ORIGINAL PAPER

UDK: 612.392.7
641.1

DOI: 10.5937/hraIsh2301020V

# Clove and nutmeg spices as sources of antioxidants

Sandra Vukovic, Sofija Kilibarda, Aleksandar Z. Kostic

Faculty of Agriculture, University of Belgrade, Belgrade, Serbia

Corresponding author:
Aleksandar Z. Kostic
Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia

E-mail address: akostic@agrif.bg.ac.rs

Received 03.04.2024.
Accepted 15.05.2024.

**Abstract**

*Spices are products intended for flavoring, seasoning, or imparting flavor, smell, and color to food. They also find application in the perfumery industry, aromatherapy, and the production of alcoholic and non-alcoholic beverages, with recognized health effects. The objects of this study were two spices, clove (Syzygium aromaticum L) and nutmeg (Myristica fragrans Houtt). The edible and commercial parts, i.e. flower buds and seeds, were obtained from the local market. The aim of the study was to investigate the effect of different solvents (warm water – 50°C and 80% acetone) and two extraction techniques (CSE – classical solvent and UAE – ultrasound-assisted extraction) on the content of bioactive compounds (total carotenoid content – TCC, total phenolic content – TPC, total flavonoid content – TFC, and total dihydroxycinnamic acid derivatives content – HCAs) and antioxidant activities (cupric reducing antioxidant capacity assay – CUPRAC, ferric reducing power assay – FRP, in vitro phospho-molybdenum total antioxidant assay – TAC and 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay – DPPH•) using spectrophotometric methods. This was done to evaluate the actual and maximum health potential of the selected spices. In clove, the content of bioactive compounds (TCC, TPC, TFC and HCAs) ranged from 155.18-193.64 μg $g^{-1}$, 17.48-29.95 mg $g^{-1}$ GAE, 0.64-1.61 mg $g^{-1}$ CE and 2.52-12.54 mg $g^{-1}$ CGAE, respectively, while for nutmeg, the ranges were as follows: 49.94-53.01 μg $g^{-1}$, 2.97-28.93 mg $g^{-1}$ GAE, 0.53-1.59 mg $g^{-1}$ CE and 1.32-6.13 mg $g^{-1}$ CGAE, respectively. The results obtained in antioxidant assays indicated the highest values for the DPPH•, followed by the TAC assay, for both spices. In general, the acetone extracts of both spices, regardless of the extraction technique used, had higher values for the examined parameters compared to the aqueous extracts. By comparing the spices, a higher content of bioactive compounds and antioxidant activity was found in clove than in nutmeg, in all tests except for CUPRAC. These findings suggest that both tested spices, especially clove, can be considered rich sources of antioxidants.*

**Key words***: antioxidant activity; bioactive compounds; clove; nutmeg.*

## INTRODUCTION

Since ancient times, spices have been the subject of trade and a valuable commodity in many parts of the world. Generally, spices are defined as products intended to flavor, season, or enhance the taste, smell, and color of food [1]. Additionally, spices have found applications in the perfume industry, aromatherapy, and both alcoholic and non-alcoholic beverage industries, with recognized health benefits. Although spices are now widely used worldwide, their usage depends on the culture, habits of the local population, and availability in the market.

*Syzygium aromaticum* L. – an evergreen tree from the Myrtaceae family, native to Indonesia, is a source of clove spices. The flower buds that are the commercial products, are harvested before the flowering stage of the *S. aromaticum* tree, dried, and sold as a whole or ground spice. In addition to Indonesia, India, Malaysia, Sri Lanka, Madagascar, and Tanzania are among the largest producers of clove today, while Brazil in South America has also been recognized as a major producer of this spice [1,2].

*Myristica fragrans* Houtt. belongs to the Myristicaceae family, and also originates from Indonesia. It is widespread in the warm and humid tropical climates of Malaysia, India, and Southeast Asia. The fruits of *M. fragrans* are the source of two popular spices: the seeds, known as nutmeg, and the seed coat, known as *mace* [1]. Nutmeg and mace have similar sensory characteristics, but nutmeg is often used in the food processing industry either whole, or more commonly, as a ground spice [3,4].

Clove and nutmeg are commonly used in cooking for the preparation of various dishes or desserts, often blended with other spices [3]. Moreover, these spices have garnered attention due to their numerous pharmacological properties.

Clove exhibits anti-fungal, anti-carcinogenic, anti-allergic, anti-viral, anti-inflammatory, and, notably, antioxidant activities, extensively documented in previously published studies [5,6]. Clove buds are a rich source of phenolic compounds such as flavonoids (kaempferol, quercetin, and its derivatives), phenolic acids (caffeic, ferulic, elagic, salicylic acids, and particularly gallic acid), and tannins [2]. Characteristic of this spice is its high essential oil content (14-20%) which consists of 36 chemical constituents, with eugenol (88.58%) and eugenol acetate (5.62%), phenol derivatives, being the most abundant [5]

The ancient use of nutmeg in treating rheumatism, pain, nausea, stomach cramps, and other illnesses attests to the medicinal effects of this spice [7]. Various biological activities such as antioxidant, antimicrobial, anti-carcinogenic, hepatoprotective, and anti-inflammatory have also been confirmed [3,8]. The chemical composition of nutmeg consists of lipids (30-50%), proteins (7.5%), sugars (28.5%), fiber (11.6%), and minerals (1.7%) [4]. The aroma and pharmacological effects stem from the essential oil, which comprises about 5-15% of nutmeg. Some authors have identified a large number of components in the essential oil of the seeds (ranging from 40-70) [9], while Naeem *et al.* [3] suggest that the predominant components are terpenes (such as α-pinene, *p*-cymene, sabinene, camphene, myrcene, and γ-terpinene), terpene derivatives (terpinol, geraniol, and linalool), and phenylpropanes (myricticin, safrole, and elmicin).

The pharmacological properties of spices are estimated based on the content and nature of their chemical compounds, but the selection of an appropriate solvent, extraction, and identification techniques can significantly influence them [10]. Common extraction procedures such as infusion, decoction, maceration, and percolation have proven to be less effective [7]. Therefore, in modern research, newer extraction methods (such as supercritical fluid extraction, microwaves, ultrasound-assisted, solvent extraction, and aqueous infusion) based on ecological and economic principles, such as time and solvent saving, and minimal harmful effects on the environment, are preferred.

In this study, classical solvent extraction (CSE) and ultrasound-assisted extraction (UAE) techniques were employed using warm water as the edible solvent and acetone as the organic solvent. These methods were applied to evaluate the bioactive compound content and antioxidant activity of selected spices. The aim was to determine both the real (aqueous extract) and maximum (acetone extract) medicinal potential.

## MATERIALS AND METHODS

### Extracts preparation

In this study, ground clove and nutmeg spices, available at a local market, were used to prepare the extracts (**Figure 1**). For this purpose, 2 g of each spice was filled with 20 mL of distilled warm water (50°C) and 80% acetone, then subjected to classical solvent extraction (CSE) and ultrasound-assisted extraction (UAE) techniques for 90 minutes. For the aqueous extracts, CSE was performed in a water bath at 50°C, while UAE was conducted in an ultrasonic bath (VAB SB 3 LD, maximum power 440 W, operating frequency 40 Hz) at maximum frequency (40 Hz), also at 50°C. For the acetone extracts, both extraction techniques were carried out at room temperature. The obtained extracts were then filtered through suitable filter paper, and the separated supernatants were stored in the dark at 4°C until further analysis.

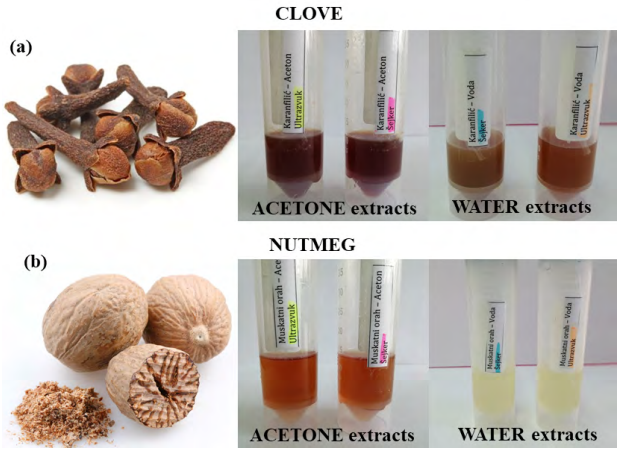


**Figure 1. (a)** Clove buds and extracts, **(b)** nutmeg seeds and extracts.

### Determination of bioactive compounds

The determination of total carotenoid content (TCC) was performed by directly measuring the absorbance of the acetone extracts at 450 nm using a spectrophotometer (model: UV-1800, Shimadzu USA Manufacturing Inc., UR, USA). The concentration of carotenoids expressed as μg per g (μg $g^{-1}$) of dry weight (DW) was calculated using the equation proposed by Fikselová *et al.* [11]:

$$TCC\ (\mu g\ g^{-1}) = A \times V \times 1000000/E \times 100 \times w,$$

A – absorbance of sample; V – volume (mL); E – extinction coefficient (2500 for acetone); w – weight of sample (g).

The total phenolic content (TPC) was determined using the Folin-Ciocalteu (FC) method [12], following the procedure: 1.25 mL of appropriately diluted extract was mixed with 0.625 mL of FC reagent (previously diluted ninefold with distilled water) and 0.625 mL of a

7.5% $Na_2CO_3$ solution. Simultaneously, a blank sample was prepared, containing distilled water instead of spice extract alongside the mentioned solutions. After incubating the reaction mixtures in the dark at room temperature for 90 minutes, the absorbance of the samples was measured at a wavelength of 765 nm. Gallic acid (GA) served as a standard to generate the calibration curve. The results are expressed as mg of gallic acid equivalents (GAE) per g of DW (mg $g^{-1}$ GAE).

The determination of total flavonoid content (TFC) in the spice samples was carried out following the method described by Kim *et al.* [13]. The reaction mixture consisted of 125 µL of the spice extract (acetone extracts were diluted in a 1:1 volume ratio, while aqueous extracts were undiluted) 625 µL of distilled water and 37.5 µL of 5% $NaNO_2$. After allowing the mixture to react at room temperature for 5 minutes, 75µl 10% $AlCl_3$ was added, and the mixture was left to react for an additional 6 minutes at room temperature. Subsequently, 250 µL 1M NaOH and 138 µL of distilled water were added to adjust the total volume to 1.25 mL. The absorbance of the samples was measured at a wavelength of 510 nm. TFC was determined using a calibration curve constructed with catechin as the standard, and the results were expressed as mg of catechin equivalents (CE) per g of DW (mg $g^{-1}$ CE).

The content of total dihydroxycinnamic acid derivatives (HCAs) was determined using the method proposed by Fraisse *et al.* [14], which was slightly modified. The procedure for preparing the reaction mixtures included the following steps: 0.2 mL of the diluted extract was mixed with 0.4 mL of 0.5 M HCl, 0.4 mL of Arnow's reagent, 0.4 mL of 2.125 M NaOH solution, and 0.6 mL of distilled water. After 20-minute incubation at room temperature, the absorbance of the samples was measured at a wavelength of 525 nm. The results were expressed as mg of chlorogenic acid equivalents (CGAE) per g of DW (mg $g^{-1}$ CGAE).

**Determination of antioxidant activity**

For the *in vitro* phospho-molybdenum total antioxidant capacity (TAC) assay [15] 0.3 mL of the appropriate dilution extract and 3 mL of intensely yellow phospho-molybdenum reagent were mixed in a glass test tube sealed with aluminum foil. The incubation of the reaction mixtures was carried out in a water bath at a temperature of 95°C for 90 minutes. After cooling, the absorbance of the samples was measured at 695 nm. Total antioxidant activity in spice extracts was determined using a calibration curve with ascorbic acid (AA) as a standard. The results are expressed as mg of ascorbic acid equivalents (AAE) per g of DW (mg $g^{-1}$ AAE).

The ferric reducing power (FRP) assay was determined spectrophotometrically following the method proposed by Nibir *et al.* [16]. In a glass test tube, 0.5 mL of the diluted spice extract was mixed with 0.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 0.5 mL of 1% potassium ferricyanide. The reaction mixture was then heated at 50°C for 20 minutes. Following incubation, 0.5 mL of 10% trichloroacetic acid was added, and the total mixture was centrifuged (4000×*g*) for 5 minutes. Subsequently, clear supernatants were mixed with 2 mL of distilled water and 0.4 mL of 0.1% $FeCl_3$. The addition of $FeCl_3$ resulted in the appearance of an emerald-green color in the reaction mixture, the intensity of which was measured at a wavelength of 700 nm. A solution of ascorbic acid (AA) with a starting concentration of 1 mg $mL^{-1}$ was used to construct a calibration curve. The results are expressed as mg of ascorbic acid equivalents (AAE) per g of DW (mg $g^{-1}$ AAE).

The Cupric Reducing Antioxidant Capacity (CUPRAC) assay [17] was conducted in the following manner: in a plastic test tube, 0.350 mL of the diluted extract was mixed with 0.350 mL of a 0.01 M $CuCl_2$, 0.350 mL of a 0.0075 M neocuproine solution (prepared by dissolving neocuproine in concentrated absolute ethanol), and 0.350 mL of 1 M ammonium acetate buffer (pH 7.0). The reaction mixtures were incubated in the dark for 30 minutes, after which the absorbance of the samples was measured at a wavelength of 450 nm. The calibration curve was prepared with ascorbic acid as the standard. The results are expressed as mg of ascorbic acid equivalents (AAE) per g of DW (mg $g^{-1}$ AAE).

The DPPH· (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed according to the method previously described by Gawron-Gzella *et al.* [18]. Namely, 105 µL of the extracts was mixed with 840 µL of 150 µM DPPH· solution. After 30 minutes of incubation at room temperature and in the dark, the absorbance of the reaction mixtures was read at 515 nm. A decrease in the purple color of DPPH· radicals compared to the blank (containing DPPH· reagent solution and solvent instead of the spice extract) indicates the inhibition of DPPH· radicals caused by the action of spice phytochemicals. The results are expressed as percentage inhibition of the DPPH· radicals, which was calculated using the following equation:

$$\% \text{ inhibition} = (Ab - As)/Ab \times 100$$

Ab – the absorbance of blank; As – the absorbance of the sample extract.

**Statistical analysis**

The statistical analysis was performed using the two-way analysis of variance (ANOVA) followed by Tukey's HSD test using R software, version 3.6.0 [19]. Statistical significance was considered at $p < 0.05$. All tests were performed in triplicate, and the results are expressed as mean ± standard deviation (SD), and the data are presented as graphs and tables.

## RESULTS AND DISCUSSION

### Bioactive compounds content

The results of TCC for clove and nutmeg measured in acetone extracts using CSE and UAE are shown in **Figure 2**.

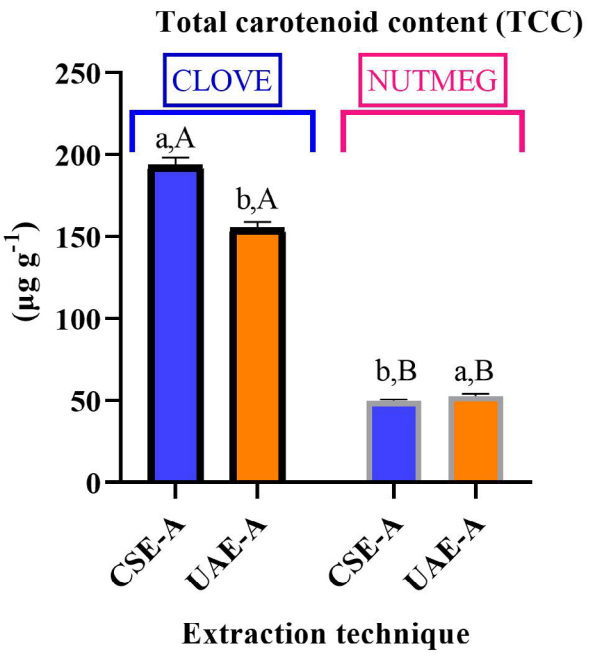


**Figure 2.** Total carotenoid content (TCC) for clove and nutmeg. CSE-Classical solvent extraction; UAE-Ultrasound assisted extraction; A-Acetone. Lowercase letters indicate the comparisons between extraction techniques; uppercase letters indicate the comparisons between the spices, achieved with the same extraction technique; different letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$).

The obtained results indicated that the extraction technique statistically significantly affects the TCC in the case of both tested spices. Namely, in the case of clove, the TCC achieved with CSE was 193.64 µg $g^{-1}$ which was statistically significantly higher than the content achieved using UAE (155.18 µg $g^{-1}$). Conversely, for nutmeg, the content obtained with UAE (53.01 µg $g^{-1}$) was statistically significantly higher than the content obtained with CSE (49.94 µg $g^{-1}$). In addition, comparing the results for both spices revealed that clove had a statistically significantly higher content of total carotenoids than nutmeg, regardless of the extraction method used (**Figure 2**).

**Figure 3** illustrates the bioactive compound content found in the aqueous and acetone extracts of clove and nutmeg, obtained through two different extraction techniques: CSE and UAE. Overall, the results indicated a statistically significant increase in the content of bioactive compounds (TPC, TFC, and HCAs) in the acetone extracts compared to the aqueous extracts for both tested spices. On the other hand, the choice of extraction technique exhibited a lesser influence on the bioactive compound content.

More precisely, in the case of clove, the highest TPC content was attained in the acetone extracts using UAE (29.95 mg $g^{-1}$ GAE), however, it did not exhibit a statistically significant difference from the content achieved in the acetone extracts using CSE (28.91 mg $g^{-1}$ GAE). The TPC content observed in the aqueous extracts obtained using CSE and UAE was 17.63 and 17.48 mg $g^{-1}$ GAE, respectively. These contents did not significantly differ from each other but were statistically significantly lower than the content obtained in the acetone extracts (**Figure 3a**).

Similar results were obtained for nutmeg, i.e. the TPC obtained in the acetone extracts with CSE and UAE (28.93 and 28.78 mg $g^{-1}$ GAE, respectively) did not exhibit a significant difference from each other. However, they were statistically significantly higher compared to the TPC obtained in the aqueous extracts (2.97 mg $g^{-1}$ GAE for CSE and 3.00 mg $g^{-1}$ GAE for UAE), which also did not differ from each other (**Figure 3a**).

In clove, a statistically significantly higher total flavonoid content (TFC) was observed with CSE compared to UAE, for both solvents (**Figure 3b**). Specifically, the TFC content in the acetone extract prepared with CSE (1.61 mg $g^{-1}$ CE) was the highest among all tested clove extracts, yet it did not show a statistically significant difference from the content obtained in the same solvent using UAE (1.49 mg $g^{-1}$ CE). Similarly, the aqueous extracts prepared with CSE (1.00 mg $g^{-1}$ CE) exhibited a higher TFC than the aqueous extract obtained with UAE (0.64 mg $g^{-1}$ CE), which was the lowest among all tested clove extracts.

In the case of nutmeg, TFC exhibited a similar trend to TPC, with no statistically significant difference observed between the same solvents, regardless of the extraction technique used (**Figure 3b**). Specifically, nutmeg contained 1.50 mg $g^{-1}$ CE TFC for CSE and 1.50 mg $g^{-1}$ CE for UAE acetone extracts, and 0.54 and 0.53 mg $g^{-1}$ CE, respectively for aqueous extracts.

The total content of dihydroxycinnamic acid derivatives (HCAs) exhibited statistically significant differences among all tested extracts of clove (**Figure 3c**). The highest content was observed in the acetone extract obtained via CSE (12.54 mg $g^{-1}$ CGAE), while the lowest content was detected in the aqueous extract (2.52 mg $g^{-1}$ CGAE) prepared using the same extraction technique (CSE). In nutmeg, the HCAs measured in the acetone extracts obtained with CSE (6.13 mg $g^{-1}$ CGAE) and UAE (5.96 mg $g^{-1}$ CGAE) did not show a statistically significant difference from each other, but were significantly higher than the content measured in the aqueous extracts, between which no significant difference was found (**Figure 3c**).

A general comparison of the content of bioactive compounds in both tested spices revealed that the aqueous extracts of clove, obtained with both CSE and

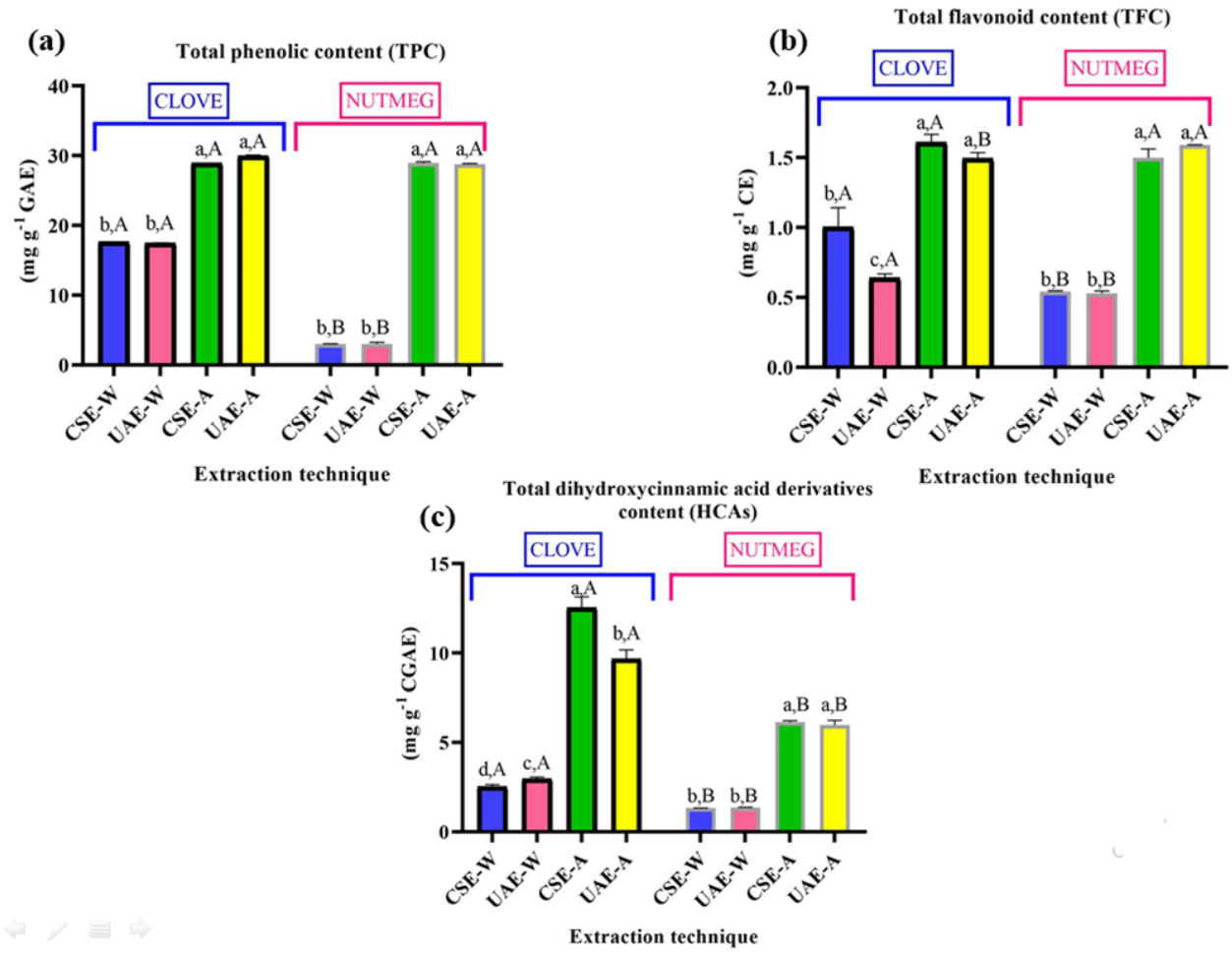


**Figure 3.** Effect of various solvents (W-water and A-acetone) and two distinct extraction techniques (CSE-classical solvent extraction and UAE-ultrasound assisted extraction) on the (a) total phenolic content, (b) total flavonoid content and (c) total content of dihydroxycinnamic acid derivatives in clove and nutmeg spices. Lowercase letters indicate comparisons between solvents and extraction techniques within each spice; uppercase letters indicate comparisons between the spices obtained with the same solvent and extraction technique; different letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$). GAE-gallic acid equivalents; CE-catechin equivalents; CGAE-chlorogenic acid equivalents.

UAE, contained statistically significantly higher levels of TPC, TFC, and HCAs compared to nutmeg. However, there was no statistically significant difference between the TPC measured in the acetone extracts of clove and nutmeg prepared with CSE and UAE, nor between the content of TFC measured in the acetone extracts prepared with CSE. The acetone extracts (both CSE and UAE) of clove contained statistically significantly higher levels of HCAs compared to the nutmeg acetone extracts. Interestingly, only in the case of the acetone extract prepared with UEA, the TFC of nutmeg was statistically significantly higher than that of clove.

The high content of bioactive compounds (TPC and TFC) in the tested spices has also been confirmed in other studies [20,21,22]. Specifically, Słowianek and Leszczyńska [23] corroborated the high TPC content measured in the clove and nutmeg extracts (154.1 mg $g^{-1}$ GAE and 6.3 mg $g^{-1}$ GAE, respectively), indicating a notable antioxidant potential, particularly in clove. The same trend of higher TPC was also identified in the study by Ali *et al.* [24], who reported 215.14 mg $g^{-1}$ GAE in the clove extracts, while nutmeg contained 14.85 mg $g^{-1}$ GAE. In the same study, a high TFC yield was also reported (5.59 mg $g^{-1}$ CE in clove and 2.22 mg $g^{-1}$ CE in nutmeg). On the other hand, results obtained by Kim *et al.* [25] indicated a significantly lower content of TPC and TFC in the aqueous clove extracts (108 μg $g^{-1}$ GAE and 75 μg $g^{-1}$ QE). These variations in results across studies are attributed to the use of different types of solvents, extraction conditions, and the geographical location of the spices' cultivation.

## Antioxidant activity

The results depicting the impact of various solvents (water and acetone) and two distinct extraction techniques (classical solvent extraction and ultrasound-assisted extraction) on the antioxidant activity of clove and nutmeg spices, measured by CUPRAC, FRP, TAC, and DPPH· assays, are presented in **Table 1**.

The acetone extracts of clove exhibited greater antioxidant activity compared to the aqueous extracts

**Table 1.** Antioxidant activity of clove and nutmeg spices.

| Extraction technique | Solvent | CUPRAC (mg g$^{-1}$ AAE) mean ± SD | | FRP ( mg g$^{-1}$ AAE) mean ± SD | | TAC (mg g$^{-1}$ AAE) mean ± SD | | DPPH· (% Inh) mean ± SD | |
|---|---|---|---|---|---|---|---|---|---|
| | | Clove | Nutmeg | Clove | Nutmeg | Clove | Nutmeg | Clove | Nutmeg |
| CSE | 80% Acetone | 1.95 ± 0.17 **b,B**** | 3.55 ± 0.05 **b,A** | 3.86 ± 0.15 **a,A** | 2.51 ± 0.01 **b,B** | 25.66 ± 1.21 **a,A** | 15.00 ± 0.32 **b,B** | 86.31 ± 1.19 **a,A** | 36.40 ± 3.29 **c,B** |
| UAE | | 2.34 ± 0.05 **a,B** | 4.25 ± 0.31 **a,A** | 3.83 ± 0.15 **a,A** | 3.46 ± 0.06 **a,B** | 24.45 ± 0.59 **a,A** | 15.84 ± 0.30 **a,B** | 85.30 ± 0.10 **a,A** | 57.49 ± 5.01 **a,B** |
| CSE | Water (50°C) | 1.72 ± 0.09 **c,B** | 2.52 ± 0.04 **c,A** | 3.30 ± 0.00 **b,A** | 0.20 ± 0.01 **c,B** | 9.75 ± 0.08 **b,A** | 2.55 ± 0.11 **c,B** | 85.26 ± 0.05 **a,A** | 14.79 ± 0.95 **d,B** |
| UAE | | 1.74 ± 0.09 **c,A** | 1.94 ± 0.16 **d,A** | 2.80 ± 0.23 **c,A** | 0.20 ± 0.00 **c,B** | 9.77 ± 0.20 **b,A** | 2.51 ± 0.06 **c,B** | 10.11 ± 0.86 **c,B** | 48.00 ± 1.72 **b,A** |

Lowercase letters indicate the comparisons between solvents and extraction techniques within the spice; uppercase letters indicate the comparisons between the spices, obtained with the same solvent and extraction technique; different letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$). CSE – classical solvent extraction; UAE – ultrasound-assisted extraction; AAE – ascorbic acid equivalents; % Inh – percentage of inhibition.

across all assays. Furthermore, statistical analysis revealed no significant difference between the antioxidant activity of the acetone extracts prepared using either CSE or UAE, except in the CUPRAC assay, where statistically significantly higher values were observed in the extracts prepared with UAE compared to CSE. The extraction technique did not have a statistically significant impact on antioxidant activity, even in the case of the aqueous clove extracts tested in the CUPRAC and TAC assays. However, in the FRP and DPPH· assays, the aqueous extracts prepared with UAE showed statistically significantly lower antioxidant activity compared to those prepared with CSE (**Table 1**).

For the acetone extracts of nutmeg, the extraction technique exhibited statistical significance across all assays with results from UAE significantly surpassing those from CSE. However, for the aqueous extracts, the extraction technique did not exert a statistically significant influence in the FRP and TAC assays. Nevertheless, in the CUPRAC assay, there was notably higher antioxidant activity in the extract prepared with CSE, whereas in the DPPH· assay, the extract prepared with UAE displayed higher activity (**Table 1**).

A general comparison of the antioxidant activity of the tested spices showed that clove extracts had a higher antioxidant activity than nutmeg extracts across all assays, except for CUPRAC.

Current findings align with those of Adaramola and Onigbinde [20], who observed the highest antioxidant activity (evaluated via the DPPH· assay) in clove buds extracted using 80% acetone, followed by 80% methanol, with the lowest activity found in the aqueous extracts. Similar trends in solvent efficiency were also noted for nutmeg [8]. The considerable health benefits are further supported by Shan *et al.* [21] and Gupta [22], who reported significantly higher antioxidant activity (DPPH· and FRP) in clove compared to other spices, including nutmeg. In many studies, UAE has been recognized as a green extraction technique, favored for its superior extraction of essential compounds [26,27]. However, in the current study, UAE did not demonstrate a significant increase in the yield of bioactive components or antioxidant activity across most assays, consistent with the findings of Alcántara *et al.* [28].

## CONCLUSION

This study aimed to investigate the impact of different solvents (warm water at 50°C and 80% acetone) and two extraction techniques (classical solvent and ultrasound-assisted extraction) on the content of bioactive compounds and antioxidant activity of clove and nutmeg. For both spices, results indicated that the choice of solvent had a significantly greater influence on the analyzed parameters than the extraction technique employed. Specifically, the acetone extracts of both spices exhibited higher TCC, TPC, TFC, and HCAs compared to their aqueous counterparts. Comparison between the two spices revealed that the aqueous extracts of clove had higher values of TCC, TPC, TFC, and HCAs compared to nutmeg, while the difference

between clove and nutmeg was less pronounced in the acetone extracts. In terms of antioxidant activity, the acetone extracts also showed better antioxidant activity for both spices compared to the aqueous extracts. In the FRP, TAC, and DPPH· assays, all tested clove extracts exhibited significantly higher antioxidant activity than those of nutmeg, whereas the reverse was observed in the CUPRAC assay. Although the acetone extracts exhibited a high content of the tested phytochemicals, it is noteworthy that the aqueous extracts represent the real potential release of these compounds when utilizing spices in food. In this regard, the tested spices, especially clove, can be recommended as a rich source of antioxidants.

## ACKNOWLEDGMENTS

This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-65/2024-03/200116).

## REFERENCES

1. UNIDO and FAO. Herbs, spices and essential oils. Post-harvest operations in developing countries. 2005.
2. Cortés-Rojas DF, de Souza CR, Oliveira, WP. Clove (*Syzygium aromaticum*): a precious spice. Asian Pac J Trop Biomed 2014;4(2):90-96.
3. Naeem N, Rehman R, Mushtaq A, Ghania B. Nutmeg: A review on uses and biological properties. Int J Chem Biochem Sci 2016;9:107-110.
4. Malik T, Sharma R, Panesar PS, Gehlot R, Tokusoglu O, Dhull S B et al. Nutmeg nutraceutical constituents: In vitro and in vivo pharmacological potential. J Food Process Preserv 2021;00:e15848.
5. Pulikottil S, Nath, S. Potential of clove of *Syzygium aromaticum* in development of a therapeutic agent for periodontal disease. A review. S Afr Dent J 2015;70(3):108-115.
6. Banerjee K, Madhyastha H, Sandur VR, Manikandanath NT, Thiagarajan N, Thiagarajan P. Anti-inflammatory and wound healing potential of a clove oil emulsion. Colloids Surf B Biointerfaces 2020;193:111102.
7. Matulyte I, Marksa M, Ivanauskas L, Kalvėnienė Z, Lazauskas R, Bernatoniene J. GC-MS Analysis of the Composition of the Extracts and Essential Oil from *Myristica fragrans* Seeds Using Magnesium Aluminometasilicate as Excipient. Molecules 2019;24:1062.
8. Gupta A, Bansal V, Babu V, Maithil N. Chemistry, antioxidant and antimicrobial potential of nutmeg (*Myristica fragrans* Houtt). J Genet Eng & Biotechnol 2013;11:25-31.
9. Sohilait MR, Sohilait HJ, Kainama H. Comparison Of Chemical Compositions Of Seed And Mace Nutmeg (*Myristica fragrans*) Essential Oils From Amboina Island, Moluccas, Indonesia And Their Antioxidant Activities. Nat Vol Essent Oil 2021;8(6):5537-5545.
10. Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci 2020;12(1):1-10.
11. Fikselová M, Šilhár S, Mareček J, Frančáková H. Extraction of carrot (*Daucus carota* L.) carotenes under different conditions. Czech J Food Sci 2008;26(4):268-274.
12. Ng A, Parker ML, Parr AJ, Saunders PK, Smith AC, Waldron KW. Physicochemical characteristics of onion (*Allium cepa* L.) tissues. J Agric Food Chem 2000;48(11):5612-5617.
13. Kim D, Kim MJ, Shin Y, Jung S, Kim YJ. Green Pepper (*Piper nigrum* L.) Extract Suppresses Oxidative Stress and LPS-Induced Inflammation via Regulation of JNK Signaling Pathways. Appl Sci 2020;10(7):2519.
14. Fraisse D, Felgines C, Texier O, Lamaison JL. Caffeoyl Derivatives: Major Antioxidant Compounds of Some Wild Herbs of the Asteraceae Family. Food Sci Nutr 2011;2(3):181-192.
15. Prieto P, Pineda M, Aguilar M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Anal Biochem 1999;269:337-341.
16. Nibir YM, Sumit AF, Akhand AA, Ahsan N, Hossain MS. Comparative assessment of total polyphenols, antioxidant and antimicrobial activity of different tea varieties of Bangladesh. Asian Pac J Trop Biomed 2017;7(4):352-357.
17. Yilar M, Bayar Y, Bayar AA, Genç N. Chemical composition of the essential oil of *Salvia bracteata* Banks and the biological activity of its extracts: antioxidant, total phenolic, total flavonoid, antifungal and allelopathic effects. Bot Serbica 2020;44(1):71-79.
18. Gawron-Gzella A, Królikowska A, Pietrzak M. Antioxidant activity of teas obtained from leaves of *Camellia sinensis* (L.) Kuntze in course of various production processes available on Polish market. Herba Pol 2018;64(2):60-67.
19. RStudio Team. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. 2020. http://www.rstudio.com/.
20. Adarmola B, Onigbinde A. Effect of Extraction Solvent on the Phenolic Content, Flavonoid Content and Antioxidant Capacity of Clove Bud. Int J Pharma Bio Sci 2016;11(3):33-38.
21. Shan B, Cai ZY, Sun M, Corke H. Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. J Agric Food Chem 2005; 53:7749-7759.
22. Gupta D. Comparative analysis of spices for their phenolic content, flavonoid content and antioxidant capacity. American International Journal of Research in Formal, Applied & Natural Sciences (AIJRFANS) 2013;4(1):38-42.
23. Slowianek M, Leszczynska J. Antioxidant properties of selected culinary spices. Herba Polonica 2016;62(1):29-41.
24. Ali A, Wu H, Ponnampalam EN, Cottrell JJ, Dunshea FR, Suleria HAR. Comprehensive Profiling of Most Widely Used Spices for Their Phenolic Compounds through LC-ESI-QTOF-MS$^2$ and Their Antioxidant Potential. Antioxidants 2021;10(5):721.
25. Kim IS, Yang MR, Lee OH, Kang SN. Antioxidant activities of hot water extracts from various spices. Int. J. Mol. Sci. 2011;12:4120-4131.
26. Šic Žlabur J, Brajer M, Voća S, Galić A, Radman S, Rimac-Brnčić S et al. Ultrasound as a Promising Tool for the Green Extraction of Specialized Metabolites from Some Culinary Spices. Molecules 2021;26(7):1866.

27. Rao MV, Sengar AS, Sunil CK, Rawson, A. Ultrasonication – A green technology extraction technique for spices: A review. Trends Food Sci Technol 2021;116(11):975–991.
28. Alcántara C, Žugčić T, Abdelkebir R, García-Pérez JV, Jambrak AR, Lorenzo JM, et al. Effects of Ultrasound-Assisted Extraction and Solvent on the Phenolic Profile, Bacterial Growth, and Anti-Inflammatory/Antioxidant Activities of Mediterranean Olive and Fig Leaves Extracts. Molecules 2020;25(7):1718.

# Začini karanfilić i muskatni oraščić kao izvori antioksidanata

Sandra Vukovic, Sofija Kilibarda, Aleksandar Z. Kostic

Poljoprivredni fakultet, Univerzitet u Beogradu, Beograd, Srbija

**Kratak sadržaj**

*Začini su proizvodi namenjeni za aromatizovanje, začinjavanje ili poboljšanje ukusa, mirisa i boje hrane. Takođe, našli su primenu u parfimerijskoj industriji, aromaterapiji, industriji alkoholnih i bezalkoholnih pića, a takođe je prepoznat i njihov lekoviti efekat. Predmet ovog istraživanja su začini karanfilić (Sizigium aromaticum L) i muskatni oraščić (Miristica fragrans Houtt), čiji se jestivi i komercijalni delovi, odnosno cvetni pupoljci i seme, dobijaju od zimzelenog drveća poreklom iz Indonezije. Cilj rada bio je ispitivanje uticaja različitih rastvarača (topla voda – 50°C i 80% aceton) i dve tehnike ekstrakcije (CSE – ekstrakcija rastvaračem i UAE – ultrazvučna ekstrakcija) na sadržaj bioaktivnih jedinjenja (sadržaj ukupnih karotenoida – TCC, sadržaj ukupnih fenola – TPC, sadržaj ukupnih flavonoida – TFC i sadržaj ukupnih derivata dihidroksicimetne kiseline – HCAs) i antioksidativne aktivnosti (CUPRAC, FRP, TAC i DPPH testovima) korišćenjem spektrofotometrijskih metoda, u cilju procene stvarnog i maksimalnog zdravstvenog potencijala odabranih začina. U karanfiliću, sadržaj bioaktivnih jedinjenja (TCC, TPC, TFC i HCA) kretao se od 155,18-193,64 μg $g^{-1}$, 17,48-29,95 mg $g^{-1}$ GAE, 0,64-1,61 mg $g^{-1}$ CE i 2,52-12,54 mg $g^{-1}$ CGAE, respektivno, dok su za muskatni oraščić rasponi bili sledeći: 49,94-53,01 μg $g^{-1}$, 2,97-28,93 mg $g^{-1}$ GAE, 0,53-1,59 mg $g^{-1}$ CE i 1,32-6,13 mg $g^{-1}$ CGAE, respektivno. Rezultati dobijeni u testovima antioksidativne aktivnosti ukazuju da su za oba začina najveće vrednosti zabeležene u $DPPH^{\bullet}$, a zatim u TAC testu. Generalno, acetonski ekstrakti oba začina su, bez obzira na korišćenu tehniku ekstrakcije, imali veće vrednosti ispitivanih parametara u odnosu na vodene ekstrakte. Upoređivanjem začina u svim testovima osim u CUPRAC testu utvrđen je veći sadržaj bioaktivnih jedinjenja i antioksidativne aktivnosti u karanfiliću nego u muskatnom oraščiću. Uzimajući u obzir navedene činjenice, oba začina, a posebno karanfilić, mogu se smatrati bogatim izvorom antioksidanata.*

**Ključne reči:** *antioksidativna aktivnost; bioaktivna jedinjenja; karanfilić; muskatni oraščić.*