Evaluation of Antioxidant Extraction by Microwave Treatment from Japanese Indigo Plant

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Research

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Abstract

Tadeai (Awa-ai) is an indigo plant that is produced in large quantities in Tokushima prefecture, Japan. The leaves of Tadeai are used for dyeing (Ai-zome), but there is no use for the other parts, i.e. flowers, stems, leaves, and roots, most of which are discarded. In this study, we search for new ways to use Tadeai including parts other than the leaves and examined the antioxidant activity of water soluble extract from Tadeai. To extract antioxidants efficiently, processing was undertaken using a microwave irradiation device. The evaluation of Tadeai extract and its antioxidant activity was performed by measuring the amount of phenolic compounds by the Folin–Ciocalteu method and the DPPH radical scavenging activity test. Compared to the sample that was not subjected to microwave treatment, the amount of phenolic compounds was observed to be double, and the antioxidant activity eight times higher. The microwave treatment conditions that showed the best results are 200 °C and 1 min, and the evaluation indicated that the part that exhibits the best results is the root. Furthermore, the differences among Tadeai varieties were also examined. The best result was for the “Kojoko Akahana;” the root of the “Kojoko Akahana” exhibited the highest antioxidant effect. Its amount of phenolic compounds and EC₅₀ value were 44.1 mg-catechin equiv./g-dry sample and 0.018 g/L, respectively. In this study, it was shown that the antioxidant activity of the parts other than the leaves is high, so it is expected that Tadeai waste will be effectively utilized as a raw material for producing antioxidants in the future.

Introduction

In recent years, foods with antioxidant properties have attracted wide attention. A person consumes oxygen when performing life activities, when reactive oxygen (ROS) is generated (Simon et al. 2000). In the process of energy production, the number of electrons in the oxygen atom is reduced to four, to form water. However, not all atoms undergo electron reduction, and partially reduced oxygen is generated, which becomes ROS. ROS includes hydroxy radicals and superoxide, and free radicals having unpaired electrons are highly unstable. When ROS species are generated in vivo, they attack biomolecules such as lipids, proteins, and nucleic acids. The hydroxy radicals are the most reactive among ROS species (Seifried et al. 2007); they are the most harmful to the living body. Lipid peroxide causes diseases such as arteriosclerosis and further causes myocardial infarction. In addition, it has been reported that the reaction with proteins affects enzymes and the like, and the reaction with nucleic acids increases the risk of developing cancer (Jang et al. 2012; Heo et al. 2014). ROS is considered to be involved in aging and many lifestyle-related diseases because of its high reactivity with biological components. Furthermore, since active oxygen is also produced by external factors and environmental factors, it has a high correlation with lifestyle-related diseases. Environmental factors that generate free radicals are air pollutants, radiation, certain drugs, ultraviolet rays, tobacco, etc. Contact with these environmental factors results in the free radicals being introduced into the living body (Simon et al. 2000). Antioxidants are effective in suppressing or eliminating ROS formation. Therefore, they are expected to prevent aging and lifestyle-related diseases. There are three types of antioxidant reactions and types. The first is a "prophylactic antioxidant" that works in generating ROS under stress from external factor. The second is

"radical scavenging antioxidants" that generate ROS and prevent attack on target molecules (such as lipids and proteins) or damage to tissues. Finally, there are three types of "repair, regenerative antioxidants" that repair damaged tissue etc. (Seifried et al. 2007). In this study, we focus on the second of these three, namely the "radical-capped antioxidants," many of which possess a phenolic hydroxyl group that functions as the free radical supplemented antioxidant (Evans et al. 1997; Krishnaiah et al. 2011; Nonadis et al. 2011; Amić et al. 2014). Since polyphenols are abundantly available in plants (Hu et al. 2000; Yu et al. 2003; Srinivasan et al. 2007; Wannes et al. 2010), they have long been useful as natural antioxidants (Lee et al. 2002; Kubola et al. 2008; Öztürk et al. 2007). The catechins contained in green tea (Katalinić et al. 2004) and resveratrol contained in red wine (Gülçin 2010), etc., belong to this group (Leja et al. 2001; Barreira et al. 2008; Wannes et al. 2010; Elfalleh et al. 2012) and are well known as polyphenols. This study evaluates polyphenolic antioxidants in plants; we extract polyphenols from non-edible plants that are not currently used as food, and evaluate whether they can be used as antioxidants.

The term "microwave" is used to refer to a radio wave of extremely short wave length, with a wavelength range of 1 mm to 1 m. The wavelengths or frequencies that can be used have been established, to prevent phenomena like radio interference. Among them, 2.45 GHz (wavelength: 12.2 cm), which is the same as that used in household microwave ovens, is frequently used for laboratory microwaves. When a substance is irradiated with microwaves, reflection, transmission and absorption of the microwaves occur, depending on the properties of the substance. The most significant change due the interaction with substances is the heat generated by microwave absorption. 2.45 GHz means that the electric field changes 2450×10^6 times per second, and this causes molecules with a dipole in the electric field such as water molecules, to rotate and vibrate (Banik et al. 2003). However, the energy of the part which cannot cope with rapid rotation is converted to heat, and the temperature rises. Thus, unlike conventional external heating, heat conduction proceeds extremely smoothly. Along with that, various reactions are promoted. In addition, since microwaves break even hydrogen bonds and some covalent bonds, it is considered that not only heating but also decomposition of plants (biomass) is possible through the use of microwaves. Cellulose breaks down hydrogen bonds between molecules (Banik et al. 2003; Borges et al. 2014). Lignin and hemicellulose are assumed to promote microwave decomposition through high temperature water (Nassar et al. 1984; Jakab et al. 1997; Swqueiros et al. 2013). In a hemicellulose-containing plant (biomass), the acetyl group bound to hemicellulose is liberated to produce acetic acid as the temperature rises. The acidity of the acetic acid and the acidity of the organic acid generated from the sugar combine to lower the pH. The action of the proton not only hydrolyzes hemicellulose and lignin into low-molecular weight sugars and polyphenols, but also reduces the degree of polymerization of cellulose.

In Tokushima, Japan, textile printing has been popular for a long time, and a large amount of Tadeai, a raw material of textile printing, has been produced. Tadeai leaves are used for printing because they contain a large amount of indicant (Campo et al. 2001), which is a precursor of indigo, a pigment derived from leaves but not other parts of the plant. By drying the leaves of Tadeai in the sun, the indicant in the leaves is enzymatically degraded and oxidized to produce indigo (Kawahito et al. 2009). The amount of indicant in the leaves decreases as the plant blooms, so the post-flowering Tadeai is not used much while

the stems, flowers, roots, etc. are not used and are mostly discarded. Therefore, the study focuses on how to effectively use parts of Tadeai that are discarded. Tadeai contains various useful ingredients in addition to indigo such as polyphenols and other substances that possess antioxidant properties (Kawamura et al. 2010; Kim et al. 2012; Heo et al. 2013). In this work, we explored effective utilization methods for parts of Tadeai that are usually discarded, including effective antioxidant extraction by microwave treatment.

Materials And Methods

Sample

Tadeai (Awa indigo plants, Awa-ai, *Polygonum tinctorium* Lour., Syn: *Persicaria tinctorial* (Aiton) H. Gross) such as “Kojoko Shirohana”, “Kojoko Akahana”, “Senbon”, and “Akakuki Kosenbon” were used as samples in this work. “Kojoko Shirohana” is the most popular Tadeai as a raw material of textile printing in Tokushima prefecture, Japan. After separating four parts, i.e. flowers, stems, leaves, and roots, each part was ground using a crusher mill (Wonder Crush Mill D3V-10, Osaka Chemical Co. Ltd.) at 25,000 rpm for 10 s and then used for obtaining water soluble extract with antioxidant activity. All chemicals were purchased from Nacalai Tesque Co., Ltd. (Kyoto, Japan) and were of analytical grade.

Microwave Treatment (mw)

Microwave treatment (MW) was carried out using an initiator⁺ instrument (Biotage Co. Ltd.) equipped with a 20 mL reaction tube, at a frequency of 2.45 GHz (Noda et al. 2019). 0.5 g of milled Tadeai was suspended in 20 mL of distilled water and then treated by MW. The processing time and processing temperature were 0–10 min and 100–200 °C, respectively.

Extraction And Separation

MW-treated samples were extracted using a distilled water with 60-fold of the sample dry weight, at room temperature for 1 d and separated into water soluble material and a residue, using filter paper (No. 131, φ185 mm, Advantec Co., Ltd, Tokyo, Japan). Next, the extract was dried by freeze drying.

Determination of free radical scavenging activity and total phenolic content

Determination of free radical scavenging activity and total phenolic content of extract was carried out (Aksoy et al. 2013). Free radical scavenging activities of solutions prepared from the extracts obtained via MW of the Tadeai sample, prepared in distilled water at concentrations of 1.0, 0.4, 0.2, 0.1 and 0.05 g/L, were determined in accordance with Mishra et al. 2012, which is based on the principle of scavenging the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. DPPH was added to the solutions prepared with the extract and stirred. Each mixture was kept in the dark for 30 min and the absorbance was

measured at 517 nm against a blank. In this work, EC_{50} , a concentration at a radical scavenging activity of 50%, of each sample was evaluated. Since the EC_{50} value is a widely used parameter to measure the free radical scavenging activity and a lower value indicates a higher antioxidant activity (Maisuthisakul et al. 2007).

The amount of phenolic compounds of extract was determined according to the Folin–Ciocalteu method (Kim et al. 2012). The Folin–Ciocalteu reagent (1 mL) was added to the extract (200 μ L) with 4 mL of distilled water; after 5 min, 10%(w/v) Na_2CO_3 was added and the mixture was stored at room temperature for 2 h. The absorbance of the mixture was measured at 760 nm against water on a UV spectrophotometer. The results were calculated using the standard calibration curve of (+)-catechin and expressed as (+)-catechin equivalents (g-catechin equiv./g-dry sample).

Severity Factor

The severity factor of thermal treatment is expressed by a correlation between processing temperature and processing time (Overend and Chornet 1987; Noda et al. 2019). The severity factor can be calculated by the following Eq. (1)

$$S = \text{Log}[t \cdot \exp\{(T - 100)/14.75\}]$$

1

where S is the severity factor, T is the processing temperature ($^{\circ}$ C), and t is the processing time (min). 14.75 is the activation energy value under conditions where process is first order kinetics and obeys the Arrhenius law.

Analysis Of Extract

Analysis of extract

The FT-IR spectral analysis of extract was performed on an FT-IR spectrophotometer (FTIR 420, JASCO Co., Ltd.) using KBr pellets (Senthilkumar et al. 2017) containing 1 % finely ground samples. Each spectrum was recorded using 32 scans ranging from 4000 to 400 cm^{-1} , with a resolution of 4 cm^{-1} in the transmission mode.

Results And Discussion

Antioxidant activity of Tadeai subjected to microwave treatment (MW)

The effect of processing temperature of MW on the extraction of antioxidant from Tadeai was examined using “Kojoko Shirohana” because it is the most popular and widely cultured Tadeai in Tokushima prefecture. MW was performed using four types of sites: flowers, stems, leaves, and roots. Figures 1 and 2 show the change of amount of phenolic compounds and EC_{50} value obtained by a processing time of 5 min, respectively. Since a lower EC_{50} indicate better antioxidant properties (Noda et al. 2019), a processing temperature of 200 °C seems to be the optimal regardless of types of sites. Therefore, a processing temperature of 200 °C for MW was used in the following experiments. The reason why the best result was obtained at 200°C is due to the degradation of high-molecular weight lignin contained in Tadeai. As the processing temperature increases, the resolution increases and the quantum of products from lignin degradation increases (Nassar et al. 1984; Borges et al. 2014), increasing the amount of phenolic compounds and decreasing EC_{50} values. It is well known that lignin is a high-molecular weight polyphenol that is abundant in plant cell walls and that many low-molecular weight phenolic compounds exhibit antioxidant properties. Therefore, it seems that MW decomposed lignin into low-molecular weight phenolic compounds that can be soluble in water and exhibit antioxidant activity.

Next, the optimal processing time was examined for the optimal processing temperature, i.e. 200 °C, determined from the above results. Figures 3 and 4 shows the change of amount of phenolic compounds and EC_{50} value obtained by a processing temperature 200 °C. The amount of phenolic compounds initially increased rapidly as the processing time increased. After that, it decreased gradually beyond a processing time of 1–2 min. On the other hand, EC_{50} value decreased for a processing time of 1–2 min and then increased or reached a constant value. In case of leaf, a longer processing time decreased the amount of phenolic compounds but did not change the EC_{50} value at all. This means that the produced phenolic compounds with antioxidant activity were degraded by a longer processing time (Jakab et al. 1997; Leopoldini et al. 2011; Farag et al. 2014). If the phenolic hydroxyl group decreases, the amount of phenolic compounds also decreases. Furthermore, regarding EC_{50} value, it is assumed that other antioxidant compounds was produced by the degradation of phenolic compounds. This point is a future subject. Since the phenolic hydroxyl group itself has an oxidizing action, the reducing power of the hydroxyl group in the reactive group is important for the antioxidant power of the phenol compound. It is inferred that these hydroxyl groups were reduced through excessive decomposition by MW over a long time.

Figure 5 shows the relationships between (a) EC_{50} vs and severity factor, (b) amount of phenolic compounds vs severity factor, and (c) amount of phenolic compounds vs EC_{50} of extracts obtained from various parts, i.e. flower, stem, leaf, and root, of “Kojoko Shirohana” by MW treatment. The two values, i.e. that EC_{50} vs severity factor and amount of phenolic compounds vs EC_{50} , were shown to be correlated negatively. On the other hand, the amount of phenolic compounds vs severity factor was shown to be correlated positively. The closer correlation coefficient, i.e. r , is to 1 or -1, the stronger the correlation. Since the higher amount of phenolic compounds the lower EC_{50} , the antioxidant activity of extract increased with increasing the amount of phenolic compounds. However, there were differences depending on which part of Tadeai was subject to analysis. Therefore, it is estimated that the extractive components, i.e.

phenolic compounds, of different parts of Tadeai are different. The root has the largest amount of phenolic compounds as well as the highest antioxidant activity because it has a low EC_{50} value. On the other hand, the leaf has the lowest amount of phenolic compounds and antioxidant activity. It can be seen that the phenolic compounds of extract obtained from the root has a higher antioxidant activity compared with those obtained from leaf, flower, and stem. In order to investigate the reason for this, the structural analysis of extract was performed by FT-IR spectrum analysis.

FT-IR spectrum of extract obtained from various parts of Tadeai

Figure 6 shows the results of FT-IR spectrum analysis of extracts obtained from various parts, i.e. flower, stem, leaf, and root, of “Kojoko Shirohana” by MW treatment. The leaves are used as a raw material for indigo dyeing. It is known that components in leaves known as indicants are involved in dyeing. Parts other than leaves are not used for indigo dyeing and are often discarded unused. For this reason, much research and analysis has been conducted on the leaves. While structures and components of the leaves have been identified, much else is unknown. Sites that have a strong structure such as stems and roots become a plant framework and contain a large amount of lignin in the cell wall. Furthermore, flowers contain a lot of pigments and fragrance components. The analysis was conducted considering these points. Since there was almost no significant difference among the four parts from 2000 cm^{-1} to 4000 cm^{-1} , and we examined the results by narrowing down the range to 2000 cm^{-1} or less where the changes appeared. The peaks at $1300\text{--}1400\text{ cm}^{-1}$ and $1200\text{--}1300\text{ cm}^{-1}$ were found to have hydroxyl groups bonded to the benzene ring and this benzene ring was found to be trisubstituted (Vautier et al. 2001; Heo et al. 2013). The peak between 1000 and 1100 cm^{-1} indicates C-O in C-OH (Oliveira et al. 2016). The peak between 1700 and 1800 cm^{-1} indicates C=O in benzene-OH, the peak between 1600 and 1650 cm^{-1} indicates C=C in benzene, the peak between 1550 and 1800 cm^{-1} indicates C=O in benzene = O and N-H in pyrrole, the peak between 1350 and 1550 cm^{-1} indicates C-H and C-C and C-O in the side chain which bound to benzene (Toledo et al. 2012; Ju et al. 2019). Comparing these differences in peak intensity of four parts, a low peak of hydroxyl groups bonded to the benzene ring, i.e. $1200\text{--}1300\text{ cm}^{-1}$, of leaf was observed. Furthermore, the peak increased in order of leaf, flower, stem, and root, and this corresponded to their antioxidant activity. It seems that the amount of hydroxyl groups bonded to the benzene ring affected the antioxidant activity because EC_{50} value of root was much lower than those of leaf as described above. From these results, it was found that other parts beside leaves of Tadeai that usually discarded unused can be candidates as a raw material of antioxidant material production. Future study will be focused on more detail structure analysis of extracts from various parts of Tadeai by using ^1H NMR and GC-MS.

Comparison Of Antioxidant Activity Among Tadeai Varieties

Figures 6 and 7 shows the differences in amount of phenolic compounds and EC_{50} value depending on Tadeai varieties. Four types of Tadeai were analyzed: “Akakuki Kosenbon”, “Senbon”, “Kojoko Akahana”,

and “Kojoko Shirohana”. Four types of Tadeai were analyzed: “Akakuki Kosenbon”, “Senbon”, “Kojoko Akahana”, and “Kojoko Shirohana”. These results indicate that the varieties and parts of Tadeais affected not only the amount of phenolic compounds but also EC₅₀ value, significantly. Except leaves, i.e. a useful raw material for indigo dyeing, the extracts from flowers, stems, and roots provided low EC₅₀ values, i.e. 0.018–0.21 g/L. Noda et al. 2019 reported that EC₅₀ value (0.26 g/L) obtained from garlic husk by MW at 200°C for 5 min, and Noda et al. 2013 reported that EC₅₀ value (0.191 g/L) obtained from raw garlic by steam explosion at 235°C for 5 min. Garlic is one of the highest antioxidant foods but the antioxidant activity of MW-treated Tadeai waste, flowers, stems, and root, was higher (lower EC₅₀ value) than those of treated garlic materials, so Tadeai waste seems to be a good raw material to extract antioxidant. Roots gave the most antioxidant activity regardless of varieties of Tadeai. Since the roots of plants have many useful components, as they have been used as traditional Chinese medicine since ancient times, it seems that the roots have a high antioxidant component. Unlike the leaves and flowers, the roots contain many components related to skeleton formation such as lignin, i.e. a resource for phenolic compounds, which is considered to be antioxidant materials. Among the varieties and the parts of Tadeai, the root of “Kojoko Akahana” shows the lowest EC₅₀ value. i.e. 0.018 g/L, and amount of phenolic compounds, 44.1 mg-catechin equiv./g-dry sample. In this experiment, all varieties were obtained at the same time after they had flowered. However, the flowering time varies depending on the variety, and the flowering time of “Kojoko Akahana” is early. Therefore, the results may change depending on the harvest time, so it will be necessary to consider this point in the future.

Conclusions

This is the first report for extracting water soluble antioxidant material from Tadeai, a Japanese indigo plant, using an environmental friendly methods, i.e. microwave treatment.

This study explored effective utilization methods for parts, i.e. flowers, stems, and roots, of the Tadeai that are usually discarded after the leaves are used as a raw material in textile printing. We believe that our study makes a significant contribution to the novel utilization of Tadeai waste because Tadeai contains various useful ingredients such as not only indigo in the leaves but also phenolic compounds that possess antioxidant properties in the other parts. The amount of phenolic compounds and the DPPH radical scavenging activity after subjecting the plant parts to microwave extraction; compared to the sample that was not subjected to microwave treatment, the amount of phenolic compounds was observed to be double, and the antioxidant activity eight times higher. Furthermore, it was found that the extracts from roots show the highest antioxidant activity.

Abbreviations

MW

Microwave treatment; DPPH:1,1-Diphenyl-2-picrylhydrazyl free radical FT-IR:Fourier transform infrared; EC₅₀:Half maximal (50%) effective concentration; ¹H NMR:Proton nuclear magnetic resonance; GC-

Declarations

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Authors' contributions

CS designed the study. AS and YN performed the experiment and wrote a part of the manuscript. CA wrote a part of the manuscript and polished the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Fig. 1

Figure 1

Change in amount of phenolic compounds with change in processing temperature. Sample is “Kojoko Shirohana” and processing time is 5 min. Treatment was performed on four types of sites: flowers, stems, leaves, and roots.

Fig. 2

Figure 2

Change in EC50 value with change in processing temperature. Sample is “Kojoko Shirohana” and processing time is 5 min. Treatment was performed on four types of sites: flowers, stems, leaves, and roots.

Fig. 3

Figure 3

Change in amount of phenolic compounds with change in processing time. Sample is “Kojoko Shirohana” and processing temperature is 200 oC. Treatment was performed on four types of sites: flowers, stems, leaves, and roots.

Fig. 4

Figure 4

Change in EC₅₀ value with change in processing time. Sample is “Kojoko Shirohana” and processing temperature is 200 oC. Treatment was performed on four types of sites: flowers, stems, leaves, and roots.

Fig. 5

Figure 5

Relationship between the two of severity factor, EC50, and amount of phenolic compounds. Treatment was performed on four types of sites: flowers, stems, leaves, and roots of “Kojoko Shirohana.” (a) EC50 vs and severity factor, (b) amount of phenolic compounds vs severity factor, (c) amount of phenolic compounds vs severity factor.

Fig. 6

Figure 6

FT-IR spectrum analysis of extracts obtained from various parts, i.e. flowers, stems, leaves, and roots, of “Kojoko Shirohana” by MW treatment. A spectrum of the extract under the conditions having the most antioxidant activity are shown. The processing temperature is 200 oC, the processing time for leaves is 2 min, and the processing time for flowers, stems, and roots is 1 min.

Fig. 7

Figure 7

Amount of phenolic compounds of extract obtained from four types of sites: flowers, stems, leaves, and roots of Tadeai varieties. The processing temperature is 200 oC, the processing time for leaves is 2 min, and the processing time for flowers, stems, and roots is 1 min.

Fig. 8

Figure 8

EC₅₀ value of extract obtained from four types of sites: flowers, stems, leaves, and roots of Tadeai varieties. The processing temperature is 200 oC, the processing time for leaves is 2 min, and the processing time for flowers, stems, and roots is 1 min.

Supplementary Files

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