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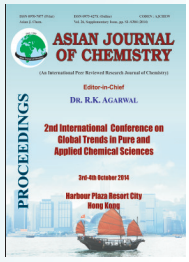

Antioxidant Activity of Ethanolic Extract in Different Parts of Nutmeg (*Myristica fragrants*)

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Myristica fragrants (nutmeg) is a tropical fruit in Banda Island. It is also used to cultivate in the South of Thailand. It is used as conventional medicine for disease prevention and treatment. However, there are no research studies about its antioxidant activities using ethanol solvent. The total phenolic content and antioxidant capacity in different parts of ethanolic extract (pulp, mace and seed) from nutmeg were evaluated. Dried samples were extracted using 95 % ethanol, while Folin-Ciocalteu assay was employed to determine total phenolic content. Whereas, antioxidant activities were determined by ferric reducing antioxidant power and DPPH assay. The results revealed that the nutmeg seed contained the highest total phenolic content, which was 465.91 ± 03 mg GAE/100 g DW. For antioxidant activity, the nutmeg seed possessed the highest inhibited DPPH (IC_{50} = 4.6 ± 15 mg/L) and the highest ferric reducing antioxidant power values (542.39 ± 22 µmol TE/100 gDW), which were significantly higher than reference standard. Phenolic compounds in nutmeg samples have exhibited strong correlation with antioxidant capacity. Therefore, nutmeg, especially nutmeg seed is potentially functional food with high antioxidants.

Keywords: Antioxidant activity, DPPH, Ferric reducing antioxidant power, Nutmeg, Total phenolic content.

INTRODUCTION

Fruit and vegetables are rich sources of antioxidants which can be applied to prevent and treat for many diseases such as cancer and cardiovascular diseases, respectively. *Myristica fragment* Houtt. or nutmeg is an aromatic evergreen tree of plant in the family Myristicaceae and commonly found in Asian medicinal ingredient[1-4]. Nutmeg is a tropical native in Banda Island and cultivated in Penang Island[5]. Moreover, they are also found in the South of Thailand[6,7]. Nutmeg can cause miscarriage in human was reported in the 19th century. Therefore, the poisoning of nutmeg has been presented in diverse cases. However, the crude of nutmeg has no approved medicinal value before used as folk medicine for illness treatment[8].

There are many parts of nutmeg including skin, pulp, mace and seed that have been widely used as traditional Ayurvedic, Chinese and Thai medicine[9]. The characteristics of these parts are shown in Fig. 1.

The fresh fruit is quite wet and the encrusted seed which is red lamella, is called "mace". There is one nutmeg seed which is brown and angiocarpous. Its flavor is so strange and it is sweet and hot.

Generally, antioxidant activity of extracts is important on nature of the extracting solvent due to the presence of antioxidant compounds with different chemical characteristics and polar solvent. For example, 80 % methanol was employed for recovery of polyphenol compounds from the nutmeg matrix[10]. The polyphenols compounds had previously been proved with water soluble components attributed to its chemical structure properties[11].

In this study 95 % ethanol was used for the extraction of polyphenols from the pulp, mace and seed of *M. fragrants* (Nutmeg). Total phenolic content of ethanolic extracts was investigated by using Folin-Ciocalteu reagent and antioxidant capacity was determined by using DPPH assayed and ferric reducing antioxidant power assayed. The relationship between total phenolic content and antioxidant capacity was also studied.

EXPERIMENTAL

Preparation of nutmeg extract: The different part of nutmeg *i.e.*, pulp, mace and seed were purchased from Ban-Ronna, Ronpiboon District, Nakhon Si Thammarat Province, Thailand. The fruits were washed with distillation water and skin was cautiously peeled while pulp and mace were separated from the seed. The parts were cut into small pieces and were dried at 50 °C for 24 h. Sample extracts were prepared by

(a) (b) (c)

Fig. 1. Different parts of nutmeg (a) fruit (b) mace (c) seed

adding 10 g of the sample into 70 mL of 95 % ethanol in beaker and kept at a room temperature overnight. The extract was filtered through Whatman filter paper no. 1 and filtrate was stored at -6 °C until used.

DPPH radical scavenging activity: The DPPH radical scavenging activity was determined according to Mahakunakorn *et al.*[12]. The initial concentration of DPPH redical was 0.5 mM in all the antioxidant redical reaction. For each ethanolic extract, five different concentrations ranging from 10 to 0.3125 ppm were tested. The absorbance at 515 nm was measured against a blank of pure 95 % ethanol. Ascrobic acid was used as a standard compound. Inhibition of DPPH redical was caculated as follows:

$$\text{DPPH scavenging effect} = [A_0 - A_1/A_0] \times 100$$

whereas A_0 and A_1 are the absorbance of control and the sample, respectively.

Ferric reducing antioxidant power assay: Ferric reducing antioxidant power was determined in sample extracts according to Benzie and Strain[13]. This method is based on the ability of the sample to reduce Fe^{3+} to Fe^{2+} ions. In the presence of TPTZ (2,4,6-tripyridyl-*s*-triazine), the Fe^{2+}-TPTZ complex exhibits blue color which is read at 593 nm. Briefly, 3 mL of working ferric reducing antioxidant power reagent was added to appropriate concentration of the sample extract in acetate buffer. After incubation for 10 min at a room temperature, the absorbance was measured at 593 nm against $FeSO_4$ as a standard.

Determination of total phenolic content: Total soluble phenolic compounds (TPC) were determined in sample extracts using the Folin-Ciocalteu reagent and the values are expressed as equivalents of gallic acid, which is most commonly used as a standard in phenolic estimations[14].

The 0.1 mL of samples was introduced to test cuvettes and then 0.3 mL of Folin Ciocalteu's reagent and 2 mL of Na_2CO_3 (15 %) were added. The absorbance of all samples was measured at 765 nm. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of dry weight.

Statistical analysis: Data were expressed on dry weight basis and were presented as mean ± SD. Data were subjected to statistical analysis (correlation between AOA and total phenolic content) using SPSS 14.0 package. Peason's correlation was used to determine the correlation between antioxidant capacity and phenol content. Significance differences were set at $p < 0.05$.

RESULTS AND DISCUSSION

Antioxidant properties: The 95 % of aqueous ethanol was used to solvent for extracting the different parts of nutmeg. This solvent is highly polar and non-toxic. In previous studies, there are not any research studies about antioxidant activities of nutmeg in ethanol solvent[15].

For determination of the antioxidant capacity, the results showed that seed extract has the highest ferric reducing antioxidant power and inhibition values, which were significantly higher than reference standard. Ferric reducing antioxidant analysis showed that the seed had the greatest reducing property and DPPH radical scavenging ability followed by mace and pulp extracts (Table-1). The antioxidant property is highly relied on their redox properties and chemical structure (the number and position of hydroxyl group)[16]. According to Shan *et al.*[17], the high antioxidant activity in nutmeg seed was contributed by caffic acid and catechin. These both groups are good antioxidant activities 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.

TABLE-1
ANTIOXIDANT COMPONENTS AND
CAPACITIES IN DIFFERENT PARTS OF NUTMEG

Parts of nutmeg	Antioxidant activity		Phenol content
	DPPH	Ferric reducing antioxidant power	Total phenolic content
	% Inhibition (IC_{50} mg/L)	µ mol TE/100 gDW	mg GAE/100 gDW
Pulp	52.41 (5.62±09)	522.21±1.1	298.19±0.8
Mace	59.28 (4.88±11)	537.83±0.8	343.73±1.6
Seed	63.13 (4.60±15)	542.39±0.2	465.91±0.3

From the determination of phenol content (TPC), the seed extract contained the highest amount of total phenolic content followed by mace and pulp extracts (Table-1). This result was not similar trend of Tan *et al.*[15], which methanolic extract of the different parts showed the highest total phenolic content in nutmeg mace while nutmeg seed had the highest ferric reducing antioxidant value.

Since abundant literature from other parts of the world indicated phenolic compounds to be important antioxidants in plant food, the total phenolic content was also determined in the present study and correlated their antioxidant activity[18,19].

The correlation between antioxidant activity (DPPH and ferric reducing antioxidant power) of the different parts of nutmeg was given in Table-2.

TABLE-2
ANTIOXIDANT ACTIVITY *vs*. TOTAL PHENOLIC CONTENT CORRELATION OF THE DIFFERENT PARTS OF NUTMEG

Correlation	r^2	r^2 (%)
Total phenolic content *vs*. DPPH	0.9134	83.43
Total phenolic content *vs*. ferric reducing antioxidant activity	0.8449	71.39
DPPH *vs*. ferric reducing antioxidant activity	0.9895	97.91

*$p < 0.05$

Pearson's correlation test revealed significant positive correlation exist between total phenolic content DPPH and ferric reducing antioxidant power assay ($p < 0.05$). It can be deduced that phenolic compounds are the main contributors to the antioxidant activity in the parts of ethanolic nutmeg extracts.

Conclusion

Nutmeg is a rich phenolic fruit and demonstrates a good antioxidant capacity with highest level found in nutmeg seed followed by mace and pulp, respectively. Ethanolic extract of nutmeg exhibited significant positive correlation exist between antioxidant activity (DPPH and ferric reducing antioxidant power) and total phenolic content. Therefore, nutmeg, especially nutmeg seed is potentially functional food with high antioxidants.

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