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# Chemical composition and biological potential of garlic by-products

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KEYWORDS
garlic; black garlic; by-product; phenolics; HPLC; antioxidant activity

KEY CONTRIBUTION

The chemical composition and biological potential of by-products from white and black garlic were investigated.

The extracts were prepared using microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE).

The highest total phenolic content and antioxidant were obtained by MAE.

The extracts showed strong antimicrobial activity, especially against Listeria monocytogenes.

Extracts from white garlic by-products showed potential as natural additives in the food industry.

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**ABSTRACT**

Garlic is a vegetable from the *Allium* genus that has been valued for its medicinal properties since ancient times. Today, alongside traditional white garlic, increasing attention is being given to black garlic (produced through the thermal processing of white garlic at a specific temperature and relative humidity), a food with equally beneficial properties and rich nutritional value that is associated with numerous health benefits and a valuable nutritional composition. During the thermal processing of garlic, valuable by-products are formed, characterized by a high content of phenolic compounds, which are known for their exceptional biological properties, particularly their antioxidant and antimicrobial activity. The aim of this research was to determine and compare the chemical composition and biological potential of extracts from white and black garlic by-products. Extracts were prepared using microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE). The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu method, while specific phenolic compounds were identified using the HPLC method. The antioxidant potential was evaluated using FRAP, ORAC, and DPPH methods, and the antimicrobial activity was assessed by determining minimal inhibitory and bactericidal concentration. The results indicate a high TPC, with rutin identified as the dominant flavonoid, and a good biological potential for all tested extracts. Extracts from black and white garlic by-products exhibited similar antimicrobial activity against all tested microorganisms, with better activity observed against Gram-positive species, particularly Listeria monocytogenes. The extract from white garlic by-products obtained through microwave-assisted extraction demonstrated the highest antioxidant activity as measured by the DPPH (43.38%) and ORAC (39.02 mM TE/L) methods, along with the highest total phenolic content (60.10 mg GAE/g), indicating its exceptional potential for application in the food industry as a natural alternative to synthetic additives.

## Introduction

Garlic is a vegetable from the genus *Allium*. It has been cultivated in Central Asia since ancient times, from where it spread to other parts of the world, and today it is a highly valued food in all parts of the world (dos Santos et al., 2022). Garlic (*Allium sativum*) is one of the oldest cultivated plants that has been used for medicinal purposes for thousands of years. In their writings, the Egyptians described the use of garlic and interpreted its effect on heart problems, headaches and bites. In Ancient Greece, it was used to treat intestinal disorders, and in India it was used for washing wounds and as an antiseptic. Garlic tea was used in China to treat headaches, fever, cholera and dysentery (Alam et al., 2016).

Compared to fresh garlic, black garlic does not have a strong and unpleasant odour and taste due to the lower content of allicin, which is converted into alkaloids and flavonoid compounds as it matures. The physico-chemical changes that take place during the thermal process are responsible for the increased bioactivity of black garlic compared to fresh garlic. Due to its increased bioactivity, black garlic is characterized by antioxidant, antibacterial, antidiabetic, anti-inflammatory, anti-cancer effects, and cardiovascular diseases risk reduction (Jing, 2020; Kimura et al., 2017).

In addition to black garlic, research has shown numerous positive effects of fresh garlic on human health, with its anti-cancer potential being among the most prominent. This is greatly contributed by the high nutritional value of the plant, as well as known bioactive compounds with sulphur such as allin. It is believed that garlic has antimicrobial, antioxidant and anti-inflammatory properties. Although the mentioned biological properties have been confirmed, the mechanisms by which the components of garlic act in the body have not yet been fully elucidated (Kimura et al., 2017).

Most of the mentioned activities are the result of research conducted on the garlic cloves, while a very small number of studies dealt with the husk, which is usually discarded and considered an industrial by-product, and makes up 25% of the total industrial production. The results of the studies indicate a rich phenolic profile and potential biological properties of the garlic husk, which could enable its use and application in the pharmaceutical or food industry (dos Santos et al., 2022).

The aim of this study was to determine the total phenolics content in the white garlic and black garlic husks as well as to investigate their biological potential in particular antioxidant and antimicrobial activity.

## Materials and methods

*Sample preparation*

Two different samples of garlic were used in this study. White garlic bulbs were collected in Katuni (Šestanovac Municipality, Croatia), while black garlic bulbs were purchased from the company Allium Nigrum j.d.o.o. (Zagreb, Croatia). For this study, the by-product samples consisted of the husks obtained by removing the outer peel of the garlic bulb and the peel around the garlic cloves (inner peel). The husks were powdered in a MultiDrive control mill (IKA-Werke GmbH & Co. KG, Staufen, Germany). The garlic husks were homogenized for 2 min at 10,000 rpm. Two novel green extraction methods were used. For ultrasound-assisted extraction (UAE), samples were extracted with 50% ethanol in 1:20 ratio. The extraction was carried out in an ultrasonic bath (DU-100, Giorgio Bormac S.r.l., Carpi, Italy) for 1 h at 60 °C and 40 kHz. After extraction, the samples were filtered and ethanol was evaporated in a rotary evaporator. For microwave-assisted extraction (MAE), samples were extracted with 50% ethanol in 1:20 ratio. The extraction lasted 5 min at 600 W in an advanced microwave extraction system (ETHOS X, Milestone Srl, Sorisole, Italy). The samples were then filtered and ethanol evaporated. After ethanol evaporation, all samples were frozen and then freeze-dried (FreeZone 2.5, Labconco, Kansas City, MO, USA). After drying, the extracts were dissolved (10 mg/mL) in 50% ethanol for further analyses.

*Determination of the phenols*

The total phenolics content (TPC) in garlic husk extracts was determined by most often used quantification method Folin-Ciocalteu (Amerine and Ough, 1981; Čagalj et al., 2022). The obtained results were expressed in mg of gallic acid equivalents per g of dry extract (mg GAE/g dry extract).

To determine the phenolic composition, HPLC was used in combination with a UV/Vis detector (Shimadzu Nexera LC-40, Shimadzu, Kyoto, Japan) and an appropriate data processing program (LabSolution, Shimadzu, Kyoto, Japan). The column used in the work was Phenomenex-C18 (250 × 4.6 mm, particle size 5 µm, Phenomenex, California, USA). The method by Sharma et al. (2014) was used with modifications. Two mobile phases were used: phase A (0.1% acetic acid), phase B (methanol). The temperature of the column was 27 °C, and the volume of the injected sample was 20 µL. The total run time was 25 minutes, following the gradient: initially, 20% B until 5 minutes; from 5 to 10 minutes, a linear increase to 80% B, maintained at 80% B until 15 minutes; from 15 to 20 minutes, 20% B until the end of the analysis. Injection was done in two repetitions and detection was performed at wavelengths of 280 nm and 360 nm. Concentrations of identified and quantified compounds are expressed in mg of compound per L of extract (mg/L).

*Antioxidant and antimicrobial activity*

For the assessment of antioxidant activity, assays based on different mechanisms were used: FRAP, DPPH and ORAC.

The FRAP (ferric reducing antioxidant power) method is based on the electron transfer mechanism, which uses antioxidants in an acidic medium to measure the reduction of the ferrous $Fe^{3+}$ ion complex into the intensely blue ferrous $Fe^{2+}$ ion complex. Antioxidant activity was determined as an increase in absorbance at 593 nm (Shahidi and Zhong, 2015). The used FRAP method was previously described by Benzie and Strain (1996) with modifications described in Čagalj et al. (2022). The FRAP results were expressed as micromoles of Trolox equivalents/L of extract (µM TE/L).

The DPPH (2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay) radical scavenging assay is one of the most commonly used antioxidant methods. DPPH is a stable, commercially available synthetic radical of dark purple colour. The method is based on the donation of electrons to antioxidants to neutralize DPPH radicals, and the reaction is accompanied by a change in the colour of the DPPH solution at 517 nm. The change in colour from dark purple to yellow is an indicator of the DPPH antioxidant efficiency of the tested antioxidants (Shahidi and Zhong, 2015). The used method was previously described by Milat et al. (2019). The results were expressed as the percentage of DPPH radical inhibition (% inhibition).

In the ORAC (oxygen radical absorbance capacity) method, the peroxyl radical reacts with fluorescein, which leads to a weakening of the fluorescence intensity, whereby the ability of the antioxidant to break the chain of peroxyl radicals is measured, where the inhibition of oxidation is monitored (Shahidi and Zhong, 2015). The method used was previously described by Burčul et al. (2018). The ORAC results were expressed as milimoles of Trolox equivalents/L of extract (mM TE/L).

The antimicrobial activity of the prepared extracts was evaluated by the broth microdilution method using 2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride (INT) as indicator. The minimal inhibitory concentration (MIC) was the lowest concentration at which no bacterial growth was detected as a reduction of colourless INT to red formazan (Elez Garofulić et al., 2021; Klančnik et al., 2010). The minimal bactericidal concentration (MBC) was the lowest concentration at which no bacterial growth was observed after cultivation of the bacterial suspension where no change in colour occurred (Elez Garofulić et al., 2021). The analyses were performed with common foodborne bacteria (*Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 14579, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853). The MIC and MBC results were expressed as mg/mL.

## Results and discussion

*Results of total phenols determination*

The content of total phenols in garlic husk extracts is shown in Figure 1. The results indicate more than two times higher proportion of phenols in extracts of white garlic husk (42.41 mg GAE/g) compared to extracts of black garlic husk (18.53 mg GAE/g) obtained by using UAE. Similar results were observed for husks extracts produced by using MAE. The TPC of white garlic husk was 60.10 mg GAE/g, while the TPC of black garlic was two times lower with 31.76 mg GAE/g. It is also possible to observe a higher proportion of phenols in the extracts obtained by using the MAE method compared to the UAE.

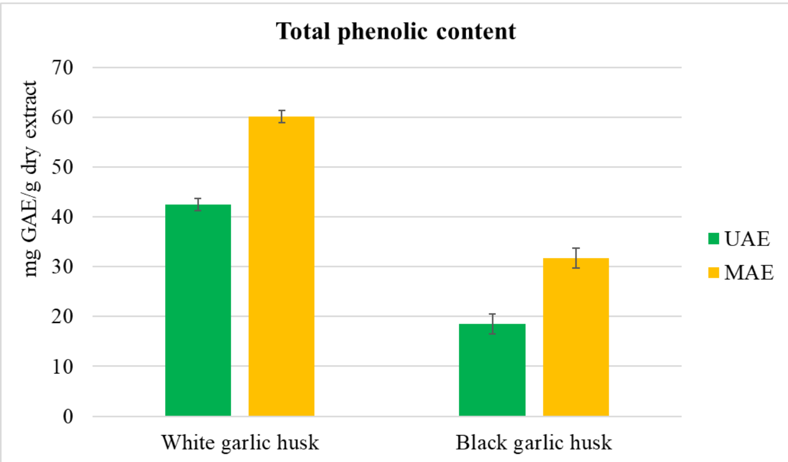


**Figure 1** Total phenolics content in garlic husk extracts (MAE – microwave-assisted extraction; UAE – ultrasound-assisted extraction; GAE – gallic acid equivalents)

Although research on garlic by-products is scarce, the available literature indicates an extremely interesting material with a high TPC compared to the edible part of this vegetable. For whole black garlic bulbs, Choi et al. (2014) reported higher values of TPC in black garlic samples (ranging from 25.81 to 58.33 mg GAE/g) compared to raw garlic samples. The authors also report an increase in the TPC in black garlic during fermentation. The increase in TPC was recorded until the 21$^{st}$ day of maturation, after which the TPC decreased and at the end of maturation period (35 days) it amounted to 48.35 mg GAE/g. The TPC in black garlic extracts is also affected by different fermentation conditions, which was confirmed by study of Kim et al. (2013), who carried out fermentation at different temperatures and relative humidity. Carreón-Delgado et al. (2023) examined the phenolic composition of garlic by-product extract prepared in methanol by stirring for 18 h at 120 rpm at 50 °C. The presented results indicate the TPC in the by-product from 28.50 to 32.47 mg GAE/g, which corresponds to results in this study for UAE extracts. The influence of different solvents and extraction methods on the TPC was discussed by several authors. Fortunata et al. (2019) reported a higher TPC in aqueous extracts of garlic by-products (64.69 mg GAE/L) compared to the ethanol extract prepared by maceration (47.04 mg GAE/L). Jang et al. (2018) reported almost 3.5 times higher proportion of total phenols in aqueous extract of black garlic by-product (147.58 mg GAE/g) compared to the ethanol extract (43.01 mg GAE/g). The used extraction method was mixing for 24 h. Using the HPLC method, the concentrations of the most abundant phenolic compounds were determined, and the obtained results are presented in Tables 1 and 2. Similar to TPC results, white garlic husk extract had higher proportion of phenolic components compared to the black garlic husk extract. Epicatechin and rutin were detected among the most dominant phenolic compounds in the white garlic husk extract. In black garlic husk extracts, epicatechin and rutin were also among the more dominant, but their concentrations were up to 6 times lower. Sunanta et al. (2023) highlighted caffeic acid, ferulic acid, quercetin, kaempferol and apigenin as some of the main phenolic compounds in garlic, while Azmat et al. (2023) reported that quercetin, kaempferol and rutin are dominant in the by-product of garlic. These studies confirm the results of our research on the detected compounds, although the authors only performed a qualitative analysis and did not quantify the detected compounds. Besides, white garlic and black garlic by-products extracts results are scarce in the literature, which further complicates the comparison of the obtained results. Although Jing (2020) emphasizes that black garlic generally has three times higher TPC in whole black garlic bulbs and six times higher TPC in peeled black garlic cloves than in fresh garlic cloves, this was not confirmed in our study.

**Table 1** Results of the HPLC method of the most abundant compounds in garlic husks extracted by ultrasonic-assisted extraction

| Phenolic compound, mg/L | White garlic husk | Black garlic husk |
|---|---|---|
| **Protocatechuic acid** | 0.16 ± 0.01 | 0.10 ± 0.00 |
| **Epicatechin** | 4.89 ± 0.29 | 1.63 ± 0.00 |
| **Rutin** | 6.47 ± 0.26 | 1.67 ± 1.02 |
| **Quercetin-3-glucoside** | 2.67 ± 0.15 | 0.82 ± 0.12 |
| **Myricetin** | 4.60 ± 0.95 | 0.66 ± 0.24 |
| **Quercetin** | 2.53 ± 0.08 | 1.11 ± 0.37 |
| **Kaempferol** | 0.73 ± 0.04 | 1.09 ± 0.00 |

**Table 2** Results of the HPLC method of the most abundant compounds in garlic husks extracted by microwave-assisted extraction

| Phenolic compound, mg/L | White garlic husk | Black garlic husk |
|---|---|---|
| **Protocatechuic acid** | 0.08 ± 0.00 | 0.11 ± 0.00 |
| **Epicatechin** | 3.57 ± 0.03 | 2.24 ± 0.28 |
| **Rutin** | 6.37 ± 0.18 | 1.00 ± 0.88 |
| **Quercetin-3-glucoside** | 2.63 ± 0.24 | 0.74 ± 0.57 |
| **Myricetin** | 2.34 ± 0.20 | 0.63 ± 0.14 |
| **Quercetin** | 1.60 ± 0.06 | 1.30 ± 0.31 |
| **Kaempferol** | 1.04 ± 0.06 | 0.93 ± 0.37 |

*Results of antioxidant and antimicrobial activity*

The results of the antioxidant activity of the extracts determined by the FRAP, DPPH and ORAC method are shown in Figure 2.

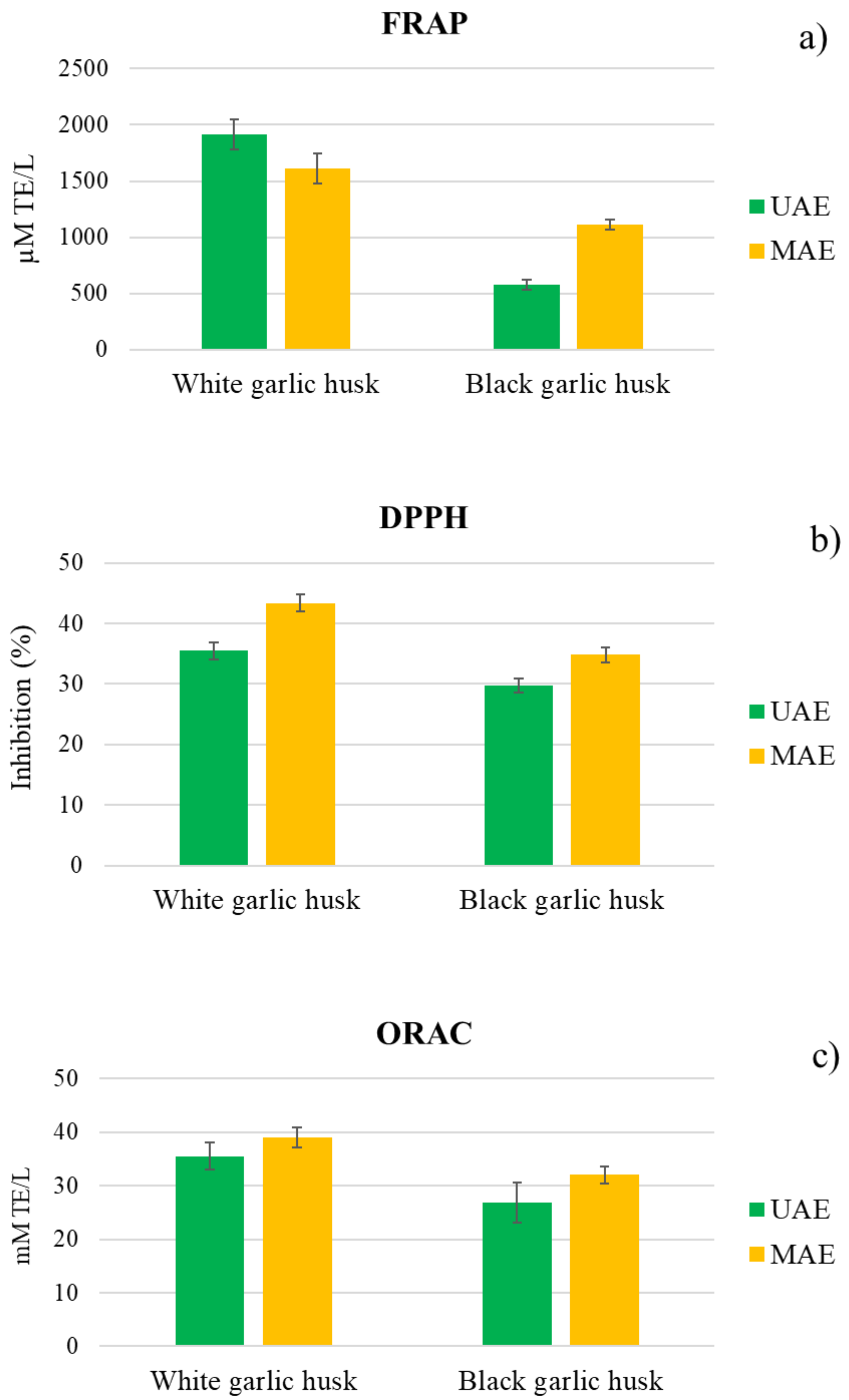


**Figure 2** Antioxidant activity measured by: a) FRAP, b) DPPH and c) ORAC method in garlic husk extracts (MAE – microwave-assisted extraction; UAE – ultrasound-assisted extraction; TE – Trolox equivalents)

The white garlic husk extract had higher reducing capacity (1915.38 µM TE/L) than black garlic husk extract (574.23 µM TE/L) when comparing samples extracted using UAE. Similar can be observed with extracts prepared by MAE, where the white garlic extract had higher reducing power, but slightly lower compared to the same sample prepared by UAE. In the FRAP values for black garlic husk extracts, almost twice the reduction capacity is observed for MAE sample (1111.54 µM TE/L) compared to the same sample extracted using UAE (574.23 µM TE/L), which is in relation to TPC results. The reducing ability of the extract, as well as the correlation between the activity and the TPC in black garlic by-product extracts, were examined by Jang et al. (2018). The influence of the used solvent (water, ethanol, chloroform) during the extraction on the reducing power of the extracts was discussed. Authors reported the highest antioxidant activity for aqueous extract.

The antiradical activity of samples prepared by MAE was higher compared to samples prepared by UAE. Using this method, the highest antioxidant capacity was recorded for the white garlic husk MAE extract (43.38%), while the black garlic husk UAE extract had the lowest activity (29.75%). In contrast to the results presented in our research, where none of the tested samples had 50% of inhibition, Jang et al. (2018) reported slightly higher values, 51.16% of inhibition for the ethanolic extract of black garlic by-products. In the research conducted by dos Santos et al. (2022) for different extracts of garlic by-products (water, 50% and 70% ethanol) recorded a high antioxidant potential with $IC_{50}$ values of 2.11 to 3.57 g of extract/L, with the aqueous extract having the best result. The results cannot be compared to our results due to the difference in measurement unit.

For the ORAC results, extracts of white garlic husk had the highest antioxidant activity, of which the extract prepared by MAE had a higher value (39.02 mM TE/L). Extracts of black garlic husk prepared using UAE and MAE showed lower antioxidant activity, 26.82 and 32.02 µM TE/L, respectively. Considering that numerous methods are used to determine the antioxidant activity, the results are presented in different ways, and different solvents and extraction parameters are used when preparing the extracts, the comparison of the results of this research with the research of other authors is very difficult. A small number of studies related to garlic by-products, especially the black garlic by-product was found. The studies were mostly conducted on the garlic cloves. Research that has so far analysed the effects of garlic by-products shows that it has positive effects on human health and an antimicrobial effect that opens up the possibility of its use as a food preservative. Applications of garlic by-products can still be found in the literature, such as in the development of edible films in combination with chitosan (Chaudhary et al., 2021). The garlic by-product is also used as an environmentally friendly adsorbent for the removal of phenolic compounds from water systems (Muthamilselvi et al., 2018). Garlic bark has also been shown to be acceptable for removing antibiotics from aqueous media (Shaikhiev et al., 2022). All mentioned studies show the possibility of utilization and future application of garlic by-products in food industry, due to the rich chemical composition and antioxidant activity.

In contrast to DPPH and ORAC, the FRAP method is based on the mechanism of electron transfer, whereas DPPH and ORAC assess the ability to neutralise free radicals primarily through the release of hydrogen atoms. As different antioxidants act via different mechanisms, it is not unusual for the FRAP method to show different activity values compared to DPPH and ORAC. As different antioxidants act via different mechanisms, it is not unusual for FRAP to show different activity levels compared to DPPH and ORAC. Some phenolic compounds in the extracts may have strong reducing power but limited free radical scavenging activity, especially when steric or solubility factors limit their interaction with the radicals. These methodological differences likely explain why DPPH and ORAC did not show the same trends or correlation with total phenolic content (TPC) as FRAP. Similar discrepancies between antioxidant methods have also been reported in previous studies (Shahidi and Zhong, 2015; Jang et al.

2018). The results of the antimicrobial activity of the extracts determined by MIC and MBC are shown in Table 3. White garlic husk extract produced by using UAE showed no significant inhibition against *E. faecalis*, while extract produced by using MAE exhibited inhibitory activity in the concentration of 10 mg/mL. No bactericidal effect was recorded (MBC >10 mg/mL). Black garlic husk extracts produced by both UAE and MAE methods exhibited antimicrobial activity. It can be concluded that the black garlic husk extracts were more effective against *E. faecalis* than white husk extracts, regardless of the extraction method. White and black garlic husk UAE extracts had low inhibition against *B. cereus*. In contrast, MAE extracts for both husks showed higher activity, with MIC values of 5 mg/mL and MBC values of 10 mg/mL. These results show that MAE is better choice over UAE in extracting antimicrobial compounds that were efficient against *B. cereus*.

The results for L. monocytogenes exhibited the greatest variation among all tested organisms. White garlic husk UAE extract showed moderate activity, whereas black garlic husk UAE extract was more effective. However, MAE extracts for both white and black garlic husks showed the strongest activity, with MIC and MBC values of 2.5 mg/mL, indicating that MAE significantly enhanced the antimicrobial potential of both husks.

Regardless of husk type or extraction method, no inhibitory or bactericidal activity was recorded against *E. coli*. Similarly, white garlic husk UAE extract demonstrated no inhibition against *P. aeruginosa*, while black garlic husk UAE extract had both inhibition and bactericidal activity, but in rather high concentration. White and black garlic husk MAE extracts exhibited some inhibition, but no bactericidal effect was recorded. Overall, MAE produced extracts with higher antimicrobial activity compared to UAE, particularly against *B. cereus* and *L. monocytogenes*. Black garlic husk extracts showed higher antimicrobial activity than white husk extracts. These results make MAE a better extraction method for isolating antimicrobial compounds from white garlic husks. Research shows higher antimicrobial activity of garlic extracts against Gram-positive bacteria (Jing, 2020), which was confirmed in this study.

**Table 3** Results of the antimicrobial activity of garlic husks extracts measured using MIC (Minimum inhibitory concentration) and MBC (Minimum bactericidal concentration) method, expressed in mg/mL. (MAE – microwave-assisted extraction; UAE – ultrasound-assisted extraction)

| | ***Enterococcus faecalis*** | | ***Bacillus cereus*** | | ***Listeria monocytogenes*** | | ***Escherichia coli*** | | ***Pseudomonas aeruginosa*** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** |
| **White garlic husk UAE** | >10 | / | 10 | >10 | 10 | 10 | >10 | / | >10 | / |
| **Black garlic husk UAE** | 10 | 10 | 10 | >10 | 5 | 5 | >10 | / | 10 | 10 |
| **White garlic husk MAE** | 10 | >10 | 5 | 10 | 2,5 | 2,5 | >10 | / | 10 | >10 |
| **Black garlic husk MAE** | 10 | 10 | 5 | 10 | 2,5 | 2,5 | >10 | / | 10 | >10 |

## Conclusion

The results obtained show that the extracts of black and white garlic husk had good phenolic content and biological potential. The highest TPC of 60.10 mg GAE/g of extract was recorded for the white garlic husk extract obtained by microwave-assisted extraction. The most abundant compound determined by the HPLC method was the flavonoid rutin, and it was the most present in samples of white garlic husks prepared by both UAE and MAE. The highest antioxidant activity tested by FRAP was recorded for the white garlic husk sample obtained by UAE, while the highest ORAC and DPPH results were recorded for the white garlic husk sample obtained by MAE. Garlic by-products show the potential for the use in food industry as natural antioxidants and antimicrobials, but additional research is needed to confirm these effects in food matrices and challenge tests against pathogenic bacteria.

***Author Contributions:*** A.T.: Formal analysis, Data curation, Writing - original draft preparation, D.S.: Conceptualization, Formal analysis, Investigation, Methodology, Data curation, Writing - original draft preparation, M.Č.: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft preparation, R.F.M.: Formal analysis, Investigation, Data curation, Writing - original draft preparation, M.V.B.: Formal analysis, Investigation, Data curation, Writing - original draft preparation, V.Š.: Conceptualization, Resources, Data curation, Writing - review and editing, Supervision, Project administration, Funding acquisition.
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