich in phenolic compounds (ajwain, summer savory and oregano) was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). The most abundant compounds were phenolic monoterpenes, such as thymol and carvacrol. The polyphenol characteristics of the analysed WR and SR extracts were defined by High Performance Liquid Chromatography (HPLC). The HPLC analysis indicated the presence of chlorogenic acid, catechin, *p*-hydroxy benzoic acid, ferulic acid, rutin and hesperidin as major components in WR. Total phenolic and total flavonoid contents were determined using Folin-Ciocalteu and aluminum chloride assays, respectively. The antioxidant potential was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assays. The WRO (water residue from oregano) and WRS (water residue from savory) extracts exhibited higher DPPH radical scavenging activity and reducing power than butylated hydroxytoluene (BHT). The antibacterial activity of WR and SR extracts was evaluated on the example of their efficiency against six bacterial strains. The antibacterial activity of WRO extract against *Salmonella typhimurium* 14028 and *Serratia marcescens* 37 exceeded those of the standard ampicillin. Meanwhile, WRS extract exhibited greater antibacterial activity than ampicillin against *Bacillus cereus* 33018.

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УТИЛИЗАЦИЯ ОСТАТКОВ ДИСТИЛЛЯЦИИ ЭФИРНЫХ МАСЕЛ АЖГОНА, ЧАБЕРА САДОВОГО И ОРЕГАНО

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КЛЮЧЕВЫЕ СЛОВА: АННОТАЦИЯ

водная дистилляция, водный остаток, сухой остаток, фенольные и флавоноидные соединения, антиоксидантный и антибактериальный

Водная дистилляция (ВД) является традиционным методом экстракции эфирных масел (ЭМ). Этот метод даёт значительный объем водного остатка (ВО) и твёрдого остатка (ТО), получаемых в качестве побочных продуктов. Целью данного исследования было изучение эффективности использования побочных продуктов дистилляции, а именно — ВО и ТО в качестве антиоксидантных и антимикробных действующих веществ. Химический профиль ЭМ, экстрагированных из некоторых ароматических растений, богатых фенольными соединениями (ажгон, чабер садовый и орегано), был проанализирован с помощью метода газовой хроматографии-масс-спектрометрии (ГХ-МС). Наиболее часто встречающимися соединениями оказались фенольные монотерпены, такие как тимол и карвакрол. Полифенольные характеристики проанализированных ВО и ТО экстрактов были определены с помощью метода высокоэффективной жидкостной хроматографии (ВЭЖХ). Анализ по методу ВЭЖХ показал присутствие хлорогеновой кислоты, катехина, *p*-гидроксibenзойной кислоты, феруловой кислоты, рутина и гесперидина в качестве основных компонентов в ВО. Общее содержание фенольных соединений и флавоноидов определяли с помощью анализа Фолина-Чокальтеу и анализа на содержание хлорида алюминия соответственно. Антиоксидантный потенциал оценивали с помощью анализа 2,2-дифенил-1-пикрилгидразила (ДФПГ) и анализа восстанавливающей железом антиоксидантной активности (ВЖАА). Экстракты из водного остатка орегано (ВОО) и водного остатка чабера (ВОЧ) проявляли более высокую активность в отношении радикалов ДФПГ и восстанавливающую способность, нежели бутилированный гидрокситолуол (БГТ). Антибактериальная активность экстрактов ВО и ТО оценивалась на примере их эффективности в отношении шести штаммов бактерий. Антибактериальная активность экстракта ВОО в отношении *Salmonella typhimurium* 14028 и *Serratia marcescens* 37 превышала антибактериальную активность стандартного ампициллина. При этом экстракт ВОЧ проявлял более высокую антибактериальную активность, нежели ампициллин, в отношении *Bacillus cereus* 33018.

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1. Introduction

WD is the most popularly utilized method to obtain EOs from the aromatic plants [1]. Large amounts of plant material are used in traditional distillation procedures for the extraction of essential oils, which in their turn generate residues that should be valorized and replenished rather than devalued and depleted [2]. The WD process produces two kinds of wastes: the aromatic water residue (the non-distilled aqueous phase or wastewater, often called decoction), in which the plant material was immersed [3] and the plant solid residue that is recovered at the end of distillation [4,5]. The WR is habitually discarded, though it may be rich in non-volatile phenolic compounds, which possess interesting biological properties [6]. A high quantity of solid residue rich in phenolics is left in the water, which constitutes a major problem for the EO industry [7]. Different methods are used to recover several valuable substances from these wastes such as polyphenols and polysaccharides [8–10]. Reducing "waste" to "secondary raw material" can resolve some or all of the environmental and economic problems [11]. Consequently, the recovery of the relevant substances with biological activities is considered in this industry [9]. Nowadays, the demand for natural-based products is increasing sharply [12]. Polyphenols have been proven to have positive antioxidant, antibacterial and anticancerous properties [13]. Using plant polyphenol-rich extracts is a promising trend in the food industry [14]. Polyphenols may show synergism with antibiotics [15].

Number of studies on the recovery of phenolic compounds from the by-products remaining after the essential oil distillation of aromatic plants are very limited [16,17].

Ajwain (*Trachyspermum ammi*) Sprague, syn. *Carum copticum* (L.) Link, is an aromatic herb belonging to the *Apiaceae* family [18]. This annual plant is growing in the East of India, Iran, Pakistan, and Egypt [19]. Its fruit (seeds) are widely used as a spice and food seasoning. Thymol is present in high concentration in ajwain EO. Depending on the concentration, these phenolic compounds act as either bacteriostatic or bactericidal agents [20].

Summer savory (*Satureja hortensis* L.) is a leafy spice. It belongs to the *Lamiaceae* family [21]. It has antimicrobial, antioxidant, anticancer, and anti-inflammatory properties because of its bioactive components such as carvacrol, thymol, *p*-cymene, and γ -terpinene [22].

One of the most well-known aromatic plants in the *Lamiaceae* family is oregano (*Origanum vulgare* L.) which has a distinct well-recognizable flavor [23,24]. Its essential oil is extracted from the leaves [25]. It is mainly composed of carvacrol and thymol [26]. It is used as an alternative to chemical preservatives due to its antioxidant and antimicrobial activities.

To the best of our knowledge, no information about the polyphenols profile, antioxidant and antibacterial properties of the ajwain and summer savory water distillation by-products is available. The aim of this work is to investigate the efficiency of these EO water distillation by-products as natural bioactive substances.

2. Materials and methods

2.1. Materials

The dried fruits of ajwain (*Trachyspermum ammi*), and dried leaves of summer savory (*Satureja hortensis* L.) and oregano (*Origanum vulgare* L.) were purchased from Harraz Company for Medicinal Plants, Cairo, Egypt. The dried fruits and leaves were ground using a hammer mill to a fine powder. The antimicrobial study was conducted using three-gram positive bacterial strains (*Bacillus subtilis* 765 NRRL Northern Regional, *Bacillus cereus* 33018, *Staphylococcus aureus* 25923), and three-gram negative bacteria strains including *Salmonella typhimurium* 14028 Rockvill, Maryland, USA, *Escherichia coli* 15 and *Serratia marcescens* 37 FS (Food Science Department, Faculty of Agriculture, Cairo University).

2.2. Chemicals and reagents

Folin-ciocalteu reagent, gallic acid, quercetin, DPPH, ferric chloride, butylated hydroxytoluene (BHT), anhydrous sodium sulphate, *n*-alkanes (C_8 – C_{24}) and solvents of analytical and HPLC-grade were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Extraction of essential oil and preparation of water residue

Fifty grams of the investigated materials (ground to fine powder) were subjected to WD with distilled water using Clevenger apparatus for 3 h according to European Pharmacopoeia procedure [27]. At the end of extraction time, the obtained EO was dried over sodium sulphate anhydrous and stored in a dark glass bottle at 4 °C until conducting an analysis. The WD was performed for each plant material in triplicates. The mean value of the yield (%) was calculated on dry weight basis. The mixture of WR and SR was allowed to cool to room temperature and then was filtered under vacuum through a Whatman paper No. 1. The SR was air dried at room temperature up to constant weight and stored at 4 °C until usage.

A portion of the separated WR was stored in dark vials at –20 °C and used for the determination of polyphenols content and antioxidant activity. The other portion was concentrated under reduced pressure using a rotary evaporator N-1000 (EYELA, Japan) at 40 °C before freeze-drying (Modulyo Freeze Dryer, Edward, England) prior to HPLC analysis and antimicrobial activity examination.

2.4. Extraction of solid residue

Solid residue dried samples were extracted with 80 % ethanolic solution at a ratio of 1:20 (w/v) using a high-speed Heidolph homogenizer (Type DR22054, Germany) for 30 min as reported by Helmy et al. [28]. After filtration, the solvent was removed in a rotary evaporator N-1000 (EYELA, Japan) under vacuum at 40 °C and the concentrated extract was stored at –20 °C for further analysis.

2.5. Essential oil analysis by GC–MS

The GC–MS (TRACE GC Ultra Gas Chromatograph, THERMO Scientific Corp., USA) was used to assess the volatile substances profile of ajwain, summer savory, and oregano EOs as described by Morsy and Hamad [29]. Identification was performed by retention index (RI) in reference to *n*-alkanes (C_8 – C_{24}), MS spectra, which were compared with spectra from the NIST library. The percentage composition of the volatiles was calculated using the normalization method and the GC peak areas.

2.6. Total phenolic and flavonoid contents

The WR and SR extracts were analyzed spectrophotometrically for total phenolic content (TPC) using the Folin-Ciocalteu reagent as described by Gulluce et al. [30]. Absorbance was measured at 750 nm. Gallic acid was used as a reference standard. The results were expressed as mg of gallic acid equivalent (GAE)/g dry weight of the extracted material. Total flavonoid content (TFC) of the investigated samples was determined using the colorimetric aluminum chloride method according to Zhishen et al. [31]. Absorbance was measured at 510 nm. The results are expressed in mg of quercetin equivalent (QE)/g of dry weight of the extracted material.

2.7. Antioxidant activity

2.7.1. DPPH assay

The DPPH radical scavenging activity of the various extracts was assessed as described by Pereira et al. [32]. The disappearance of DPPH was detected spectrophotometrically Unico UV-2000 Spectrophotometer (United Products and Instruments, USA) at 517 nm. BHT was used as a standard. The IC_{50} value is the concentration of the extract required to reduce 50 % of DPPH radicals.

2.7.2. FRAP assay

FRAP was determined at 700 nm in the investigated samples according to the method of Vijayalakshmi and Ruckmani [33] using spectrophotometer. BHT was used as a positive control sample of the assay. The IC_{50} values of various samples were determined as the concentration of the extracts in which the absorbance reaches the 0.5 value.

2.8. Antibacterial capacity

The antibacterial activity of ajwain, summer savory and oregano WD wastes (lyophilized WR and SR ethanolic extracts) was determined using the well diffusion method according to Sağdıç [34]. Before the determination of antimicrobial activity, 0.1 g of the freeze-dried WR was dissolved in 5 mL distilled water. The antibacterial activity of the extracts was tested against gram-positive bacteria (*Bacillus subtilis* 765 NRRL Northern Regional, *Bacillus cereus* 33018 and *Staphylococcus aureus* 25923), and gram-negative bacteria (*Salmonella typhimurium* 14028 Rockvill, Maryland, USA, *Escherichia coli* 15 and *Serratia marcescens* 37 FS). 0.1 mL of each microorganism (1.0×10^7 CFU/mL) was spread over the surface of plate count agar medium (Oxoid, England). Wells (5 mm in diameter) were filled with 50 μ L of the samples with test LAB strains. Plates were incubated at 37 °C for 48 h and ampicillin (10 μ g/well) was used as a positive control sample [35]. The inhibition zones were measured in mm.

2.9. Statistical analyses

All analyses were carried out in triplicate, except for GC–MS and HPLC (single determination). Statistical analysis was carried out using ANOVA and Tukey's test at $p < 0.05$ significance level, using XLSTAT.

3. Results and discussion

3.1. Chemical composition of ajwain, summer savory and oregano EOs defined by GC–MS

The yields of ajwain, summer savory and oregano essential oils were equal to 2.2 ± 0.06 , 2.8 ± 0.11 and 1.2 ± 0.07 %, respectively (Table 1). These results are consistent with those found by Skubij and Dzida [36], Morsy et al. [37] and Manaa et al. [38], respectively. GC–MS analysis of ajwain, summer

savory and oregano EOs revealed the presence of 16, 21 and 21 compounds, respectively, comprising > 99% of the total EO (Table 1 and Figure 1).

The chemical profile of ajwain EO shows a predominant of oxygenated monoterpenes (particularly thymol as a phenolic monoterpene), followed by monoterpene hydrocarbons, and then oxygenated sesquiterpene. The aromatic constituents in ajwain EO were composed mainly of thymol (65.12%), *p*-cymene (27.15%) and γ -terpinene (5.65%), while carvacrol was present in a scant amount (0.13%). This result is in agreement with the result obtained by Anwar et al. [39], Saadat et al. [40] and Das et al. [41]. Other studies have reported different percentages of these components due to the geographical location and collocation time [42].

The identified volatile components in summer savory essential oil were mainly oxygenated monoterpenes. The predominant summer savory EO compounds were carvacrol (81.86%), γ -terpinene (4.20%), thymol (3.64%), *p*-cymene (3.12%) and trans- β -caryophyllene (2.95%). Summer savory is an important herb due to eminent components of its essential oil (EO) like carvacrol, γ -terpinene, *p*-cymene, and thymol [43,44]. Carvacrol acts as a quality index in summer savory essential oil quality assessment [45].

Table 1. Volatile components relative percentage (%)¹ of ajwain (A), summer savory (S) and oregano (O) obtained by HD and detected by GC-MS

Таблица 1. Относительное процентное содержание летучих компонентов (%)¹ ажгона (А), чабера садового (S) и орегано (О), полученных методом водной дистилляции и обнаруженных по методу ГХ-МС

Compounds	RI ²	A	S	O
Monoterpenes hydrocarbons				
α -Thujene	931	0.04	0.37	0.07
α -Pinene	939	0.21	0.36	0.11
Camphene	956	ND	0.05	0.06
β -Pinene	980	0.55	0.07	ND
β -Myrcene	991	0.11	0.65	0.13
α -Phellandrene	1005	ND	0.21	ND
Δ -3-Carene		0.05	ND	ND
α -Terpinene	1018	0.07	0.87	0.30
<i>p</i> -Cymene	1026	27.15	3.12	1.90
R (+)-Limonene	1033	0.05	ND	ND
γ -Terpinene	1048	5.65	4.20	1.28
Cis-Sabinene hydrate	1068	ND	0.15	ND
Trans-Sabinene hydrate	1072	ND	0.10	0.09
Oxygenated monoterpenes				
Cis-Limonene oxide	1079	0.12	ND	ND
Terpinolene	1088	ND	0.10	0.08
Endo-Borneol	1167	ND	0.05	0.66
Terpinen-4-ol	1177	0.12	0.31	0.38
α -Terpineol	1188	0.09	0.12	0.09
D-Carvone	1252	ND	ND	0.10
Bornyl acetate	1285	0.03	ND	ND
Thymol	1419	65.12	3.64	1.26
Carvacrol	1430	0.13	81.86	89.34
Sesquiterpenes hydrocarbons				
Alloaromadendrene	1432	ND	ND	0.14
α -Humulene	1454	ND	0.19	0.13
Trans- β -caryophyllene	1468	ND	2.95	2.39
Ledene	1489	ND	ND	0.12
Oxygenated sesquiterpenes				
β -Bisabolene	1510	ND	0.10	0.24
Caryophyllene oxide	1581	ND	0.20	0.58
(+) Spathulenol	1585	0.15	ND	ND
Total oxygenated compounds (%)		65.76	86.38	92.73
Total non-oxygenated compounds (%)		33.88	13.29	6.72
Monoterpenes Hydrocarbons		33.88	10.15	3.94
Oxygenated Monoterpenes		65.61	86.08	91.91
O/H ratio ³		1.94	8.48	23.33
Total identified compounds (%)		99.64	99.67	99.45
% yield		2.2 $\pm$ 0.06	2.8 $\pm$ 0.11	1.2 $\pm$ 0.07

¹ Relative area percent (peak area relative to total peak area).

² Retention indices (RI) using a TR-5MS column.

³ O/H ratio represented Oxygenated monoterpenes/Monoterpenes hydrocarbons
ND = not detected.

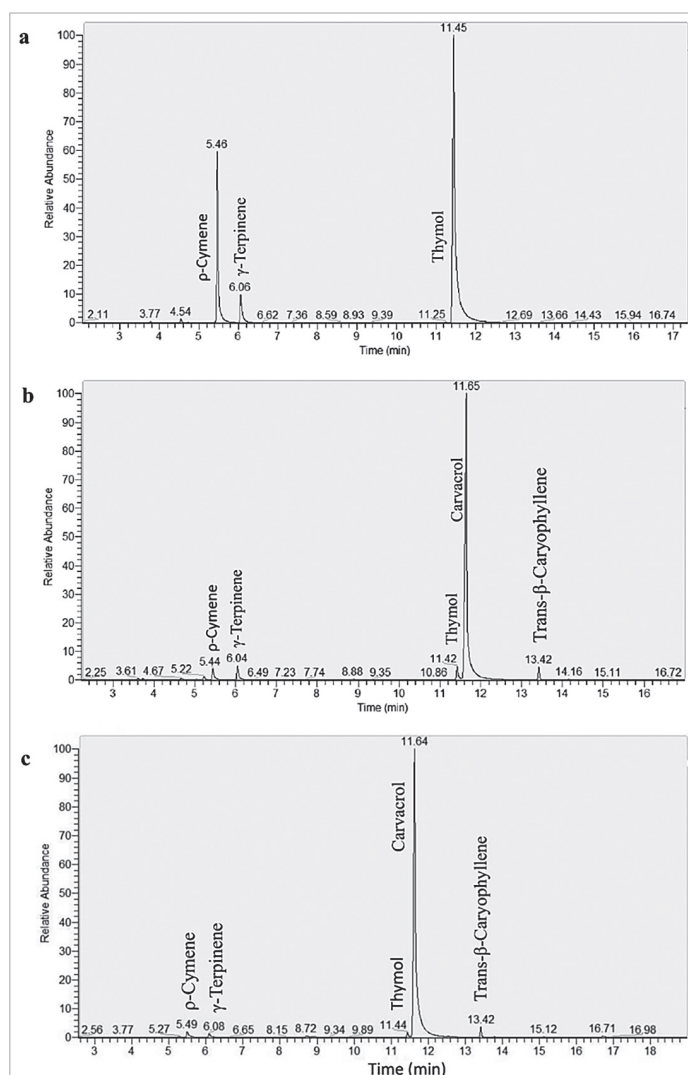


Figure 1. GC-MS chromatograms of volatile constituents of a: ajwain, b: summer savory and c: oregano

Рисунок 1. ГХ-МС хроматограммы летучих компонентов а: ажгона, б: чабера садового и в: орегано

The main identified constituents in oregano EO were carvacrol as monoterpenoid polyphenol (89.34%), trans- β -caryophyllene (2.39%), *p*-cymene (1.90%), γ -terpinene (1.28%) and thymol (1.26%). These results are consistent with the findings of Meerasri et al. [46].

The concentration ratio of oxygenated monoterpenes to monoterpene hydrocarbons (O/H ratio) in the essential oils of ajwain, summer savory and oregano were approximately 1.94:1, 8.48:1 and 23.33:1 based on the GC-MS analysis results (Table 1). It is important to highlight that oxygenated monoterpenes are more valuable and have a stronger odor than monoterpene hydrocarbons [47].

3.2. Total phenolic and flavonoid contents

TPC and TFC (total flavonoid content) of the investigated extracts are illustrated in the Figure 2. Statistical analysis showed that the highest TPC (50.3 $\pm$ 0.48 mg GAE/g DM) was recorded for the water residue obtained from summer savory (WRS), followed by the water residue obtained from oregano (WRO) (39.3 $\pm$ 0.30 mg GAE/g DM). The efficient recovery of antioxidant phenolic components from oregano was achieved with 1:20 g/mL [48].

Meanwhile, the TPCs of the water residue obtained from ajwain (WRA) processing, the solid residues obtained from summer savory (SRS) and the solid residue from oregano (SRO) were not significantly different ($p>0.05$). The lowest TPC was noticed in the solid residue from ajwain (SRA) (3.5 $\pm$ 0.01 mg GAE/g DM). However, the highest TFC was found in the WRO (45.5 $\pm$ 0.39 mg QE/g DM), followed by the WRS (32.3 $\pm$ 0.03 mg QE/g DM), SRS (17.6 $\pm$ 0.03 mg QE/g DM), and the WRA (11.1 $\pm$ 0.31 mg QE/g DM). The lowest levels of TFC were recorded for SRA and SRO without significant ($p>0.05$) difference. Irakli et al. [49] found that TPC and TFC values of oregano solid residue ethanolic extract were equal to 90.49 mg GAE/g and >125 mg CATE/g, respectively. Mishra et

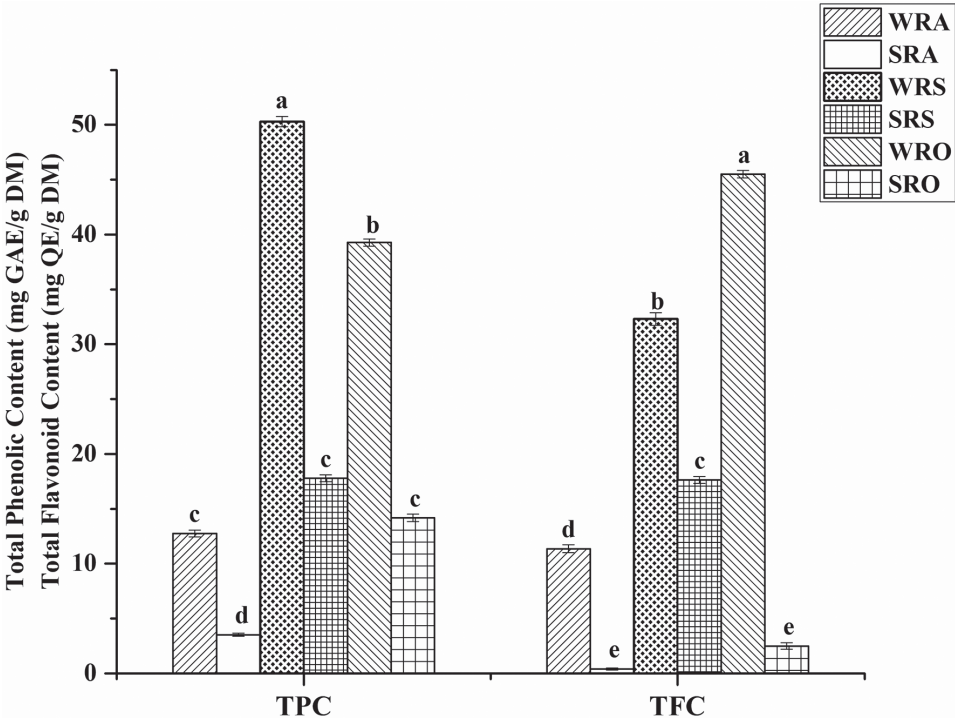


Figure 2. Total phenolic content (mg GAE/g dried material) and total flavonoid content (mg QE/g dried material) from the water distilled by-products. WRA: water residue from ajwain, WRS: water residue from summer savory, WRO: water residue from oregano, SRA: solid residue from ajwain, SRS: solid residue from summer savory and SRO: solid residue from oregano. The values refer to mean $\pm$ standard deviations (SD) of three parallel measurements

Рисунок 2. Общее содержание фенолов (мг. экв. ГК/г сухого материала) и общее содержание флавоноидов (мг. в КЭ/г сухого материала), содержащихся в побочных продуктах, полученных путем водной дистилляции. БОА: водный остаток ажгона, БОЧ: водный остаток чабера садового, БОО: водный остаток орегано, ТОА: твердый остаток ажгона, ТОЧ: твердый остаток чабера садового и ТОО: твердый остаток орегано. Значения равны среднему значению результатов трех параллельных измерений $\pm$ стандартное отклонение (SD)

al. [50] reported that TPC of the aqueous ajwain extracts was found to be 6.2 ± 0.4 g GAE/100 g dried extract. TFC in the WR of ajwain seeds was equal to 0.04 mg QE/g DWW [19].

3.3. HPLC of polyphenols

Compared to the SR extracts, the WR residue possessed a higher content of phenolic compounds. The longer Clevenger system distillation duration is thought to promote both the breakdown of more labile polyphenols and the leaching of less soluble polyphenols into the DWW [51].

Some phenolic compounds could be solubilized and discarded in the wastewater used for the hydrodistillation thus changing the phenolic composition of the nondistilled plant [52]. Results are presented in the Table 2. Chlorogenic acid, catechin, vanillic acid, ρ -hydroxybenzoic acid, ferulic acid, syringic acid, and caffeic acid were the main phenolic compounds found in WRA. The lowest phenolic components found in SRA were ferulic acid, chlorogenic acid, syringic acid and caffeic acid. The predominant phenolic components in WRS were catechin, syringic acid, chlorogenic acid, ρ -hydroxybenzoic acid, vanillic acid, caffeic acid,

Table 2. Polyphenol compounds identified in the investigated by-products
Таблица 2. Полифенольные соединения, обнаруженные в исследуемых побочных продуктах

Compounds	(µg/mL)					
	WRA	SRA	WRS	SRS	WRO	SRO
Phenolic compounds						
Catechol	ND	ND	234.63	25.73	ND	ND
<i>p</i> -Hydroxy benzoic acid	271.25	ND	831.52	ND	352.17	ND
Chlorogenic acid	781.51	2.21	2320.71	14.00	106.60	19.41
Vanillic acid	274.16	ND	559.00	ND	253.72	ND
Caffeic acid	43.45	1.23	404.35	ND	37.78	ND
Syringic acid	60.81	1.31	3523.47	6.65	108.98	34.72
<i>p</i> -Coumaric acid	ND	ND	134.35	ND	92.89	ND
Ferulic acid	116.34	3.95	350.50	21.01	624.23	14.02
<i>o</i> -Coumaric acid	9.84	ND	16.22	3.95	37.92	ND
Flavonoid compounds						
Catechin	277.85	ND	5744.63	13.42	1534.24	47.76
Resveratrol	ND	7.61	437.01	43.86	210.08	ND
Quercetin	ND	ND	3080.48	76.27	24324.16	120.06
Hesperidin	84.83	13.84	13841.74	115.73	227.10	132.66
Rosmarinic acid	ND	ND	1802.59	12.92	1207.43	16.76
Rutin	302.04	ND	321.21	9.61	42.78	2.69
Myricetin	34.77	2.23	119.81	6.44	ND	6.38
Apigenin	7.77	4.43	27.51	5.26	41.18	1.18

ND: not detected

ferulic acid, catechol and *p*-coumaric acid. The highest phenolic components in WRO were catechin, ferulic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid and chlorogenic acid. Meanwhile, the SRO extract featured lower values of phenolic compounds (catechin, syringic acid, chlorogenic acid and ferulic acid) compared to the WRO. Meanwhile, the abundant phenolic compounds in WRO were catechin, ferulic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid and chlorogenic acid. On the other hand, the phenolic constituents (catechin, syringic acid, chlorogenic acid and ferulic acid) of SRO extract were found in low values.

The predominant flavonoids in WRO, SRO, WRS and SRS were quercetin, rosmarinic acid, hesperidin, resveratrol, rutin. HPLC analysis revealed that rosmarinic acid is the dominant phenolic compound of hydrodistillation water residues of *Salvia* species [53].

The WR obtained from fresh basil, rosemary and sage is characterized by high level of rosmarinic acid [54].

Myricetin was not found in WRO. Resveratrol was not found in SRO. The lowest values of flavonoid compounds were found in WRA (rutin, hesperidin, myricetin and apigenin) and SRA (hesperidin, resveratrol, apigenin and myricetin).

The ajwain residues (both WRA and SRA) did not contain quercetin or rosmarinic acid. On the other hand, resveratrol was detected in the WRO but not in the SRO. Conversely, resveratrol was found in the SRA but was not detected in the WRA. Regarding myricetin, a similar picture was observed in both SRO and WRO.

3.4. Antioxidant activity

The antioxidant activity of the investigated extracts was evaluated using DPPH assay (Figure 3). The WRO extract proved to be the most effective radical scavenger (IC₅₀ value = 2.95 µg GAE/mL), followed by the WRS (IC₅₀ value = 9.99 µg GAE/mL). Džamić et al. [55] reported that the water residue extract of *Hyssopus officinalis* L. subsp. *Pilifer* possessed higher antioxidant activity than its solid residue extract. Polyphenols with antioxidant properties, such as rosmarinic acid, are found in Greek oregano [56]. Thymol and carvacrol were responsible for the radical scavenging activity [57–58].

Chizzola et al. [59] and Wollinger et al. [60] found that the wastewater obtained after distillation of from *Rosemary officinalis* and *Thymus vulgaris* had a lot more antioxidants than the solid residue and the undistilled plant. Similarly, Alice et al. [61] found that the wastewater of *O. vulgare* featured a greater TPC than the aqueous extract of the undistilled plant. Despite their normally low-to-medium solubility in water, phenols dissolve better in wastewater due to temperature increases and their capacity to form hydrogen bonds with water [62,63]. In general, the antioxidant efficiency of the extracts is related to their high content of TPC. This result is in agreement with the results of de Elguea-Culebras et al. [51]. The highest antioxidant characteristics are related to the content of polyphenols such as flavonoids [64,65].

Meanwhile, the radical scavenging activity of SRS and SRO (IC₅₀ value = ~ 35.42 µg GAE/mL) is close to that of BHT (IC₅₀ value = 38.1 µg/mL). European Commission considered the SR of *Rosemary officinalis* as a food preservative (code E-392) [66].

No DPPH radical scavenging activity was recorded for SRA. On the other hand, the lowest activity was recorded for WRA (IC₅₀ value = 60.05 µg GAE/mL). The lowest level of phenolic compounds caused poor DPPH radical-scavenging activity [52].

Dorman et al. [67] stated that the antioxidant activity determined by different assays are not correlated with TPC of the extracts. It is mainly

related to the major phenolic component present in *Lamiaceae* extracts, rosmarinic acid.

A similar trend was observed in the FRAP assay. A lower IC₅₀ value means a higher antioxidant activity [68]. Data in the Figure 4 indicate that WRO and WRS showed higher reducing power (IC₅₀ values of 3.16 and 3.8 µg GAE/mL, respectively) compared to BHT (IC₅₀ value = 15.16 µg/mL), followed by WRA, SRS and SRO (IC₅₀ values of 30.40, 32.86 and 46.16 µg GAE/mL, respectively). These results indicated that WRO and WRS possess the efficiency to reduce the Fe⁺³ to Fe⁺² as good chelators. On the other hand, SRA exhibited poor reducing power (IC₅₀ value = 105.26 µg GAE/mL).

In comparison to synthetic antioxidants, the value of natural resource-based antioxidants will grow with time. This means that the water residue from hydro distillation must be regarded as a co-product of the extraction process instead of being considered a waste product. This residue can be added to a target product to increase its antioxidant activity or re-extracted to obtain pure antioxidants [57].

Mielnik et al. [69] reported that the antioxidants in WR can be used to keep stored meat products from turning rancid.

3.5. Antibacterial activity

Many aromatic plants or their oils were effective against the growth of several foodborne bacteria [70]. The antimicrobial activity of the investigated extracts was evaluated per their antimicrobial efficiency against the tested bacteria (Table 3).

As reported by el Hassouni et al. [71], the antibacterial activity of extracts can be divided into 3 categories according to the inhibition zone (iz): weak activity (iz ≤ 12 mm), moderate activity (iz from >12.0 to <20 mm) and strong activity (iz ≥ 20 mm).

The antibacterial properties of WRA and WRS were significantly (*p* < 0.05) superior in comparison to their counterparts of solid residue extracts against *Bacillus cereus* 33018 and *Staphylococcus aureus* 112. The same trend was also noticed for WRS and WRO extracts against *Escherichia coli* 15 and *Serratia marcescens* 37. The highest antibacterial activity of the investigated extracts against *Bacillus subtilis* 765 NRRL and *Salmonella typhimurium* 14028 was recorded in WRO. The antibacterial activity of WRO extract against *Salmonella typhimurium* 14028 and *Serratia marcescens* 37 exceeded those of the standard ampicillin. Meanwhile, WRS extract exhibited greater antibacterial activity than ampicillin against *Bacillus cereus* 33018. This WR is regarded as bioactive compounds with high antibacterial value [35].

On the other hand, the SRO showed moderate antibacterial activity at both G⁺ and G⁻ bacteria, whereas SRS showed weak to moderate antibacterial activity. According to Pandey and Rizvi [72], rosmarinic acid features several intriguing biological properties, including antiviral, antibacterial, anti-inflammatory, and antioxidant properties. Catechins provides bactericidal effects on gram-positive and gram-negative bacteria [73].

4. Conclusion

Our study demonstrated that hydrodistilled water residue is a better source of phenolic compounds than the solid residue. Water residue obtained after hydrodistillation featured remarkably higher antioxidant and antibacterial activities than the insoluble residue extracts. Therefore, the wastewater obtained as a distillation by-product can be utilized as a natural source of bioactive compounds with potential applications as antioxidants and preservatives in food, cosmetics and medicine industries.

Table 3. The antibacterial activity* of the investigated extracts

Таблица 3. Антибактериальное действие* исследуемых экстрактов

Microorganisms	WRA	SRA	WRS	SRS	WRO	SRO	Ampicillin (10 µg)
Gram Positive Bacteria							
<i>Bacillus subtilis</i> 765 NRRL	23.00 ^{bb} ± 1.00	20.67 ^{ab} ± 1.53	8.33 ^{dc} ± 1.53	12.67 ^{abc} ± 3.06	24.00 ^{cb} ± 1.00	12.67 ^{bc} ± 1.53	33.33 ^{aa} ± 1.53
<i>Bacillus cereus</i> 33018	24.00 ^{bb} ± 2.00	14.33 ^{bc} ± 1.53	28.00 ^{aa} ± 1.00	12.00 ^{abcd} ± 1.00	14.00 ^{cb} ± 1.00	12.00 ^{bcd} ± 1.00	8.67 ^{cd} ± 1.53
<i>Staphylococcus aureus</i> 112	29.00 ^{ab} ± 1.00	13.67 ^{bd} ± 1.53	23.00 ^{bc} ± 2.00	9.00 ^{ce} ± 1.00	17.00 ^{dd} ± 1.00	15.00 ^{abd} ± 1.00	35.00 ^{aa} ± 1.00
Gram Negative Bacteria							
<i>Salmonella typhimurium</i> 14028	14.00 ^{dde} ± 1.00	16.00 ^{bcd} ± 1.53	17.00 ^{cc} ± 1.00	12.00 ^{abe} ± 1.00	32.00 ^{aa} ± 1.00	14.00 ^{bde} ± 1.00	25.37 ^{bb} ± 0.96
<i>Escherichia coli</i> 15	17.67 ^{cd} ± 1.53	13.00 ^{bde} ± 1.00	21.67 ^{bb} ± 2.08	11.00 ^{abe} ± 1.00	17.00 ^{dbc} ± 1.00	13.00 ^{bde} ± 1.00	35.33 ^{aa} ± 2.08
<i>Serratia marcescens</i> 37	17.00 ^{cdcd} ± 1.00	20.00 ^{abc} ± 1.00	20.00 ^{bcb} ± 1.00	15.00 ^{ad} ± 1.00	27.00 ^{ba} ± 1.00	18.00 ^{acd} ± 1.00	22.33 ^{bb} ± 1.53

* Mean zone of inhibition in mm include well diameter (5 mm) ± Standard deviation (SD) where *n* = 3. Means followed by different lowercase letters in the same column and uppercase letters in the same row indicate significant differences at *p* < 0.05.

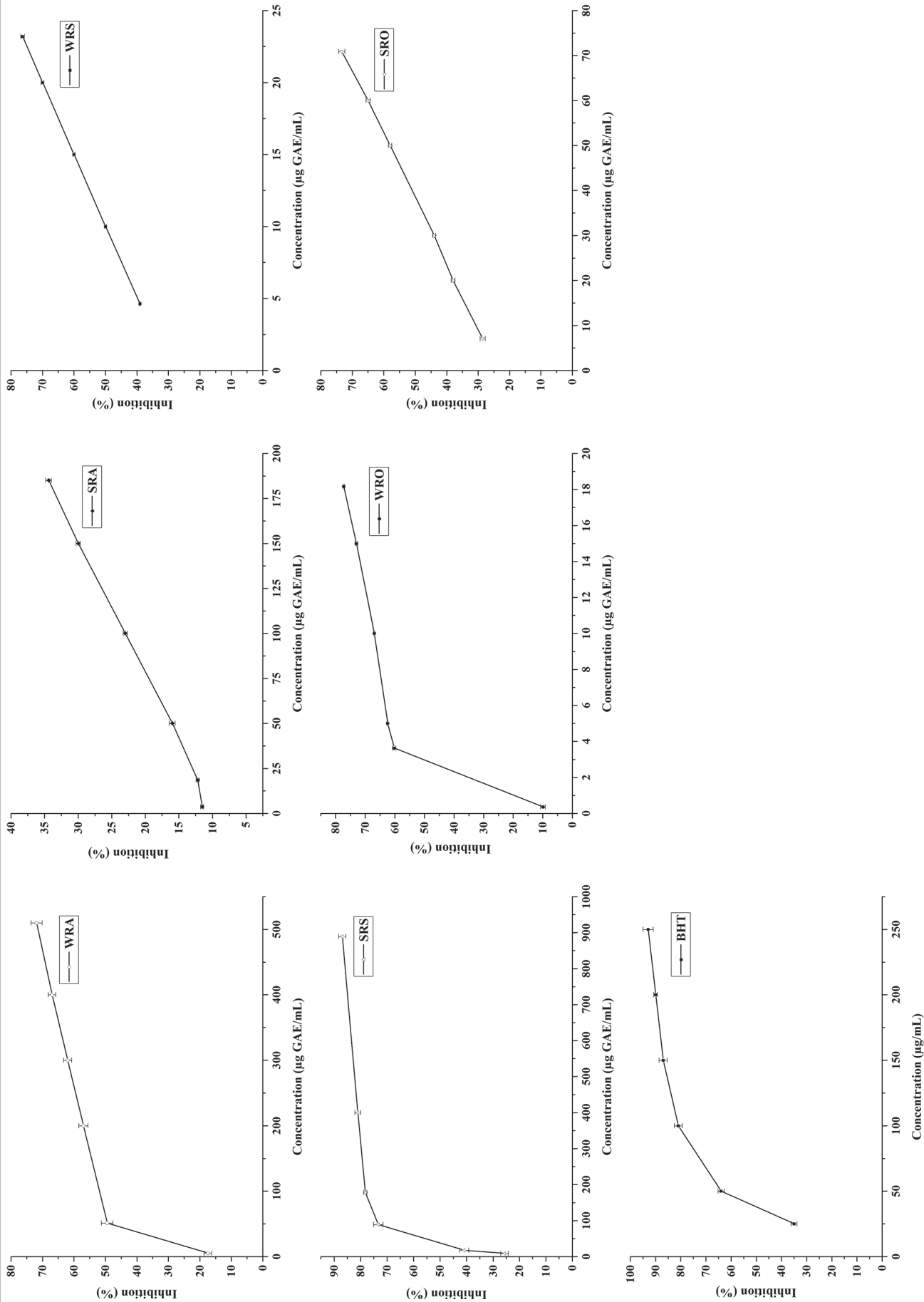


Figure 3. Antioxidant activity of the water distilled by-products and BHT as reference standard against DPPH radicals. The values refer to mean $\pm$ standard deviations (SD) of three parallel measurements

Рисунок 3. Антиоксидантная активность побочных продуктов дистилляции воды и ВБТ в качестве стандартного образца в отношении радикалов ДФПГ. Значения представлены как среднее значение результатов трёх параллельных измерений $\pm$ стандартное отклонение (SD)

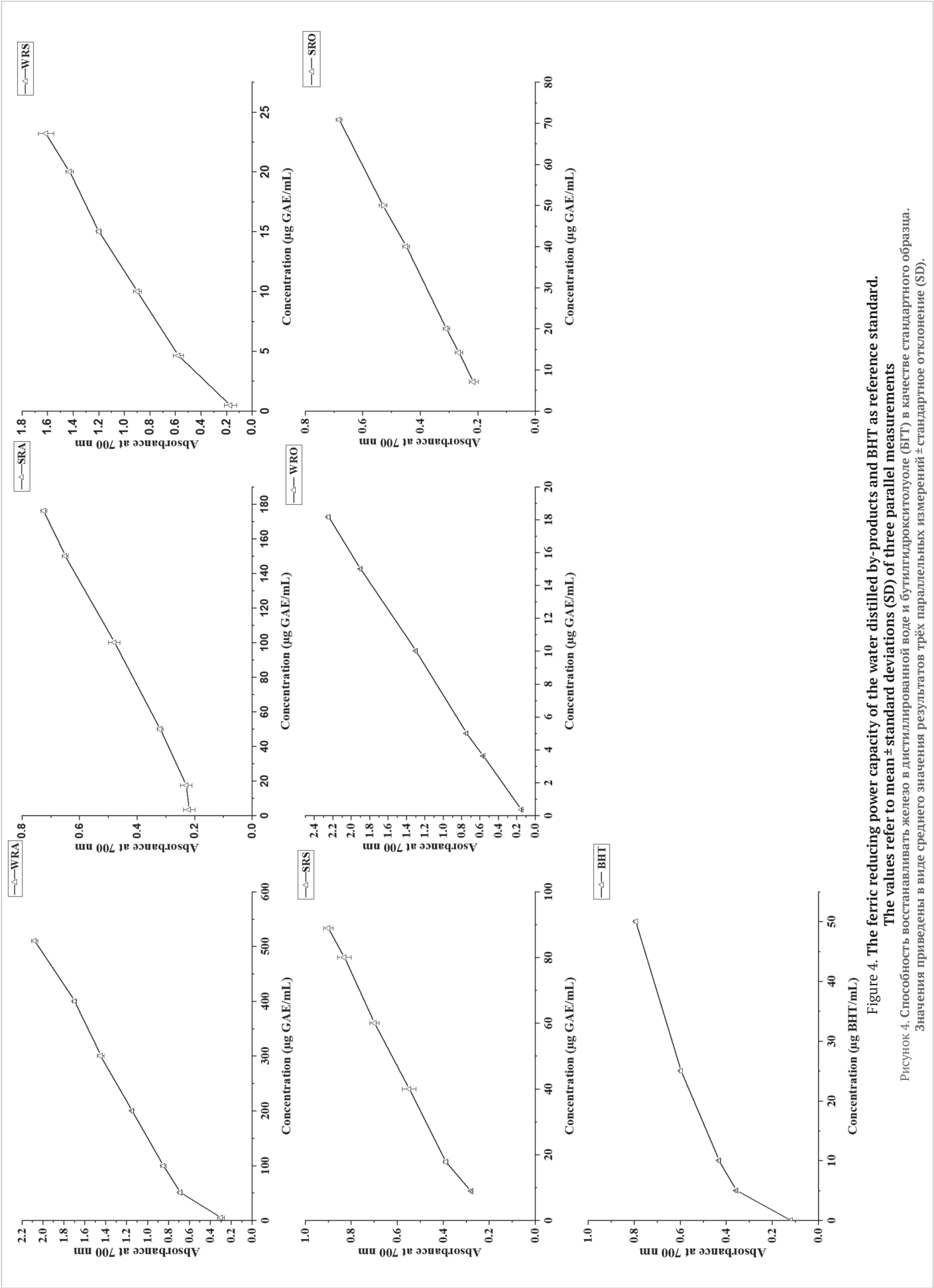


Figure 4. The ferric reducing power capacity of the water distilled by-products and BHT as reference standard.
The values refer to mean $\pm$ standard deviations (SD) of three parallel measurements
Рисунок 4. Способность восстанавливать железо в дистиллированной воде и бутилгидрокситолуоле (БГТ) в качестве стандартного образца.
Значения приведены в виде среднего значения результатов трёх параллельных измерений $\pm$ стандартное отклонение (SD).

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