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# Research Article
# Chemical Compositions and Antioxidant Activities of Flower Extracts from Chestnut (*Castanea crenata* Sieb. et Zucc)

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## Abstract

**Background and Objective:** *Castanea crenata* Sieb. et Zucc is a native species in Japan (Japanese chestnut). This species was reported to have very high antioxidant, allergic and anti-amnesic properties. The objective of the study was to evaluate the total polyphenols and antioxidant activities and analyse the bioactive compounds of this species. **Materials and Methods:** The flower of *C. crenata* was extracted by methanol and then the crude extract was fractioned by hexane, ethyl acetate, butanol and aqueous extracts. The extract and fractions were used to determine total polyphenols and antioxidant activities and to analyze bioactive components by gas chromatography-mass spectrometry (GC-MS). **Results:** The hexane fraction showed the highest amount of phenolic, flavonoid and tannin contents. This fraction also exhibited a higher reducing power at a concentration of 1 mg $mL^{-1}$ than the other fractions and standard compound BHT (butylated hydroxytoluene). Methanol extract possessed the highest antioxidant capacity and significant stronger than BHT ($p<0.05$) in DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS [2,2 -azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) methods. Significantly positive correlations were observed between phenolic and tannin contents and reducing power with $r = 0.76$ and 0.63, respectively. By gas chromatography-mass spectrometry (GC-MS) analysis, 64 components were detected in the *C. crenata* flower. Methanol and ethyl acetate were found the highest number of compounds with 24 while butanol and water fractions included 20 compounds. The lowest number of constituents were identified in hexane with 7 compounds. **Conclusion:** Methanol and ethyl acetate were the most effective solvent to extract phytochemicals in the *C. crenata* flower, followed by butanol and water and hexane showed the least efficacy.

Key words: Antioxidant activity, *Castanea crenata*, flower extract, total phenolic content, DPPH, reducing power, gas chromatography-mass

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Natural antioxidant constituents are believed to play a crucial role in human health[1]. Antioxidants are considered radical scavengers, hydrogen donors and enzyme inhibitors[2]. Therefore, antioxidants are considered extra nutritional compositions due to their existence in the human diet and their biological activity[3]. The number of 250,000 higher plant species on the surface of the earth has been estimated and about 5-10% of these terrestrial plants have ever been investigated for drug discovery[4].

Plants produce a large number of natural antioxidants, which are classified into several groups according to their biosynthetic routes and structural features such as phenolic acids, flavonoids, coumarins and stilbenes, hydrolysable and condensed tannins, lignans and lignins[5,6]. High amounts of these antioxidant compounds have been proved to prevent health problems including heart attack, oxidative stress, platelet aggregation and cancer[7].

Chestnut (Fagaceae family) is cultivated widely in Asia, Europe, North America and North Afica[8]. Several species of this tree for example *Castanea crenata*, *Castanea mollissima* and *Castanea dentata* are distributed mainly in Asia, particularly in Japan, China and Korea or the US[9]. Youn *et al.*[8] revealed that because of their rich taste and high nutritional values, chestnuts were traditional use in wedding ceremonies and other ancestral rites. *Castanea crenata* Sieb. et Zucc is a native species in Japan (Japanese chestnut). The fruit from this species was reported to have very high antioxidant, allergic and anti-amnesic properties especially inner skin parts of fruits. Moreover, flowers from *C. crenata* also were evaluated with good antioxidant activities and numerous polyphenols in ethanol and ethylacetate extracts[10]. However, methanol extract and its fractionations have not been estimated for antioxidant and identified antioxidant compounds. In this study, the antioxidant activities of methanol extract from *C. crenata* flower and its derived soluble fractions including hexane, ethyl acetate, butanol and aqueous extracts were evaluated in various methods such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), reducing power, β-carotene bleaching assays. In addition, the determination of phytochemicals from *C. crenata* flower extract and its fractions were also identified by using GC-MS.

## MATERIALS AND METHODS

**Plant materials and preparation of the extracts:** Flowers of *C. crenata* were collected from Higashihiroshima, Japan in May, 2017. All experiments of the study were conducted from May to September, 2017 in the Laboratory of Plant Physiology and Biochemistry-Transdisciplinary Science and Engineering Program (Development Science Field), Graduate School of Advanced Science and Engineering, Hiroshima University, Japan. The samples were dried in an oven at 30°C for one week, then were pulverized into fine powder.

The flower powder (25 g) was soaked in 1 L methanol (MeOH) 100% for one week at room temperature. The extract was filtered and concentrated under vacuum at 30°C until 15 g were obtained. The methanol crude extract (10 g) was dissolved in 50 mL of distilled water and extracted with hexane, ethyl acetate (EtOAc) and butanol (BuOH). The obtained extracts and aqueous solution remaining after extraction was filtered and concentrated to collect 0.1, 2.1, 4.6 and 4.2 g of hexane, ethyl acetate, butanol and aqueous extracts, respectively.

**Determination of total phenolic contents:** Total phenolic contents of extracts were evaluated by the Folin-Ciocalteu method[10]. The extract concentrations ranged between 100 and 250 μg $mL^{-1}$. Absorption was measured at 765 using a Shimadzu UV mini-1240 UV-VIS spectrophotometer. The total phenolic content was calculated as gallic acid equivalent from the calibration curve of gallic acid standard solutions (10–100 μg $mL^{-1}$) and expressed as mg Gallic Acid Equivalent (GAE) $g^{-1}$ extract.

**Determination of total flavonoid contents:** The total flavonoid contents were measured based on the previous procedure[11]. A volume of 1 mL reaction contained 0.5 mL of extract and 0.5 mL of 2% $AlCl_3$ was incubated at room temperature for about 15 min. The absorbance of the reaction at 430 nm was recorded. Rutin was used as the standard.

**Determination of total condensed tannin contents:** The total condensed tannin contents were estimated by the vanillin/HCl method of Rebaya *et al.*[12]. A volume of 0.4 mL of extract was added to 3 mL of vanillin (4% in methanol) and 1.5 mL of concentrated hydrochloric acid. After 15 min of incubation, the absorbance was read at 500 nm. The amount of total condensed tannin was expressed in terms of mg (+)- catechin equivalent (CE) $g^{-1}$ extract.

**An antioxidant assay using the DPPH radical scavenging:** The radical scavenging activity was evaluated as described previously Elzaawely *et al.*[13]. One millilitre of extract and standard BHT was mixed with 0.5 mL of DPPH 0.5 mM and 1 mL of 0.1 M acetate buffer (pH 5.5).

**ABTS radical scavenging activity:** The antioxidant capacity assay was carried out using an improved ABTS method as described by Mikulic-Petkovsek *et al.*[14] with some modifications. The 2,2¢-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS) solution was generated by the reaction of 7 mM of ABTS and 2.45 mM of potassium persulfate solution (in equal quantities) after incubation at room temperature in the dark for 16 hrs. The ABTS solution was then diluted with methanol to obtain an absorbance of 0.700±0.05 at 734 nm. Briefly, the reaction consisted of 1000 µL ABTS and 120 µL extract was incubated at room temperature for 30 min. The absorbance of the reaction at 734 nm was measured. BHT was used as the standard. ABTS radical scavenging activity was calculated by the equation:

$$\text{ABTS radical scavenging activity (\%)} = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$$

where, $A_{control}$ is the absorbance of reaction without sample and $A_{sample}$ is the absorbance of reaction with sample. The lower absorbance of the reaction mixture indicated a higher ABTS radical scavenging activity.

**Reducing power:** The reducing power was assayed by using the method described previously[10]. The volume of 0.2 mL each extract or BHT was added to 0.5 mL phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The above reaction mixture was then incubated at 50°C for 30 min. After this step, trichloroacetic acid (0.5 mL, 10%) was added to the mixture to stop the reaction and then centrifuged at 4000 rpm for 10 min. Supernatant (0.5 mL) was diluted with 0.5 mL of distilled water and 0.1 mL $FeCl_3$ solution (0.1%) was added. The reaction was thoroughly mixed and measured the absorbance at 700 nm. The $IC_{50}$ values were calculated at which the absorbance was 0.5. Lower $IC_{50}$ indicates higher reducing power.

**GC-MS analysis:** Methanol extract and other fractions were evaporated and diluted in acetone at 100 ppm for analysis by using a GC-MS system (single quadrupole, ISQ Thermo, Austin, TX, USA) with DB5MS column (30 m, 0.25 mm, 0.25 µm) (Agilent Technologies, J and W Scientific Products, Folsom, CA, USA). The GC oven temperature program was as follows: 50°C hold for 6 min, raised at 10°C per min to 250°C and hold for 3 min. The injector was set at 250°C and the detector at 280°C. The flow rate of carrier gas (helium) was maintained at 1.0 mL $min^{-1}$. The mass range was scanned from 20-650 amu. Identification of compound was obtained by using the JEOL's GC-MS Mass Center System software, version 2.65a (JEOL Ltd., Tokyo, Japan).

**Statistic analysis:** The data were analyzed by one-way ANOVA using the Minitab 16.0 software (Minitab Inc., State College, PA, USA) and SPSS 20.0. Upon significant differences, means were separated using Tukey's test at $p<0.05$ with three replications and expressed as the Mean±Standard Deviation (SD).

## RESULTS

**Total phenolics, flavonoids and tannin contents in various extracts of *C. crenata*:** Total phenolic contents were evaluated and represented in the following order: Hexane>BuOH>EtOAc and MeOH>water. The highest value was obtained for hexane fraction (534.40±30.10 mg GAE $g^{-1}$ extract), water fraction possessed the lowest amount of total phenolic content (143.56±13.49 mg GAE $g^{-1}$ extract) ($p<0.05$) (Fig. 1).

Total flavonoid contents were determined in methanol extract and different fractions with the order hexane>EtOAc> MeOH>BuOH> water. The total flavonoid contents varied from 3.87-165.56 mg RE $g^{-1}$ extract. Hexane and EtOAc had very high flavonoid contents (Fig. 2).

The total tannin contents of methanol extract and different fractions from the *C. crenata* flower were represented in Fig. 3. Hexane fraction had the highest total tannin content which was 5-14 folds greater than the other fractions.

**Antioxidant activities of *C. crenata*:** The antioxidant activities of methanol extract and its fractions were illustrated in Table 1. In the DPPH assay, MeOH and BuOH extracts were not only the highest antioxidant capacities compared with the other fractions but also significantly stronger than BHT (standard compound). In the case of ABTS assay, MeOH extract also revealed the strongest antioxidant property whereas the lowest activity was obtained in water fraction.

Table 1: Antioxidant activities of *C. crenata* flower extracts by DPPH and ABTS radical scavenging expressed as $IC_{50}$ values

| | DPPH (µg $mL^{-1}$) | ABTS (µg $mL^{-1}$) |
|---|---|---|
| MeOH | 14.70±1.77$^{e}$ | 127.68±1.79$^{d}$ |
| Hexane | 22.72±0.63$^{c}$ | 158.43±12.14$^{bc}$ |
| EtOAc | 27.12±0.38$^{b}$ | 171.40±2.78$^{b}$ |
| BuOH | 15.95±0.14$^{de}$ | 154.18±1.13$^{c}$ |
| Water | 46.98±1.15$^{a}$ | 253.15±6.62$^{a}$ |
| BHT | 17.53±0.91$^{d}$ | 47.82±2.56$^{e}$ |

MeOH: Methanol, EtOAc: Ethyl acetate, BuOH: Butanol, BHT (butylated hydroxytoluene) is a positive control and different letters in the same row indicate significant differences ($p<0.05$)

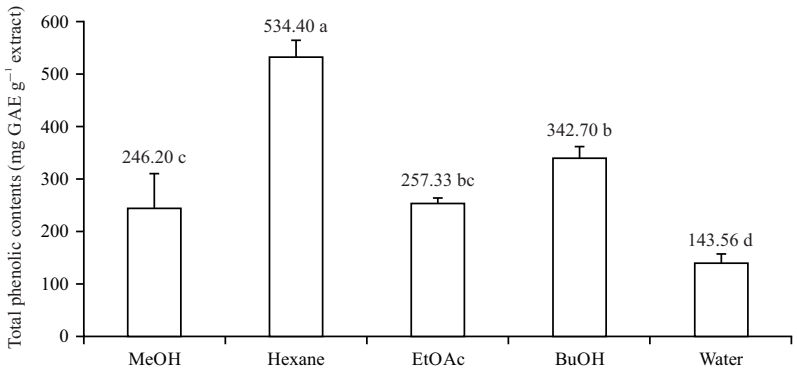


Fig. 1: Total phenolic contents in extracts of *C. crenata*

Each value represents the Mean±Standard deviation (n = 3), Values with the same letters (a, b, c, d) are not significantly different (p<0.05), MeOH: Methanol, EtOAc: Ethyl acetate and BuOH: Butanol

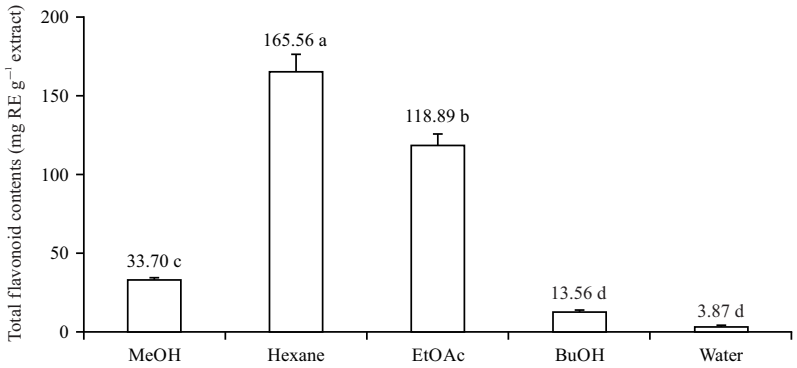


Fig. 2: Total flavonoid contents in extracts of *C. crenata*

Each value represents the Mean±Standard deviation (n = 3), Values with the same letter are not significantly different (p<0.05), MeOH: Methanol, EtOAc: Ethyl acetate and BuOH: Butanol

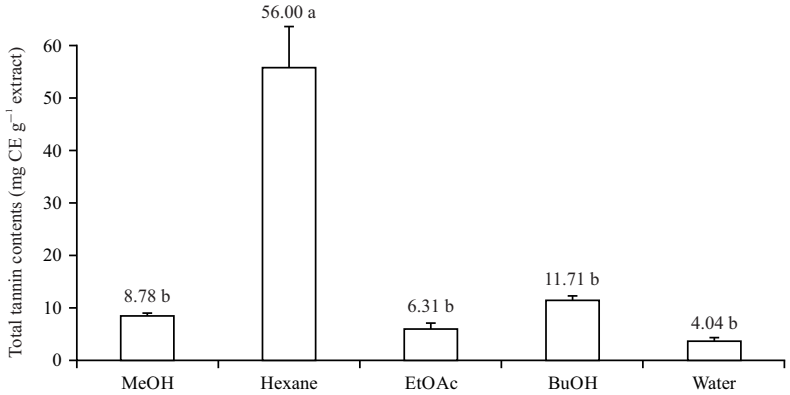


Fig. 3: Total tannin contents in extracts of *C. crenata*

Each value represents the Mean±Standard deviation (n = 3), Values with the same letter are not significantly different (p<0.05), MeOH: Methanol, EtOAc: Ethyl acetate and BuOH = Butanol

The reducing power was proportional to concentrations of extracts and the values obtained for all extracts were represented in Fig. 4. It was found that at 1 mg mL$^{-1}$, the absorbance values were higher than 2.0 for MeOH extract and all fractions. Hexane fraction was greater reducing power than other extracts and BHT at a concentration from 0.6-1 mg mL$^{-1}$.

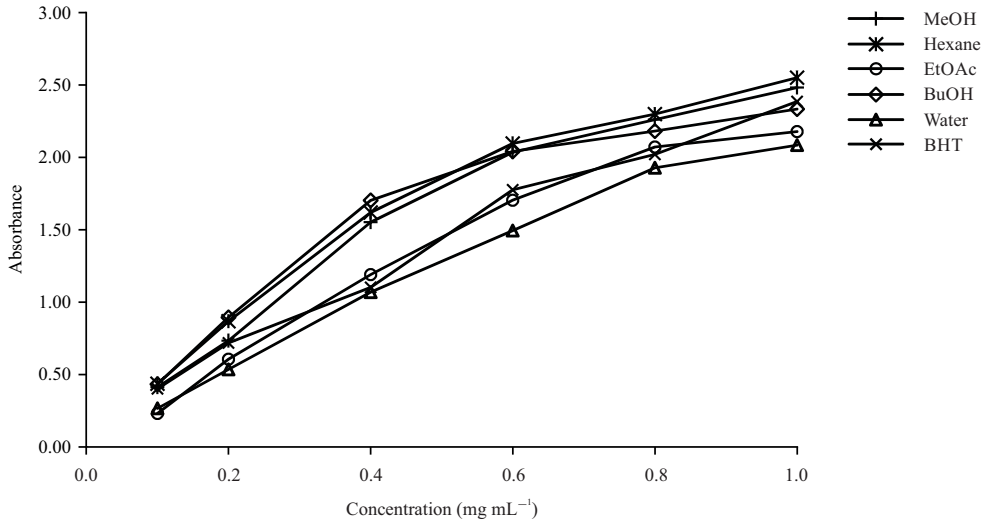


Fig. 4: Reducing power of *C. crenata* flower extracts
MeOH: Methanol, EtOAc: Ethyl acetate, BuOH: Butanol and BHT (butylated hydroxytoluene) is a positive control

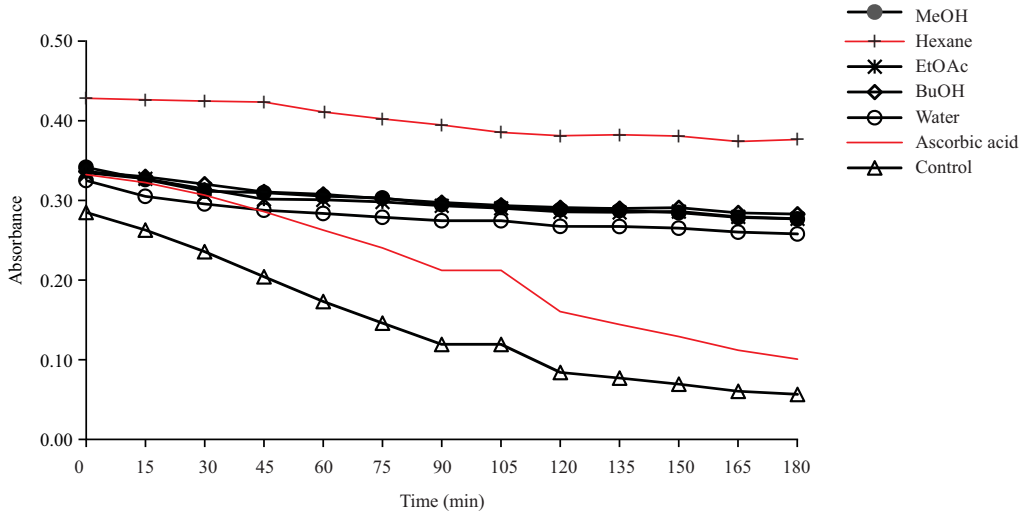


Fig. 5: Antioxidant activity of *C. crenata* flower extracts by β-carotene bleaching method
Ascorbic acid is a positive control, MeOH: Methanol, EtOAc: Ethyl acetate and BuOH: Butanol

Table 2: Pearson correlation between polyphenol and antioxidant activities

| Correlations | TPC | TFC | TTC | DPPH | ABTS |
|---|---|---|---|---|---|
| TFC | 0.71** | | | | |
| TTC | 0.89** | 0.73** | | | |
| DPPH | 0.25 | -0.13 | 0.04 | | |
| ABTS | 0.39 | 0.17 | 0.16 | 0.91** | |
| Reducing power | 0.76** | 0.19 | 0.63* | 0.71** | 0.63* |

**,*Correlations are significant at the 0.01 and 0.05 levels, respectively. $1/IC_{50}$ values of DPPH, ABTS and Reducing power were used to examine the Pearson correlation between antioxidant activities and polyphenols. TFC: Total flavonoid content, TTC: Total tannin content, DPPH: 2,2-Diphenyl-1-Picrylhydrazyl, ABTS: 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-sulfonic acid

The percentage of lipid peroxidation inhibition of extracts was indicated in Fig. 5. Methanol extract and all fractions of *C. crenata* flower inhibited oxidation and the results of all extracts were higher than ascorbic acid as a positive control. Among methanol extract and fractions, hexane fraction exhibited the highest inhibition and water fraction exhibited the lowest one.

**Pearson correlation among total phenolic, flavonoid and tannin contents and antioxidant activities:** The Pearson correlation among total phenolic, flavonoid and tannin contents and antioxidant activities was carried out (Table 2). Total phenolic and tannin contents significantly positive correlated with reducing power (r = 0.76 and 0.63, respectively), whereas total and free radical scavenging DPPH and ABTS were low correlations.

**Components of *C. crenata* flower extracts:** In this study, GC-MS was used to analyze and identify the chemical components in methanol and different fractions, as shown in Table 3. A total number of 64 compounds were identified. Of which, the methanol and ethyl acetate consisted of 24 constituents while butanol and water fractions included 20 compounds. The lowest number of constituents were identified in hexane with seven compounds. Methanol and ethyl acetate was the most effective solvent to extract phytochemicals in the *C. crenata* flower, followed by butanol and water. Hexane showed the least efficacy. Catechon and 1α,2β,3α,5β-cyclohexanetetrol are the compounds detected in four solvent extracts such as methanol, ethyl acetate, butanol and water, eight components were identified in three extract solvents including furfural, 1,2-cyclopentanedione, 2-Furanmethanol, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 5-Hydroxymethylfurfural, Phenylethyl alcohol, β-D-Glucopyranose, 1,6-anhydro.

Table 3: Chemical constituents from the methanol extract and its fractions of *C. crenata* flower quantitated by GC-MS analysis

| | | Extracting solvents | | | | |
|---|---|---|---|---|---|---|
| Chemical constituents | Molecular weight (g mol$^{-1}$) | MeOH | Hexane | EtOAc | BuOH | Water |
| 2-Hydroxy-gamma-butyrolactone | 102.03 | + | - | - | + | - |
| 2(5H)-Furanone | 84.02 | - | - | - | + | + |
| Benzene, (butoxymethyl) | 164.12 | + | - | - | + | - |
| Furfural | 96.02 | + | - | - | + | + |
| Maltol | 126.03 | + | - | - | - | - |
| *trans*-2,6-Dimethyl-2,7-octadiene-1,6-diol | 170.24 | - | - | + | - | - |
| (3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one | 258.20 | - | - | + | - | - |
| (Z,Z)-α-Farnesene | 204.19 | - | - | + | - | - |
| 1-Eicosanol | 298.32 | - | - | + | - | - |
| 1-Penten-3-ol | 86.07 | + | - | - | - | - |
| 1,2-Cyclopentanedione | 98.04 | + | - | - | + | + |
| 1,2-Ethanediol, monoacetate | 104.05 | + | - | - | + | - |
| Pyrogallol | 126.03 | + | - | + | + | - |
| 1,3-Dioxol-2-one,4,5-dimethyl | 114.03 | + | - | - | + | - |
| 1α,2β,3α,5β-Cyclohexanetetrol | 148.07 | + | - | + | + | + |
| 2-Furanmethanol | 98.03 | + | - | - | + | + |
| 2-Methoxyhydroquinone | 140.05 | - | - | - | - | + |
| 2-Methylpentanoic acid | 116.08 | - | - | + | - | - |
| 2-Propanamine, N-methyl-N-nitroso | 102.08 | + | - | - | - | - |
| 2,3,4,5-Tetrahydropyridazine | 84.07 | + | - | - | - | - |
| 2,5-Dimethyl-4-hydroxy-3(2H)-furanone | 128.05 | - | - | - | + | + |
| 2,5-Furandicarboxaldehyde | 124.02 | - | - | - | + | - |
| 3-Deoxy-d-mannoic lactone | 162.05 | - | - | - | - | + |
| 3-Furancarboxylic acid, methyl ester | 126.03 | - | - | - | + | - |
| 3-Methyl-2-(2-oxopropyl) furan | 138.09 | - | - | + | - | - |
| 3-Oxapentanol-1, 4-[(2,3-dimethyl)phenyl]- | 194.13 | - | - | + | - | - |
| 4-Cyclopentene-1,3-dione | 96.02 | - | - | - | - | + |
| 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl | 144.04 | + | - | - | + | + |
| 5-Hydroxymethylfurfural | 126.03 | + | - | - | + | + |
| Benzene, (butoxymethyl)- | 164.12 | - | - | + | - | - |
| Benzyl alcohol | 108.06 | - | - | + | + | - |
| Butanedioic acid, dimethyl ester | 146.06 | - | - | + | - | - |
| Butyrolactone | 86.04 | - | - | - | - | + |
| Campesterol | 400.37 | - | + | - | - | - |
| Catechol | 170.13 | + | - | + | + | + |
| Cholesta-4,6-dien-3β-ol | 384.34 | - | + | - | - | - |
| Coniferol | 180.08 | - | - | + | - | - |
| Coumarin | 120.06 | + | - | - | - | - |
| Cycloheptanone | 112.09 | - | - | - | - | + |
| Cyclopentane | 86.07 | + | - | - | - | + |
| Cyclopropyl carbinol | 72.06 | + | - | - | - | - |
| D-Alanine, N-propargyloxycarbonyl-, isohexyl ester | 255.15 | - | - | - | - | + |
| Dihydroxyacetone | 90.08 | - | - | - | - | + |

Table 3: Continue

| Chemical constituents | Molecular weight (g mol$^{-1}$) | Extracting solvents | | | | |
|---|---|---|---|---|---|---|
| | | MeOH | Hexane | EtOAc | BuOH | Water |
| Dimethyl oxalate | 118.03 | + | - | - | - | - |
| Ethanol, 2-butoxy- | 118.10 | - | - | + | - | - |
| Glycerin | 92.05 | - | - | - | + | - |
| Hydroquinone | 110.04 | + | - | - | - | + |
| Isobutyl nitrite | 103.06 | - | - | + | - | - |
| Methyl 2-hydroxy-3-phenylpropanoate | 180.08 | - | - | + | - | - |
| Methyl 3β-hydroxy olean-18-en-28-oate | 470.38 | - | - | + | - | - |
| Methyl carbamate | 75.03 | - | - | - | - | + |
| Oxalic acid, dimethyl ester | 118.03 | - | - | + | - | - |
| Palmitic acid | 256.24 | - | + | - | - | - |
| Phenylethyl alcohol | 122.07 | + | - | + | + | - |
| Phenylethyl acetal | 270.16 | - | - | + | - | - |
| Propanedioic acid, dimethyl ester | 132.04 | - | - | + | - | - |
| Propanoic acid, 2-oxo-, methyl ester | 102.03 | - | - | - | - | + |
| Stigmast-4-en-3-one | 412.37 | - | + | - | - | - |
| Stigmastan-3,5-diene | 396.38 | - | + | - | - | - |
| Sucrose | 342.12 | + | - | - | - | - |
| Tributyl acetylcitrate | 402.23 | - | + | - | - | - |
| β-D-Glucopyranose, 1,6-anhydro | 162.05 | + | - | + | + | - |
| β-D-Glucopyranoside, methyl 3,6-anhydro | 176.07 | - | - | + | - | - |
| γ-Sitosterol | 414.39 | - | + | - | - | - |

GC-MS: Gas chromatography-mass spectrometry, +: Detected and -: Not detected

## DISCUSSION

The antioxidant activities and bioactive sustains of the *C. crenata* flower were evaluated in this study. Previous work has been mentioned on the profile of bioactive compounds and antioxidant capacities in several parts of *C. crenata* including leaf, flower, outer skin, inner skin and kernel[8,10]. In terms of *C. crenata* shells, Youn *et al.*[8] suggested that methanol was an effective solvent compared to water, the total phenolic compound in methanol and water extract reached 205 and 171 mg GAE g$^{-1}$, respectively. In comparison with these results, it was illustrated that the flower has higher phenolic content than the shell. On the other hand, Tuyen *et al.*[10] reported that the inner skin of *C. crenata* in the previous study showed the greatest total phenolic compound with 1034 mg GAE g$^{-1}$. The quantity of flavonoid from flower extracts was significantly higher than in barks and inner skins, moreover, the difference in flower flavonoid was recorded between Tuyen's (147.41 mg RE g$^{-1}$ extract) and our data (165 mg RE g$^{-1}$ extract). Thus, such data reflected the role of solvent used for the extraction procedure and hexane was a useful chemical for flavonoid isolation. Furthermore, when comparing with results from Youn *et al.*[8], it was clear that the difference in tannin concentration in flower and shell, so the solvent played an important role in the extraction process.

Free radical scavenging is commonly considered a measurement of antioxidant activity. In contrast with the yield of secondary compounds, the antioxidant activity from methanol extract was dominant over hexane and other extracts. The previous study concluded that 34.2% of DPPH and 78.8% of ABTS radicals were scavenged by the methanol extract at the concentration of 100 μg mL$^{-1}$ while these data of the water extract were only 13.7 and 33.1%, respectively[8]. Another considerable result in this research is the $IC_{50}$ values for DPPH and ABTS scavenging of flower extract is much lower compared with inner skin (23.81 and 270.73 μg mL$^{-1}$, respectively). Thus, the *C. crenata* flower is an organ that accumulated secondary compounds with high antioxidant activity.

Chestnut shell extract showed an excellent radical-scavenging activity and prevented free radical-induced DNA deconstruction. The antioxidant activity was highly linked with the level of phenolic and flavonoid contents[15]. The correlation between phenolic, flavonoid and tannin quantity with antioxidant capacity has also been reported from other plant extracts, for example, eight wild vegetables from Nepal including *Alternanthera sessilis*, *Basella alba*, *Cassia tora*, *Digera muricata*, *Ipomoea aquatica*, *Leucas cephalotes*, *Portulaca oleracea* and *Solanum nigrum*. The correlation between antioxidant capacity and the amount of phenolic and flavonoid compounds in the extract was high[16]. Catechon and

1α,2β,3α,5β-cyclohexanetetrol, the two compounds detected from methanol and other three fractions except for hexane fraction by GC-MS analysis, were demonstrated as antioxidant candidates. A study by de La Cruz *et al.*[17] showed the role of the catechol group in the antioxidant and neuroprotective effect of olive oil in the rat brain. More interestingly, a synthetic derivative of cyclohexane carrying cyclohexane 1,3-dione was observed for reducing power[18]. Pyrogallol is one of those compounds found in methanol extract and ethyl acetate and butanol fractions. The pyrogallol dimerization using the DPPH method was reported by applying the chemical analyzed method[19]. Results suggest *C. crenata* flower extract contains rich sources of natural antioxidants with potential applications in pharmaceutical industries. Further investigations regarding more biological activities and isolation of individual compounds should be conducted.

## CONCLUSION

The presence of total phenolic, flavonoid, tannin contents and antioxidant activities of methanol extract and its fractions of *C. crenata* flowers was reported for the first time. The methanol extract and hexane fraction possess stronger antioxidant activities than other fractions and positive control BHT. There were 64 compounds detected in the *C. crenata* flower by using GC-MS. The highest number of components were indicated in methanol extract and ethyl acetate were with 24 components, the lowest number of compounds were found in a hexane fraction. The finding of this research also suggested that methanol and ethyl acetate were the most effective solvent to extract phytochemicals in the *C. crenata* flower.

## SIGNIFICANCE STATEMENT

The antioxidant activities and chemical compounds of *C. crenata* were reported for the first time. The hexane fraction exhibited a higher reducing power at a concentration 1 mg $mL^{-1}$ than the other fractions and standard compound BHT (butylated hydroxytoluene). Methanol extract possessed the highest antioxidant capacity and significant stronger than BHT ($p<0.05$) in DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) methods. A total of 64 components were detected in the *C. crenata* flower. Methanol and ethyl acetate were the most effective solvent to extract phytochemicals in the *C. crenata* flower.

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