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Antioxidant activity of galangal: effects of cooking methods

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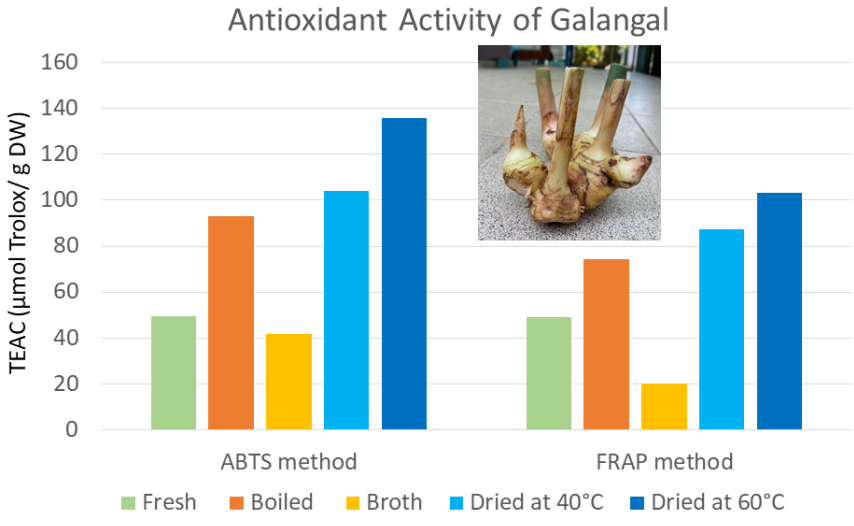
Abstract: Many herbal ingredients in Thai cuisine not only have unique flavor and aroma, but also contain antioxidative phytochemicals. Galangal or greater galangal (*Alpinia galangal*) known as "Khaa" in Thailand is such an ingredient. It is added to various dishes. The question on how boiling and heating affect its antioxidant activity was addressed in this work. Diced fresh galangal was subjected to boiling and heating (40 and 60°C). The fresh and processed galangal was subsequently extracted with 70% ethanol. Antioxidant activities of ethanolic extracts and galangal broth (solution left after filtering out boiled galangal) were assayed by two methods, ABTS (2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphoic acid) and FRAP (Ferric reducing antioxidant power). The fresh galangal possessed antioxidant activities of 49.70 and 48.94 μmol Trolox/ g dry weight by ABTS and FRAP assays, respectively. It was found that 'cooking' increased antioxidant activity by 1.52 – 2.73 times of fresh counterpart, except for galangal broth which was 0.41-0.84 times of fresh one, depending on assay. The high correlation (0.965) was observed between two assay methods.

Graphical abstract:



Keywords: galangal; greater galangal; *Alpinia galangal*; antioxidant; ABTS; FRAP; herb; spice

1. Introduction

Free radicals are atoms with one or more unpaired electrons, for example reactive oxygen species and reactive nitrogen species. Being unstable and reactive, they can react with other atoms or biomolecules in the cells in chain reactions, affecting cell signaling

and gene expression, causing cell damage or even inducing apoptosis. On the opposite side, there are antioxidants protecting cells from oxidative damage. Antioxidant, hence, its name, is defined as any substance significantly delays or inhibits oxidation by acting as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelator. Normally, cells in our body have natural antioxidants both being enzyme and non-enzyme. However, imbalance can still occur, especially when we are exposed more to exogenous sources of radicals, for example pollutants or toxins [1,2].

Galangal or greater galangal (*Alpinia galangal*) known as "Khaa" in Thailand belongs to the family Zingiberaceae (ginger) [3]. Owing to its aromatic odors and spicy flavor, galangal rhizome is a major ingredient in many authentic Thai cuisine, including Tom Yum (hot and sour Thai soup) and Tom Kha Gai (Thai coconut soup). It is used in folk medicinal recipe, especially for remedy of gastrointestinal disease [3,4]. Galangal is rich in phenolic compounds and essential oils with variety of biological activities, such as antimicrobial, antitumor, antiulcer, antiallergic, antioxidant, anti-inflamatory and anticancer activities [5]. Plant extracts, including galangal, as natural sources of antioxidants, are added to lipid containing food to reduce lipid oxidation, consequently causing rancidity and nutritionally lower food quality. Dried galangal powder was added to cooked ground pork or raw minced beef to extend their shelf-life due to its antimicrobial properties [4]. In addition, galangal essential oil and galangal extract were incorporated into biodegradable films and proved to extend food shelf-life, so called active films [6,7].

Even though there are several studies on antioxidative properties of galangal, it is not clearly shown how antioxidant activity is changed upon cooking or preservation and how much antioxidant activity is present in soup when galangal rhizome is traditionally used as splice in making soup. When used as splice in Thai soup, some eat the boiled pieces while other just sip the soup. While some spice and herb processing companies preserve it as dried galangal which is a good culinary substitute wherever fresh galangal is not available. This study was aimed to measure antioxidant activities of fresh and processed galangal by two assay methods with different chemistry behind the assay.

2. Materials and Methods

2.1. Chemicals and reagents

2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulphoic acid) or ABTS and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxyluc acid (Trolox) were obtained from Sigma-Aldrich. Ferric chloride was purchased from Loba-chemie and 2,4,6-tri(2-ryridyl)-*s*-triazine (TPTZ) was from Fluka.

2.2. Galangal preparation

Galangal rhizome was purchased from local market in Chiang Mai, Thailand. The same sample is used for all preparation methods. Galangal was cleaned with tap water and diced to approximately 0.5x0.5x0.3 cm. This was considered as fresh galangal and kept at 4 °C until use. A portion (188.53 g which is equivalent to 15 g dried galangal) of diced galangal was boiled in 300 mL boiling water for 10 min. Boiled galangal was filtered through Whatman filter paper No.1 while the broth was measured to be 180 mL and kept in dark bottle until use. Separately, 188.53 g diced galangal was dried in oven set at 40 °C for 24 hours, such that constant weight was obtained (approx. 15 g). In addition, the same amount of diced galangal was dried in 60 °C oven for 12 hours. Preparation scheme is shown in Figure 1.

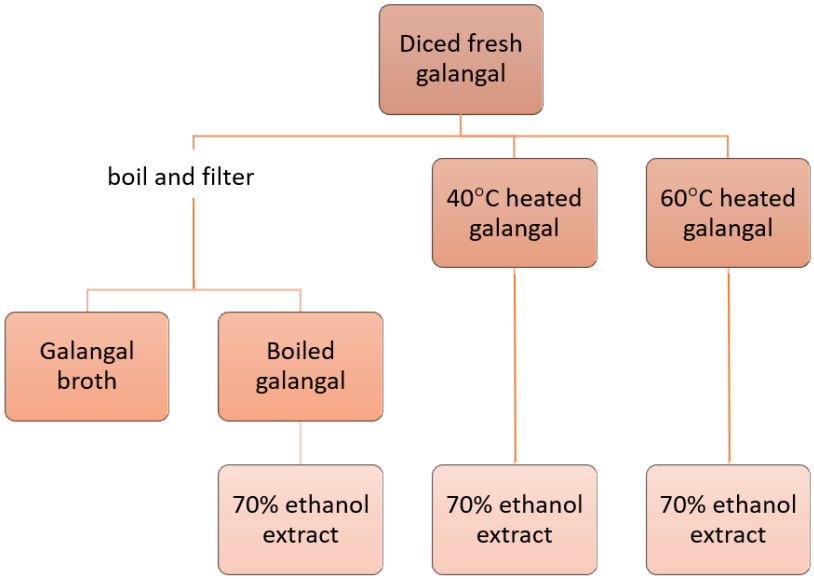


Figure 1. Scheme of galangal preparation and extraction

2.3. Galangal moisture content

Moisture content is determined via a thermogravimetric approach, i.e., by loss on drying. Diced fresh rhizome was subjected to 60 °C oven and heated until the constant weight was obtained, approximately 12 hr. The weight loss due to evaporation of moisture was then recorded. Moisture content is calculated according to the following equation:

$$\%\ moisture = \left(\frac{Fresh\ weight - Dried\ weight}{Fresh\ weight}\right) \times 100$$

2.4. Galangal extraction

In every galangal sample, same amount of sample which is equivalent to 5 g dry weight were used, i.e., 62.84 g of fresh galangal and boiled galangal and 5 g of dried galangal at 40 and 60 °C. Each sample was extracted with 50 mL 70% ethanol at room temperature for 24 hours, modified from Wong-Paz et al. [8]. The extract filtrate was then stored in dark bottle at 4°C until next step. Galangal broth, on the other hand, is directly used (Figure 1).

2.5. ABTS assay

Antioxidant capacity was evaluated by ABTS method modified from [9]. ABTS reagent was prepared by mixing 3.7 mM ABTS in 99.9% ethanol and 2.45 mM potassium persulfate in 1:1 volume ratio and allowed to stand in dark for 16 hr. ABTS radical cation ($ABTS^{+\bullet}$) was produced and diluted with 99.9% ethanol so that the absorbance at 734 nm was in the range of 0.700±0.02. Then, 4.85 mL of ABTS solution is thoroughly mixed with 0.15 mL diluted galangal extract. After standing at room temperature for 6 min., the progress of reaction is immediately followed by recording absorbance at 734 nm using spectrophotometer. For blank, 70% ethanol is used instead of galangal extract. Inhibition percentage is calculated according to the following equation:

$$\%\ inhibition = \left(\frac{Abs_{(blank)} - Abs_{(sample)}}{Abs_{(blank)}}\right) \times 100$$

A standard curve was obtained with synthetic antioxidant Trolox standards (0 – 0.30 mM). The resulting % inhibition of each sample was compared to that of calibrated standard curve. Antioxidant activity was reported as µmol Trolox equivalent antioxidant capacity (TEAC) per gram dry weight (DW).

2.6 FRAP assay

Ferric reducing antioxidant power (FRAP) was assessed according to Benzie and Strain [10]. FRAP reagent was prepared by mixing 300 mM acetate buffer pH 3.6, 20 mM ferric chloride and 10 mM TPTZ in 40 mM HCl in a volume ratio of 10 : 1 : 1. Three mL of freshly prepared FRAP reagent was then added to 0.15 mL diluted galangal extract. After incubating at 37 °C for 10 min., absorbance was measured at 593 nm. For blank, 70% ethanol was used instead of galangal extract. Trolox, a water-soluble analog of vitamin E, at concentrations of 0 – 0.30 mM was used as a reference to plot a standard curve. The resulting absorbance of each sample was compared to that of the standard curve. Antioxidant activity or Trolox equivalent antioxidant capacity (TEAC) was reported as μmol Trolox per gram DW.

2.7 Statistical analysis

All experiments were carried out in duplicates and three repeated samples were subjected in colorimetric assays. The data ware analyzed using ANOVA (analysis of variance) and Tukey HSD at 95% confidence interval. Correlation coefficient of the two assays was calculated using Microsoft Excel software in Microsoft 365 App for enterprise.

3. Results

3.1. Effect of cooking methods on antioxidant activity

From our study, 15 g constant weight of galangal rhizome was obtained from 188.53 g fresh rhizome. Thus, moisture content was approx. 92%. However, in different processed galangal samples, moisture contents would be different. To avoid the factor of moisture difference in each processed sample, all galangal samples were begun from the same weight of fresh galangal and the values were reported on dry weight basis.

Table 1 shows antioxidant activities assayed by ABTS method and FRAP method. Ethanolic extract of fresh galangal contained 49.70 and 48.93 μmol Trolox per g DW, assayed by ABTS and FRAP, respectively.

Table 1. The antioxidant activities of fresh and processed galangal extract assayed by ABTS and FRAP methods

Ethanolic extract	TEAC (μmol Trolox/ g DW)	
	ABTS[1]	**FRAP**[1]
Fresh galangal	49.70 ± 3.44 [b]	48.94 ± 0.28 [b]
Boiled galangal	92.97 ± 5.07 [c]	74.39 ± 1.04[c]
Galangal broth	41.63 ± 3.40 [a]	20.18 ± 0.24 [a]
Dried galangal at 40°C	104.16 ± 3.40 [d]	87.34 ± 0.92 [d]
Dried galangal at 60°C	135.80 ± 4.04 [e]	103.24 ± 2.36 [e]

[1] Different letter within the same column denotes statistically significant difference at P value < 0.05.

Comparing the equivalent amount of galangal reported as a dry weight basis, antioxidant activities of all galangal extracts in the same assay were significantly different as shown in Table 1. Processed galangal extracts had higher antioxidant activities (1.52 - 2.73 times) than a fresh counterpart, except galangal broth containing lower activity.

3.2. Effect of assay methods on antioxidant activity

There are two mechanisms of antioxidants to break the reaction chain of free radicals by hydrogen atom transfer (HAT) and single electron transfer (SET). FRAP is an example of assay based on SET mechanism while ABTS involves both HAT and SET [11].

ABTS method is based on inhibition of production of ABTS radical cation in aqueous medium. The ability of hydrogen-donating antioxidants to scavenge ABTS $^{+\bullet}$ is exhibited by lowering the absorbance. Inhibition percentage is correlated to TEAC using a standard curve. While FRAP method is based on absorbance measurement of intensely blue colored Fe (II) complex produced by reduction of corresponding tripyridyltriazine Fe (III) complex in acidic medium. The reducing capacity, thus, reflects the total antioxidant capable to transferring electron [11,12].

Figure 2 shows comparative antioxidant activities assessed by two assays. Slightly lower antioxidant activities by FRAP method were found in every processed sample of galangal. Even though the TEAC values were significantly different in every sample except fresh galangal, two methods followed the same trend in every sample with high correlation coefficient of 0.965.

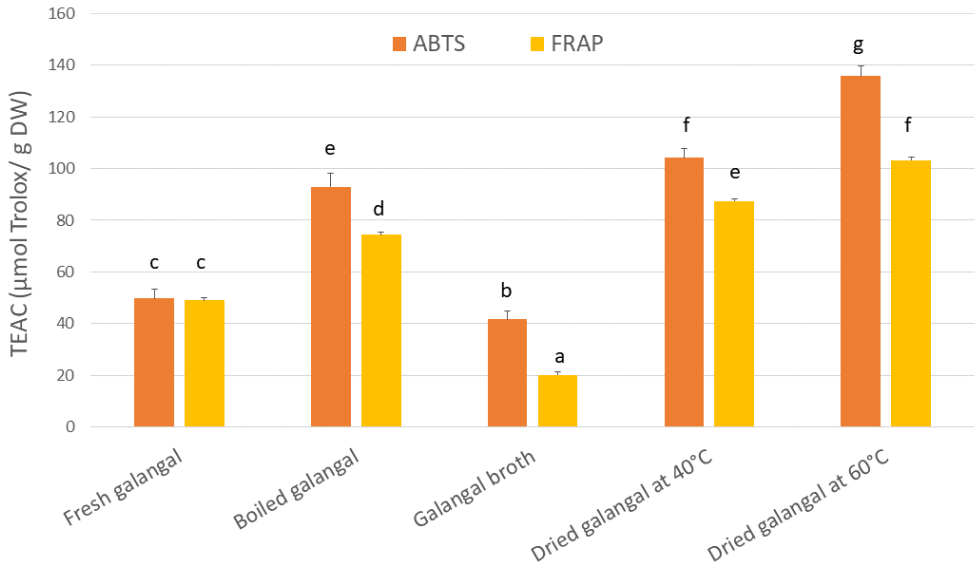


Figure 2. Comparative antioxidant activities of fresh and processed galangal extract assayed by ABTS and FRAP methods. All data regardless the assay methods and sample preparation methods were statistically analyzed. Different letter above each bar denotes statistically significant difference at P value < 0.05.

4. Discussion

Galangal is a rich source of antioxidants. Antioxidative activities of fresh galangal ethanol extract were found to be 49.70 and 48.93 µmol Trolox per g DW, assayed by ABTS and FRAP, respectively (Table 1). These were higher than values reported by Halee and Ratanapun [13] where the antioxidant activities of fresh galangal methanolic extract were reported to be 7.75 and 10.31 µmol Trolox per g DW, respectively. Besides using different solvent to extract the plant, the difference could be due to different cultivating area. They used galangal bought from Kumpheang Phet province [13]. In another previous study, the ethanolic extract of galangal was demonstrated to contain highest antioxidant activity compared with water extract and the essential oil [14]. It can be implied that active compounds in galangal were moderately polar since they were preferably extracted by ethanol. Previous work has shown the strong correlation between antioxidant capacity and total phenolic content in plants, including galangal [15,16]. In galangal ethanolic extract, phenolic components capable of being antioxidant were reported to be catechin, myricetin, *p*-coumaric acid and two other unknown substances [14].

Regarding to processing methods, higher antioxidant activities were found in almost all processed galangal ethanolic extracts (Table 1). In oven-dried ginger rhizome (60 °C

for 19 hours), related species to galangal, higher antioxidant activity was also noticed [17]. It was suggested that, during drying process, polyphenol oxidase was possibly inactivated such that phenolic compounds were not readily oxidized. In addition, heat treatment could cause cell wall destruction [18]. Total phenolic content, consequently, was higher compared to fresh counterpart. Heating, however, affected positively and negatively on carotenoids and phenolic content and consequently on antioxidant capacity in orange maize depending on heating time and moisture content [19]. Antioxidant activities by ABTS method and FRAP were reported to be 593.90 and 771.00 μmol Trolox/g DW in freeze-dried galangal [15]. These higher antioxidant activities might result from different drying method. Freeze drying was reported to cause cell disruption during pre-freezing step, thus release cellular component more effectively [17].

However, galangal broth contained less antioxidant activities than fresh counterpart, i.e. 0.41 (FRAP method) and 0.84 (ABTS method) times of fresh galangal extracted with 70% ethanol. This is supported by previous study, in which water extract contained antioxidant activity 0.84 times of 50% ethanol extract (assayed by ORAC or oxygen radical absorbance capacity method) [14]. When used in making Tom Yum or Tom Kha Gai, boiled galangal pieces would contain higher antioxidant activity than the soup. To encourage consuming boiled galangal, instead of just sipping soup, galangal should be sliced and cut into small and thin pieces. In addition, galangal slice could be added into cocktail recipe resembling ethanol extraction.

In this study two antioxidant assay methods based on different mechanisms were used. FRAP is based on SET mechanism while ABTS involves both HAT and SET [11]. ABTS assessed significantly higher antioxidant activities in all processed galangal than FRAP method (Figure 2). This implied that compounds capable of being antioxidants, which needed to be characterized in further experiments, acted via both mechanisms. Despite the difference in absolute value, the two methods showed the strong correlation in agreement with previous work performed in 19 splices commonly consumed in China [15]. Considering the precision of technical step, FRAP method is more reliable since there is no need to prepare radical as in ABTS method.

5. Conclusions

In this work, ethanolic extract of galangal rhizome exhibited reasonably high level of antioxidant activity. When culinarily used, boiling resulted in higher antioxidant activity if the boiled galangal was consumed. However, in soup, antioxidant was lower. Alternatively, dried galangal could be used in cooking as it contained even higher antioxidant than fresh counterpart. Bioactive compounds in galangal were capable of being antioxidants act via both single electron transfer and hydrogen atom transfer mechanisms.

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Conflicts of Interest: The authors declare no conflict of interest.

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