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Comparison of antioxidant components and antioxidant capacity in different parts of nutmeg *(Myristica fragrans)*

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Abstract

This study aimed to determine and compare antioxidant components and antioxidant capacity in different parts (skin, pulp, mace and seed) of nutmeg. Freeze dried samples were extracted using 80% methanol, while Folin-Ciocalteu assay was employed to determine total phenolic content, aluminium chloride assay was applied to determine total flavonoid content and ascorbic acid was assessed by titrimetric method. Antioxidant activities were evaluated by ferric reducing antioxidant power and trolox equivalent antioxidant capacity (TEAC) assays. Results revealed that nutmeg seed contained the highest TPC followed by mace, skin and pulp. Similar observation was also found for TFC. The highest ascorbic acid content was found in nutmeg mace, while the lowest was in its pulp. For antioxidant activity, nutmeg seed possessed the highest FRAP and TEAC values, while nutmeg pulp had the lowest as compared to other parts. Phenolic compounds in nutmeg samples have exhibited strong correlation with antioxidant capacity. Therefore, nutmeg is a potential functional food with high antioxidants, especially nutmeg seed. Phenolic compounds in nutmeg samples have exhibited strong correlation with antioxidant capacity. Therefore, nutmeg is a potential functional food with high antioxidants, especially nutmeg seed.

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Introduction

Fruits and vegetable are sources rich in antioxidants good for prevention and treatment of diseases such as cancer and cardiovascular diseases. Nutmeg (*Myristica fragrans* Houtt.) is an evergreen tree, has also been supported with its strong antioxidant activity attributed to the phytochemicals present naturally (Bamidele *et al*., 2011).

Nutmeg is a tropical fruit native to Banda Island but also cultivated in Penang Island (Weiss, 2002). Skin, pulp, mace and seed are parts of nutmeg which have been widely used as traditional Ayurvedic, Chinese and Thai medicine (Somani et al., 2008).

Epidemiological evidences have supported the consumption of fruits to reduction of oxidative stress-related diseases. Nutmeg has been shown to possess strong antioxidant activities, act as good preservative agent and offer benefits in some medical treatments.

Exploration of antioxidant properties in nutmeg is significant since consumption of plant-based food is favorable for reduction of oxidative-stress related diseases (Carlsen *et al*., 2010). Thus, this study aimed to determine and compare the antioxidant component and capacity in different parts of nutmeg.

Materials and Methods

Preparation of nutmeg extract

The mature fruit of nutmeg were purchased from a nutmeg orchard in Balik Pulau, Penang, Malaysia using convenience sampling and transported the same day to laboratory. The fruit were washed (distilled water), skin was cautiously peeled while pulp and mace were separated from the seed. The parts were cut into small pieces and frozen at –80°C for a day before being freeze dried. Each part of the fruits was grinded into fine powder. The powder was kept to constant weights in a desiccator at room temperature (23°C) (Cai *et al*., 2004). Sample extracts were prepared by adding 2 g of the dried sample into 50 ml of 80% methanol in a conical flask and kept at room temperature overnight, shaken on an orbital shaker at 50 rpm. The extract was filtered through Whatman filter paper no.1 under vacuum and filtrate was stored at –80°C until used.

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Determination of antioxidant components

Total phenolic content was determined by adopting Folin-Ciocalteu method (Velioglu *et al*., 1998). Basically, 0.2 ml of extracts was added with 1.5 ml of Folin-Ciocalteau reagent and mixture was allowed to stand at room temperature for 5 min. Then, 1.5 ml of sodium carbonate solution (6%) was added into the mixture. Absorbance was measured using spectrophotometer at 725 nm after 90 min standing at room temperature. Results were expressed as gallic acid equivalent (GAE) in mg per 100 g dry weight (DW).

Aluminium chloride method was selected in determining total flavonoid content. The assay was adopted with minor modification (Zhisen *et al*., 1999; Liu *et al*., 2007). Briefly, 2 ml of sample extract was added with 10 ml of 80% methanol and 1 ml of 5% sodium nitrite. After 5 min, the solution was added with 1 ml 10% aluminium chloride and allowed to stand for 6 min. Next, 10 ml of 1 M sodium hydroxide and 1 ml of 80% methanol was mixed thoroughly using vortex. The absorbance was measured using spectrophotometer at 510 nm. Rutin was used to make the calibration curve and the flavonoids content was expressed as milligram rutin equivalent (RE)/g DW.

The ascorbic acid content was determined using AOAC titration method (AOAC, 1990). Firstly, 200 g of sample was weighed and an equal volume of 6% metaphosphoric acid was blended to the homogenous slurry. Next, 10 ml of the homogenous mixture was measured and transferred into a 100 ml volumetric flask. Next, the mixture was diluted to 100 ml with 3% metaphosphoric acid. Then, the solution was centrifuged at 4000 rpm for 12 min. Then, 10 ml of the supernatant was transferred into Erlenmeyer flask and titrated immediately with a standard solution of DCPIP to a faint pink end point which persisted for 15 s. The ascorbic acid content was calculated in mg per 100 g sample based on equation 1.

$$\text{Acid ascorbic} = \frac{X \times Y \times 100}{W} \quad (1)$$

where;

X = ml dye used for titration of aliquot of diluted sample

Y = ascorbic acid equivalent of dye solution expressed as mg/ml dye solution

W = weight in g of sample in aliquot of the filtrate of diluted sample used for titration

Determination of antioxidant capacity

Total antioxidant potential of sample was determined using ferric reducing antioxidant power (FRAP) assay by Benzie and Strain (1996) and Surveswaran *et al*. (2006). The assay was based on the reducing power of an antioxidant compound. The FRAP reagent was prepared by adding 10 volume of 300 mM acetate buffer at pH 3.6, 1 volume of 10 mM TPTZ prepared in 40 mM hydrochloric acid and 1 volume of 20 mM ferrous chloride. The mixture was diluted to 1/3 with 80% methanol and pre-warmed at 37°C for 10 min. Then, 3 ml of FRAP reagent was mixed with 0.1 ml of sample extract. The mixture was shaken and incubated at 37°C for 8 min and the absorbance was read at 593 nm. A standard curve was made with Trolox equivalents (TE)/g DW of sample.

Determination of radical scavenging activity was carried out using the ABTS method or trolox equivalent antioxidant capacity (TEAC) by Re *et al*. (1999) and Lu *et al*. (2010). ABTS was dissolved in water to a concentration of 7 mM. ABTS radical cation ($ABTS^{\bullet+}$) was prepared by addition of the ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark room for 16 h before use. The $ABTS^{\bullet+}$ solution was diluted with 80% ethanol to an absorbance of 0.700±0.02 at 734 nm. The filtered sample was diluted with 80% methanol to give 20–80% inhibition of the blank absorbance with 0.1 ml of the sample. The $ABTS^{\bullet+}$ solution (3.9 ml, absorbance of 0.700±0.02) was added to 0.1 ml of samples and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6 min, and the absorbance was immediately recorded at 734 nm using a UV spectrophotometer. The absorbance of the resulting oxidized solution was compared to that of Trolox standard. Results were expressed as µmol TE per one gram DW of the powders.

Statistical analysis

Data was presented as mean ± standard deviation of triplicates. SPSS version 15.0 was used for statistical analysis. One-way ANOVA (Tukey's test) was used to determine the significance difference for the different parts of nutmeg. Pearson correlation was used to determine the correlation between antioxidant components and antioxidant capacity. Significance differences were set at $p<0.05$.

Results and Discussion

Antioxidant properties

Generally, yield and antioxidant activities of extracts are relying on nature of the extracting solvent due to the presence of distinct antioxidant compounds with different chemical characteristics and polarities Polar solvent such as 80% methanol was employed for recovery of polyphenol compounds from the nutmeg

Table 1. Antioxidant components and capacities in different parts of nutmeg

Parts of fruit	TPC (mg GAE/g DW)	TFC (mg RE/g DW)	AAC (mg/100 g)	FRAP (μmol TE/g DW)	TEAC (μmol TE/g DW)
Skin	114.47±6.3[a]	306.30±5.53[a]	2.86±0.08[a]	28.07±0.02[a]	50.02±1.15[a]
Pulp	25.26±0.79[b]	117.24±9.64[b]	2.05±0.03[b]	13.04±0.25[a]	48.22±0.30[a]
Mace	37.39±0.40[c]	245.50±13.18[c]	2.85±0.02[c]	18.19±0.27[a]	49.79±1.04[a]
Seed	18.80±0.39[d]	1345.75±18.26[d]	2.45±0.24[c]	190.83±17.33[b]	50.99±0.23[b]

Values are expressed as mean ± standard deviation. Different alphabet indicates significant difference at the level of $p<0.05$. DW: dry weight

Table 2. Pearson's correlation (r2) between analysis parameters

	TPC	TFC	AAC
FRAP assay	0.972**	0.991**	–0.096
TEAC assay	0.656*	0.667**	0.507*

** Correlation was significant at the 0.01 level (1-tailed).
* Correlation was significant at the 0.05 level (1-tailed).

matrix (Sultana *et al*., 2009). Polyphenols compound had previously been demonstrated with its water soluble components attributed to its chemical structure properties (Fuhrman and Aviram, 2006). According to Abdullah (2009), the extraction of polyphenols in nutmeg was optimized by using 80% methanol along with its high antioxidant capacities compared with hexane, chloroform and 70% acetone.

From the determination of phenol content (TPC), seed extract contained the highest amount of total phenolic content followed by mace, skin and pulp extracts (Table 1). The major types of total phenolic content found in nutmeg were largely derived from essential oils and lignans. Nutmeg essential oils contained sabinene, safrole, terpinen-4-ol, elemicin and myristicin while Myrisfragransin was the lignan in nutmeg. Lignan were the phenolic compounds through the formation of two cinnamic acids (Thompson, 1998). As for total flavonoid content (TFC), it was found that the seed possessed the highest amount of TFC followed by the skin. The pulp appeared to have the lowest TFC while the mace remained the third high in TFC value.

In the family of *Myristicaceae* species, the abundant flavonoid compounds retrieved were mostly chalcones, flavanoes, flavones, dihydroflavanols, flavonols, flavans, flavan-3-ols, virolanes, virolanols, dihydrochalcones, isoflavones and pterocarpans (Valderrama, 2000). However, the high TFC found in seed of nutmeg was due to the epicatechin which was also a type of flavonoid, normally found in teas, wine and chocolate (Gopalarkishnan and Mathew, 1983; Preedy, 2009). It is among the class of flavan-3-ols with the molecular formula of $C_{15}H_{14}O_6$, which may prevent substances in the bloodstream from oxidizing and clogging the arteries, known as heart-healthy flavonols (Preedy, 2009).

Ascorbic acid content (AAC) was found to be low in all parts of nutmeg. Each parts of nutmeg had relatively the same amount of AAC apparently. Actually, the level of vitamin C in fruits was attributed by the synthesis of sugar in fruits. Ascorbic acid was formed from sugar during photosynthesis. Therefore, plants which received the highest amount of sunlight would be able to form more ascorbic acid and contributed to higher content of vitamin C (Okiei *et al*., 2009). Nutmeg is not naturally sweet in taste, causing the low AAC in its fruit.

In determining the antioxidant capacity, seed extract appeared to have the highest FRAP and TEAC values. This showed that seed had the greatest reducing property and radical scavenging ability compared to other parts of nutmeg. Antioxidant properties of fruit were contributed by various phytochemicals, mainly vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans, simple phenols and phenolic acids. The antioxidant property is highly relied on their redox properties and chemical structure (the number and position of hydroxyl group) (Gupta and Rajpurohit, 2011). According to Shan *et al*. (2005), the high antioxidant activity in nutmeg seed was contributed by caffic acid and catechin. These two groups are good antioxidants due to their catechol structure which is able to donate phenolic hydrogen or electrons to acceptors such as reactive oxygen species or lipid peroxyl groups easily.

Correlation analysis

Pearson's correlation test revealed significant positive correlations exist between TPC, TFC, FRAP and TEAC assays ($p<0.05$). AAC exhibited significant correlation with TEAC assay but no significant correlation was observed for AAC and FRAP assay. It can be deduced that phenolic compounds were the main contributors to the antioxidant activity in nutmeg parts.

Conclusion

Nutmeg is a fruit rich in phenolic constituents and demonstrated good antioxidant capacity with highest level found in its seed but in inconsistent manner for the skin, pulp and mace. Nutmeg is potentially used for supplement and pharmaceutical exploration due to its high antioxidant properties, economical price and readily available. Non-spectrophotometric methods such as HPLC and gas chromatography are recommended for future analysis of nutmeg to reveal the embedded antioxidants. The use of aqueous ethanol (80%) is recommended as it is non-toxic to human.

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