The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2023), 47(1), 95-109

ORIGINAL RESEARCH PAPER

IN VITRO ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF MACE FROM *MYRISTICA FRAGRANS* HOUTT

HASBULLAH[1, 2], DIDAH NUR FARIDAH[1, 3], DIAS INDRASTI[1, 3], FITRIYA NUR ANNISA DEWI[4, 5], NURI ANDARWULAN[1, 3*]

[1]*Department of Food Science and Technology, IPB University, Bogor, Indonesia.*
[2]*Faculty of Agriculture, Khairun University, Ternate, Indonesia.*
[3]*South-East Asia Food and Agricultural Science and Technology (SEAFAST) Center, IPB University, Bogor, Indonesia.*
[4]*School of Veterinary Medicine and Biomedical Science, IPB University, Bogor, Indonesia.*
[5]*Primate Research Center, IPB University, Bogor, Indonesia.*
[*]Corresponding author: andarwulan@apps.ipb.ac.id

Received on 31 January 2023
Revised on 8 May 2023

Abstract

This research investigated the antioxidant and antidiabetic properties of mace from nutmeg (*Myristica fragrans* Houtt) using in vitro experiments. The nutmeg samples, including distilled water extract (EA), methanol extract (EM), methanol fraction (FM), hexane fraction (FH), and chloroform fraction (FC), were analysed for yield, total phenolic content, antioxidant activity (using *1,1-diphenyl-2-picryl-hydrazyl radical scavenging activity*-DPPH RSA and ferric reducing antioxidant power-FRAP), and α-amylase inhibitory activity. The highest yield was found in EM (25.39±0.62% db), while the highest phenolic content was achieved in FC (127.18±3.64 mg GAE/g dry fraction). In terms of antioxidant, the highest activity by DPPH assay was found in FM phenolic (IC_{50}=2.56±0.02 mg/L) and FC phenolic (IC_{50}=2.79±0.05 mg/L), while EA phenolic demonstrated the highest activity using FRAP assay (9.16±0.86 g TE/100 g phenolic). The EA phenolic also showed the greatest inhibition against α-amylase (IC_{50}=360.18±6.83 mg/L). A positive correlation was found between the ability to reduce the ferric ion of mace samples and its α-amylase inhibitory activity. The results suggest that phenolic from nutmeg mace extract/fraction of obviously exerted antioxidant activity (4.9 times higher than ascorbic acid) and antidiabetic activity (4.2 times higher than acarbose). For this reason, the mace of nutmeg (*Myristica fragrans* Houtt) can be a considerable source of natural antioxidants and antidiabetic.

Keywords: amylase, diabetes mellitus, DPPH, FRAP, hyperglycaemia

https://doi.org/10.35219/foodtechnology.2023.1.06

Introduction

Diabetes mellitus (DM) has a high prevalence over the globe, estimated to increase from 14.6% to 16.9% by 2030. The incidence is predicted to double in 2034 (Alshammari *et al*., 2020). The excessive carbohydrate intake is believed to be a major cause of hyperglycaemia which is closely associated to DM. Postprandial hyperglycaemia represents a condition in which the level of blood glucose remains high after a meal (Poovitha and Parani, 2016). Such conditions can be reduced by inhibiting the carbohydrate digestion in digestive tracts. To achieve this goal, the use of inhibitors for carbohydrate hydrolysis enzymes (α-amylase and α-glucosidase) could be an effective option. Phytochemical compounds can be an alternative when synthetic inhibitors and antidiabetic agents have unwanted side effects (Ali *et al*., 2006; Tran *et al*., 2020). The oxidative stress, a state showing the imbalance between pro-oxidants and antioxidants, can be a supreme mechanism for the development of DM and its complication. Therefore, by consuming an antioxidant-rich diet, the further stage of DM could be retarded. In general, plants contain bioactive compounds, especially phenolics that act as antioxidant agents (Shan *et al*., 2005). Nutmeg (*M. fragrans* Houtt) comprises phytochemicals that possess pharmacological effects, including antioxidant and antidiabetic (Ha *et al*., 2020; Tran *et al*., 2020).

Nutmeg is considered an indigenous plant of Indonesia, particularly Maluku Islands, and it has a high diversity. The plant has been cultivated in many parts of the world such as India, Malaysia, Sri Lanka, and Caribbean Islands, primarily Grenada and Trinidad (Rema and Krishnamoorthy, 2012; Zhang *et al*., 2015; Santoso *et al*., 2018). Apart from being a spice and flavor enhancer in food, nutmeg has also been used in traditional medicinal practices (Rema and Krishnamoorthy, 2012).

Mace, a part of the nutmeg fruit which has a high economic value besides the seeds and is important in the food, cosmetic and pharmaceutical industries. Mace is an outer cover of nutmeg seed, and it is red in ripe fruit. Traditionally, mace has been utilized as a folk medicine for various diseases such as urinary incontinence, coronary disease, central neurological disease, sexual and womb disorder, and intestinal disease (Sultana *et al*., 2018). The biological activity of mace includes antioxidant, anti-inflammatory, anticancer, anti-cariogenic, hepatoprotective, and osteoblast proliferation stimulation (Nagja *et al*., 2016; Mendhekar *et al*., 2017). Phenolic compounds, primarily phenylpropanoid, lignan, and neolignane, are major chemical constituents in mace and they show bioactivities (Zhang *et al*., 2015). Besides, other phytochemicals are present in mace, including tannin, alkaloid, saponin, flavonoid, steroid/terpenoid, and quinone (Ismiyarto *et al*., 2009; Arrizqiyani *et al*., 2017). Lignans and other phytochemicals can act as antioxidants and antidiabetics especially by controlling the blood glucose levels (Pan *et al*., 2007; Han *et al*., 2008; Xu *et al*., 2008; Katare *et al*., 2012; Meutia, 2013; Malik, 2016; Topal, 2018; Lee *et al*., 2019; Worawalai and Phuwapraisirisan, 2019; Afandi, 2020; Barre and Mizier-Barre, 2020; Zhao *et al*., 2020; Afandi *et al*., 2021; Draganescu *et al*., 2021).

Our systematic review of the effects of nutmeg on the glycaemic status of mice and rats found that the test samples prepared from mace and methanol had the best ability to reduce blood glucose (Hasbullah *et al*., 2023). However, studies exploring mace's antidiabetic effects are still limited. Therefore, the antidiabetic properties of various fractions of mace-methanol extract are interesting for further study. In addition, the antidiabetic activity of mace water extract also needs to be investigated to identify opportunities for the safer application of mace extract into food products.

This research aimed to investigate the in vitro antioxidant and antidiabetic activities of mace from *M. fragrans* Houtt. The yield, total phenolic content, DPPH RSA, FRAP, and α-amylase inhibitory activity of each mace extract and fraction were evaluated. The correlation between each bioactivity was also determined.

Materials and methods

Plant sample preparation

Dried mace was collected from the village of Dorpedu in Ternate City, Province of North Maluku, Indonesia. The species of the plant was identified by Herbarium Bogoriense, Research Center for Botany–Indonesian Institute of Sciences.

The dried mace was pulverized by blender and sieved with a 30-mesh. The powder was immediately packaged in a plastic container (with silica gel) and stored in a freezer.

Determination of moisture content

The moisture content of mace was carried out by a gravimetric method. Aluminium dish was dried in oven at 105°C for 3 h, cooled in a desiccator, and weighed (W2). The mace powder was placed on the dish and weighed (W). The sample was oven-dried at 105°C until it reached constant weight (W1). The moisture content was calculated as follows:

$$\% \text{ Moisture content (db)} = \left[\frac{(W - W2) - (W1 - W2)}{(W1 - W2)}\right] \times 100 \qquad (1)$$

Extraction and fractionation

The extraction was adopted after the method of Aminudin *et al.* (2020) with a slight modification. The mace powder (25 g) was extracted with 250 mL of methanol (Merck, Germany) or distilled water (a ratio of 1:10) and sonicated for 30 min at 30°C. The extract was filtered through V60 filter paper immediately after maceration and sonication. The filtrate was collected and the residue was extracted similarly to the former protocol (2 times extraction). The filtrate was re-filtered by using Whatman No.1 and concentraasted by a Rotavapor® R-300 rotary evaporator (Buchi, Switzerland).

Fractionation was performed using liquid-liquid fractionation using two solvents with different polarities in a funnel. Methanol filtrate (after filtration by Whatman No.1) was fractionated respectively with n-hexane (Merck, Germany) and chloroform (Merck, Germany) (a ratio of 1:1). Each fraction was concentrated with a rotary evaporator.

The dry extract and fraction were obtained by removing the solvent residue through nitrogen flushing (for EM, FH, and FC). Meanwhile, EA and FM samples were dried with a freeze dryer (Labconco, Missouri, USA). The yield of each extract and fraction was determined by the following equation:

$$\text{Yield (\%)} = \frac{\text{weight of dry extract or fraction}}{\text{weight of dried mace}} \quad (2)$$

Total phenolic content determination

The total phenolic content of the mace extracts and fractions was determined by Folin-Ciocalteau method (KC *et al*., 2020) with a slight modification. In brief, 0.25 mL of the samples' solution, 1.25 mL of Folin-Ciocalteu 10%, and 1.25 mL of Na_2CO_3 7.5% were mixed thoroughly and incubated at room temperature for 60 min under dark conditions. The absorbance was measured with a Shimadzu UV-Vis 2450 spectrophotometer (Tokyo, Japan) at the wavelength of 765 nm. The result was plotted on a standard curve of gallic acid, and the phenolic content was expressed as mg of gallic acid equivalent per g of dry extract or fraction (mg GAE/g dry sample).

The sample solution of each extract and fraction were prepared in methanol at a concentration of 1000 mg/L. A blank solution was prepared similarly, but the sample solution was replaced with 0.25 mL of methanol. Analysis was made in triplicates. The standard curve for gallic acid was prepared in a series of concentrations (20–100 mg/L), The concentration level of gallic acid solution and its absorbance value were plotted onto a graph, respectively as the x-axis and y-axis to obtain the standard curve and its linear equation.

In vitro antioxidant activity

DPPH assay is a common method for determining the antioxidant activity (Simamora *et al*., 2018), and it is strongly correlated to the FRAP method (Maesaroh *et al.*, 2018). Both of these in vitro test methods describe the antioxidant activity of a sample through different mechanisms of action and they were used to provide information about the mechanism of antioxidant action of mace extract and fraction samples.

DPPH Assay

The procedure for the DPPH RSA assay was assessed in accordance to the method of Febrinda *et al.* (2013) with some modifications. Each mace extract and fraction were prepared in methanol at various concentrations (5–1000 mg/L). The DPPH, 0.1 mM in methanol (1 mL), was mixed with each solution of extract or fraction (2

mL). The mixture was homogenized using a vortex and incubated at room temperature under dark conditions for 30 min. The absorbance (Abs) was measured at the wavelength of 516.2 nm by a Shimadzu UV-Vis 2450 spectrophotometer (Tokyo, Japan) (the maximum wavelength for DPPH 0.1 nM).

The blank solution consisted of 1 mL DPPH solution and 2 mL of methanol. Ascorbic acid was used as a comparison. The experiment was performed in triplicates. The percentage of inhibition was determined as follows:

$$\% \text{ Inhibition} = [1 - \frac{\text{Abs sample}}{\text{Abs blank}}] \times 100 \quad (3)$$

The antioxidant effect assessed by DPPH RSA was expressed as IC_{50}, the concentration of sample required to scavenge 50% of DPPH radicals (Shekhar and Anju, 2014). The value of IC_{50} was based on the phenolic content of each sample.

Ferric Reducing Antioxidant Power

FRAP assay followed a method prescribed by Fernandes *et al.* (2016) with slight modification. Antioxidant was able to reduce $Fe3^+$ ions and turn them into $Fe2^+$ ions, resulting a blue complex with a presence of *2,4,6-tripyridyl-s-triazine* ($Fe2^+$/TPTZ). This led to the rise of absorbance at 595 nm. Briefly, FRAP reagent (1800 µL), distilled water (180 µL), and sample (20 µL) were added to each well, mixed, and incubated at 37°C. The absorbance was measured at 595 nm, following 30 min of incubation.

FRAP reagent was prepared by mixing buffer acetate (300 mM, pH 3.6), TPTZ 10 mM in HCl 40 mM, and $FeCl_3$ 20 mM at a ratio of 10:1:1 (v/v/v). Each extract or fraction was prepared in ethanol at a concentration of 50 mg/L. Various levels of Trolox were made to obtain the standard curve. The result was expressed as gram Trolox equivalent per 100 g phenolics of sample (g TE/100 g phenolic). The analysis was made at triplicate.

In vitro antidiabetic activity

The inhibition of α-amylase was quantified by using starch-iodine assay *(*Cheng *et al*., 2015) with modification. The mace extract or fraction (25 µL) was mixed with 0.5 mL of starch solution 0.125% in a reaction tube and incubated at 37°C for 5 min. Subsequently, 25 µL of enzyme solution (0.5 mg/mL) was added and incubated at 37°C for 7.5 min. HCl 1 N (0.45 mL) was added to terminate the reaction, then added with iodine 0.01 N (0.5 mL) and distilled water (5 mL). Absorbance was measured using a Shimadzu UV-Vis 2450 spectrophotometer (Tokyo, Japan) at the wavelength of 660 nm. Acarbose was used as a control. Inhibition was determined as follows:

$$\% \text{ Inhibition} = (1 - \frac{\text{Abs blank} - \text{Abs sample}}{\text{Abs blank} - \text{Abs enzyme}}) \times 100 \quad (4)$$

The inhibitory activity was presented as IC_{50}, which was estimated through a regression equation obtained from the standard curve by plotting the concentration of the sample (x-axis) and the percentage of inhibition (y-axis) onto a graph. The IC_{50} value means the amount of sample required to inhibit 50% of amylase activity. All IC_{50} values were based on the total phenolic content of each extract or fraction.

Statistical analysis

All data (performed in triplicate) were analysed statistically on SPSS version 26 and expressed as mean ± SD. The analysis was performed by one-way ANOVA, followed by the Duncan test ($p<0.05$). Pearson's correlation coefficient was used to determine the correlation between IC_{50} DPPH RSA, FRAP, and IC_{50} inhibition of α-amylase. Interpretation of the correlation coefficient followed the method used by Schober and Schwarte (2018).

Results and discussion

Yield and total phenolic content

The yield and total phenolic content of the extract and fraction were presented in Table 1. The yield of EM (25.39±0.62% db) was higher than that of EA (6.31±0.91% db). Meanwhile, the yield of FC (14.93±1.55% db) was higher compared to FM (4.31±0.05% db) and FH (4.16±0.48% db). The difference in the solvents used for the extraction and fractionation was responsible for different levels of yield ($p<0.05$). The yield followed this order: EM>FC>EA>FM>FH.

Table 1. Yield and total phenolic content of extracts and fractions of mace from *M. fragrans* Houtt.

Sample	Yield (% db)	Total Phenolic Content (mg GAE/g dry sample)
EA	6.31±0.91 [b]	24.532.86 [a]
EM	25.39±0.62 [d]	108.51±9.84 [d]
FM	4.31±0.05 [a]	79.85±5.20 [c]
FH	4.16±0.48 [a]	44.75±3.40 [b]
FC	14.93±1.55 [c]	127.18±3.64 [e]

The different letter shows the result are significantly different ($p<0.05$). Legend: chloroform fraction (FC); distilled water extract (EA); dry basis (db); gallic acid equivalents (GAE); methanol extract (EM); methanol fraction (FM); n-hexane fraction (FH).

The yield of mace extract and fraction obtained in this study (4.16–25.39%) was still higher than that obtained from other studies. The yield of mace ethanol extract obtained by the solid liquid microwave assisted extraction method ranges from 3.63%–6.13% (Wijayanti *et al*., 2018). The yield of macerated mace extract using 95°C water was 10.42% (Santoso *et al*., 2018). The yields of mace methanol extract and mace ethyl acetate extract obtained by maceration were 0.048% (w/w)

and 0.218 % (w/w), respectively (Simamora *et al*., 2018). The yield of mace methanol extract obtained from 3 times maceration with methanol during 20 hours was 30.92% ± 1.09 (Assa *et al*., 2014). The yield of mace aqueous methanol (80%, v/v) was 17.4±1.5% (Sulaiman and Ooi, 2012).

The total phenolic content in each of mace extracts and fractions differed significantly ($p<0.05$), ranging from 24.53±2.86–127.18±3.64 mg GAE/g dry samples. In this case, the EM showed the highest content of phenolic (108.51±9.84 mg GAE/g dry extract), significantly different in comparison to EA (24.53±2.86 mg GAE/g dry extract) ($p<0.05$). The polarity of each solvent affects the yield of the extract as well as the amount and type of phenolic compounds it contains. Compared to distilled water, the use of methanol and ethanol was reported more effective in collecting phenolic compounds from plants (Cai *et al*., 2004; Soehendro *et al*., 2015; Simamora *et al*., 2018; Dechayont *et al*., 2019).

Knowing the total phenolic content of samples extracted with various solvents with different polarities may be useful for predicting the dominant group of phenolic compounds in the sample. Hasim *et al.* (2017) reported that dragon fruit (*Hylocereus undatus*) vine contained a considerable amount of semi-polar phenolics since the total phenolic content in the samples extracted by acetone was higher than those extracted by methanol and ethyl acetate. Based on the fractionation result of EM, FC was attributed to the highest total phenolic (127.18±3.64 mg GAE/g dry fraction), being significantly higher ($p<0,05$) than FM and FH, reaching 1.59 and 2.84 times. Based on this finding, mace is richer in semi-polar phenolic compounds. Overall, the total phenolic in both extract and fraction of mace differed, following this order: FC>EM>FM>FH>EA.

The total phenolic content of the mace extract and fraction obtained in this study ranged from 24.53–127.18 mg GAE/g dry sample. In another study, the total phenolic content of mace extract (mg GAE/g dry sample) were 0.37–0.52 (Wijayanti *et al*., 2018), 0.48 (Santoso *et al.,* 2018), 11.56–13.43 (Simamora *et al*., 2018), 37.39 (Tan *et al*., 2013) and 48.2 (Sulaiman and Ooi, 2012).

In vitro antioxidant activities

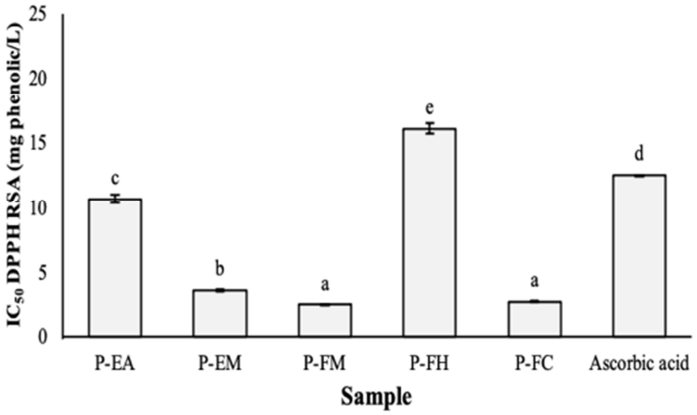
DPPH radical scavenging activity

DPPH assay is a simple, rapid, and affordable method, and applied in many uses, aiming to evaluate antioxidant activity of plant extracts (Banothu *et al*., 2017; Sahreen *et al*., 2017). The transfer of hydrogen from antioxidant to DPPH is a very slow process, and considered a marginal reaction pathway, while the transfer of electron from deprotonated antioxidant to DPPH occurs at a more rapid action which substantially dictates the reaction rate. Therefore, the chemical reaction underlying DPPH assay is based on electron transfer, although it also involves the hydrogen transfer (Banothu *et al*., 2017).

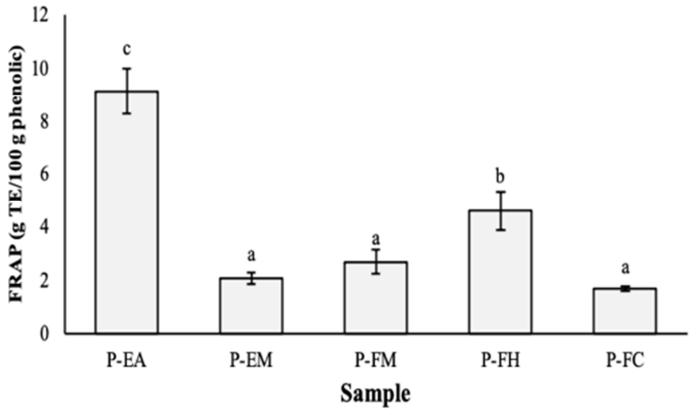
DPPH RSA is expressed as IC_{50}, indicating that lower IC_{50} means more antioxidant effect. Figure 1A shows DPPH RSA of the phenolics from mace extracts and fractions. The phenolic from methanol fraction (P-FM) exhibited the highest DPPH RSA (IC_{50}=2.56±0.02 mg/L), followed by phenolic from chloroform fraction (P-

FC), methanol extract (P-EM), distilled water extract (P-EA), and n-hexane fraction (P-FH), resulting in IC_{50} of 2.79±0.05 mg/L, 3.67±0.05 mg/L, 10.74±0.35 mg/L, and 16.14±0.40 mg/L, respectively. Statistically, the DPPH RSA between P-FM and P-FC did not differ significantly, but both samples were higher than P-EM, P-EA, and P-FH ($p<0.05$). All extracts and fractions (except for P-FH) showed higher levels of DPPH RSA compared to the ascorbic acid (IC_{50}=12.55±0.07 mg/L) ($p<0.05$).

The IC_{50} of DPPH RSA of phenolic from mace extract and fraction in this study ranged from 2.56–16.14 mg/L. Other studies also determined the IC_{50} of DPPH RSA of mace extracts at 1510 mg/L (Santoso *et al.,* 2018), 201.97 mg/L (Assa *et al.,* 2014), and 363.1 mg/L (Sulaiman and Ooi, 2012).



A)



B)

Figure 1. DPPH radical scavenging activity (A) and ferric reducing antioxidant power (B) of phenolic from mace extract and fraction. The different letter shows the result are significantly different ($p<0.05$). Legend: phenolic of distilled water extract (P-EA); phenolic of methanol extract (P-EM); phenolic of chloroform fraction (P-FC); phenolic of methanol fraction (P-FM); phenolic of n-hexane fraction (P-FH).

Some studies have reported a positive correlation between phenolic compounds and antioxidant effect (Shan *et al.*, 2005; Zhou *et al.*, 2009; Yao *et al.*, 2010; Assa *et al.*, 2014; Fernandes *et al.*, 2016; Sahreen *et al.*, 2017; Bhardwaj *et al.*, 2019). Such correlation depends highly on the amount of samples tested, the range of total phenolic content and antioxidant activity, and the test methods used (Cai *et al.*, 2004). The discrepancy in free radical scavenging activity is caused by the stoichiometry of reactions between antioxidant compounds within extract and various radicals. Besides, the diversity might be influenced by other factors including the stereo selectivity of radicals or differential solubility, which in some cases, it occurs in a crude extract containing a myriad of antioxidant constituents.

Ferric Reducing Antioxidant Power

Like the DPPH procedure, FRAP assay is considered as an easy, inexpensive, and quick protocol, but it is often applied to measure antioxidant effect for a simple electron-donating compound (Benzie and Strain, 1996). Figure 1B exhibits the results of FRAP test of extract and fraction of mace. In this work, P-EA showed the highest, and the value of FRAP followed the order: P-EA>P-FH>P-FM>P-EM>P-FC, reaching 9.16±0.86, 4.62±0.73, 2.72±0.44, 2.10±0.22, and 1.71±0.10 g TE/100 g phenolic ($p<0.05$).

Acidic condition in the FRAP test can attenuate the reduction ability of the antioxidant compound due to acid protonation. Therefore, the ionized form of compounds could affect their antioxidant effect (Maesaroh *et al*., 2018). Although many studies reported a significant correlation between phenolic and antioxidant, the antioxidant effect of each phenolic compound relied markedly on the oxidation-reduction reaction and its chemical structure (number and position of hydroxyl groups) (Gupta and Rajpurohit, 2011). Some research studies also reported a significant positive correlation between total phenolics and FRAP (Zhou *et al*., 2009; Banothu *et al*., 2017; Bhardwaj *et al*., 2019).

In vitro α-amylase inhibitory activity

DM can be caused by dysfunction of pancreatic cells and/or insulin resistance with impaired glucose tolerance (Danaei *et al*., 2011). Impaired glucose tolerance may be associated with regularly high postprandial glucose spikes in the blood *(*Livesey *et al*., 2008; Manzano and Williamson, 2010). The main source of postprandial glucose is from hydrolysis of starch, which involves two enzymes: α-amylase and α-glucosidase. Starch is hydrolysed by α-amylase in saliva and pancreas, resulting in maltose and oligosaccharide by cleaving α-1,4 glycosidic bonds (Hanhineva *et al*., 2010; Williamson, 2013). Meanwhile, α-glucosidase in membrane surface of brush-border of intestinal cells hydrolyses oligosaccharides to produce glucose which is then transferred into blood by transporter sodium-dependent glucose transporter type 1 (SGLT1) and glucose transporter type 2 (GLUT2) (Scheepers *et al.*, 2004). DM is also associated with the consumption of high glycaemic index foods. Afandi (2020) found that the upmost determinant factor for the decline of glycaemic index was the inhibition of α-amylase, reducing glycaemic index at 4.5-fold more effective than the inhibition of α-glucosidase.

The α-amylase inhibition by the mace extract and fraction was measured by starch-iodine method (Cheng *et al*., 2015), and presented as IC_{50}. The lower value of IC_{50} indicates the higher inhibitory effect against α-amylase. P-EA had the highest inhibition of α-amylase (IC_{50}=360.18±6.83 mg/L), then followed by P-FM (IC_{50} =435.69±4.46 mg/L), P-EM (IC_{50} =603.12±29.20 mg/L), P-FH (IC_{50} =1021.98±6.12 mg/L) and P-FC (IC_{50}=1575.84±40.21 mg/L), as exhibited in Figure 2. The phenolics from the extract and fraction of e (except for P-FC) showed the higher inhibition against α-amylase, reaching up to 1.47-4.18-fold higher than acarbose (IC_{50}=1504.89±13.32 mg/L), and this differed significantly ($p<0.05$).

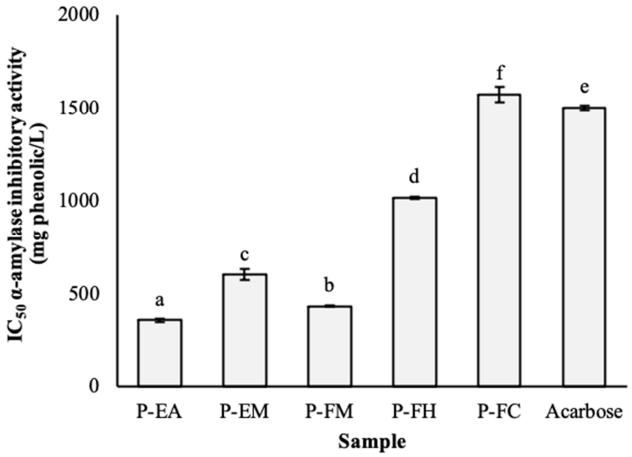


Figure 2. α-amylase inhibitory activity of phenolic from mace extract and fraction. The different letter shows the result are significantly different ($p<0.05$). Legend: phenolic of distilled water extract (P-EA); phenolic of methanol extract (P-EM); phenolic of chloroform fraction (P-FC); phenolic of methanol fraction (P-FM); phenolic of n-hexane fraction (P-FH).

Phenolic compounds were reported as being able to delay the action of α-amylase and α-glucosidase (Afandi *et al*., 2021). The activity of α-amylase inhibition by a particular extract was determined by not only total phenolic, but also types of phenolic compounds (Oboh *et al*., 2014). However, in case of crude extract/fraction, the presence of other chemical constituents might also contribute to α-amylase inhibitory activity.

Correlation between antioxidant and α-amylase inhibitory activity of mace

The scavenging activity of radicals by phenolic of mace extract/fractions showed no positive correlation with the ferric ions reducing. This can be common as different antioxidant test methods can give different results for similar antioxidant compounds (Maesaroh *et al.*, 2018). Additionally, the dissimilarity in bioactivity can be altered by the difference in types and amount of the compounds. Hence,

regarding the current case of the crude extract/fraction, antioxidant properties might result from other chemical components such as vitamin, carotenoid, terpenoid, and alkaloid (Gupta and Rajpurohit, 2011). On the other hand, the value of IC_{50} for α-amylase inhibition by mace extract and fraction weakly correlated with the IC_{50} value of DPPH RSA, thus it could be neglectable. Meanwhile, FRAP value negatively correlated with IC_{50} value of α-amylase inhibition, reaching at moderate (R=-0.491), as depicted in Figure 3. In other words, the ability of the samples to reduce ferric ions is positively correlated with its ability to inhibit the activity of α-amylase in hydrolysing starch. This finding provides a plausible reason that compounds contributing to the α-amylase inhibition are similar to those reducing ferric ions, but dissimilar to those scavenging DPPH radicals. To clarify this, further works should be considered.

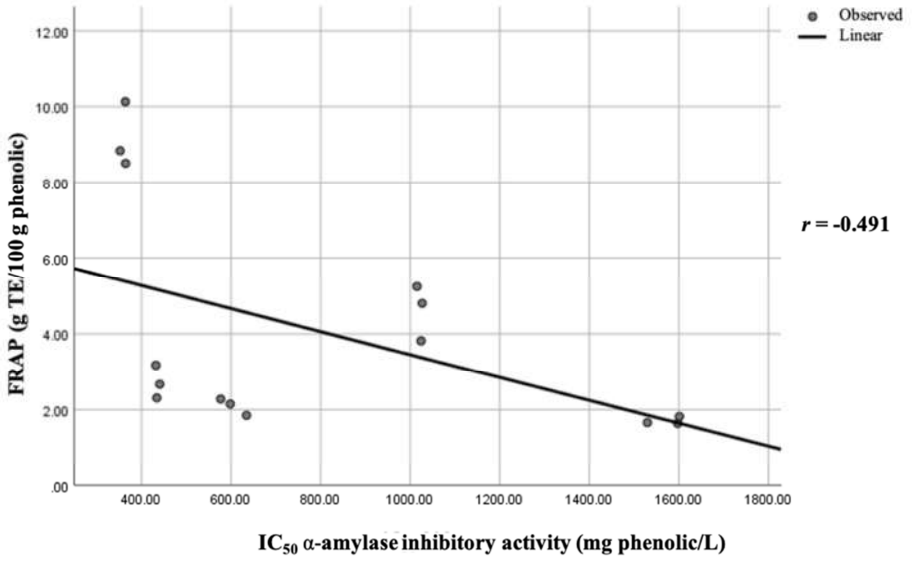


Figure 3. The correlation between FRAP and IC_{50} α-amylase inhibitory activity of phenolic from mace extract/fraction. Significant correlation at the level of 0.05 (2-tailed).

Conclusions

The highest yield was achieved at extraction by methanol. FC sample showed the greatest content of total phenol; thus, it could be estimated that phenolic compounds in nutmeg mace were mostly semi-polar components. DPPH RSA values for phenolic mace extract and fraction (except for P-FH) exceeded ascorbic acid (1.17–4.9 times higher), where the highest activity was possessed by P-FM and P-FC. In terms of FRAP assay, the values ranged from 1.71±0.10–9.16±0.86 g TE/100 g phenolic, and P-EA showed the highest FRAP value. The α-amylase inhibitory activity of mace extracts and fractions (except for P-FH) was higher than acarbose (1.47–4.18 times higher), and P-EA demonstrated the highest. A positive

correlation was found between the reduction of ferric ions and α-amylase inhibition at a moderate level. In general, our experiment concluded that phenolic content in mace could exert antioxidant and antidiabetic effects via in vitro, reaching 4.9 times higher than ascorbic acid and 4.2 times higher than acarbose, respectively. Therefore, the use of mace as a source of natural antioxidants and antidiabetics is quite promising.

Acknowledgments

This study was provided financial support by Directorate General of Higher Education, Research and Technology–Ministry of Education and Culture-Research and Technology Republic of Indonesia through the Doctoral Dissertation Research scheme (grant code 1/E1/KP.PTNBH/2021).

References

Afandi, F.A. 2020. Meta-analysis of Factors Affecting the Glycemic Index of Starch-Based Foods and Its Verification Using Food Model. *Disertation, Institut Pertanian Bogor University*.

Afandi, F.A., Wijaya, C.H., Faridah, D.N., Suyatma, N.E., Jayanegara, A. 2021. Evaluation of Various Starchy Foods: A Systematic Review and Meta-Analysis on Chemical Properties Affecting the Glycemic Index Values Based on In Vitro and In Vivo Experiments. *Foods*, **10**(2), 364.

Ali, H., Houghton, P.J., Soumyanath, A. 2006. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus. *Journal of Ethnopharmacology*, **107**(3), 449–455.

Alshammari, M., Windle, R., Bowskill ,D., Adams, G. 2020. Diabetes treatment guidelines and nurses' adherence to them: A case of the UK and Kuwait. *Nursing & Healthcare International Journal*, **4**(1), 000214.

Aminudin, A., Andarwulan, N., Palupi, N.S., Arifiantini, I. 2020. Characteristics and antioxidant activity of kebar grass (Biophytum petersianum) extract. *Biosaintifika*: *Journal of Biology & Biology Education*, **12**(2), 178–185.

Arrizqiyani, T., Sonjaya, N., Asty, A. 2017. Optimizing the potential of nutmeg plants as antibacterial *Escherichia coli* using the extraction method. *Implementasi Penelitian Dan Pengabdian Masyarakat Untuk Peningkatan Kekayaan Intelektual*, 375–382.

Assa, J.R., Widjanarko, S.B., Kusnadi, J., Berhimpon, S. 2014. Antioxidant potential of flesh, seed and mace of nutmeg (*Myristica fragrans* Houtt). *International Journal of ChemTech Research*, **6**(4), 2460–2468.

Banothu, V., Neelagiri, C., Adepally, U., Lingam, J., Bommareddy, K. 2017. Phytochemical screening and evaluation of in vitro antioxidant and antimicrobial activities of the indigenous medicinal plant Albizia odoratissima. *Pharmaceutical Biology*, **55**(1), 1155–1161.

Barre, D.E., Mizier-Barre, K.A. 2020. Lignans' potential in pre and post-onset type 2 diabetes management. *Current Diabetes Reviews*, **16**(1), 2–11.

Benzie, I.F.F., Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP Assay. *Analytical Biochemistry*, **236**(1), 70–76.

Bhardwaj, P., Thakur, M.S., Kapoor, S., Bhardwaj, A.K., Sharma, A., Saxena, S., Chaurasia, O.P., Kumar, R., Bhardwaj, P., Thakur, M.S., Kapoor, S., Bhardwaj, K., Saxena, S. 2019. Phytochemical Screening and antioxidant activity study of methanol extract of stems and roots of *Codonopsis clematidea* from trans-himalayan region. *Pharmacognosy Journal*, **11**(3), 536–546.

Cai, Y., Luo, Q., Sun, M., Corke, H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences*, **74**(17), 2157–2184.

Cheng, Q., Cai, S., Ni, D., Wang, R., Zhou, F., Ji, B., Chen, Y. 2015. In vitro antioxidant and pancreatic α-amylase inhibitory activity of isolated fractions from water extract of Qingzhuan tea. *Journal of Food Science and Technology*, **52**(2), 928–935.

Danaei, G., Finucane, M.M., Lu, Y., Singh, G.M., Cowan, M.J., Paciorek, C., Lin, J.K., Farzadfar, F., Khang, Y.H., Stevens, G.A., Rao, M., Ali, M.K., Riley, L.M., Robinson, C.A., Ezzati, M. 2011. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: Systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *The Lancet*, **378**(9785), 31–40.

Dechayont, B., Phuaklee, P., Chunthorng-Orn, J., Poomirat, S., Juckmeta, T., Phumlek, K., Mokmued, K., Ouncharoen, K. 2019. Antimicrobial, anti-inflammatory, and antioxidant activities of the wood of *Myristica fragrans*. J*ournal of Herbs, Spices & Medicinal Plants*, **26**(1), 49-60.

Draganescu, D., Andritoiu, C., Hritcu, D., Dodi, G., Popa, M.I. 2021. Flaxseed lignans and polyphenols enhanced activity in streptozotocin-induced diabetic rats. *Biology*, **10**(1), 1–13.

Febrinda, A.E., Astawan, M., Wresdiyati, T., Dewi, Y.N. 2013. Antioxidant and alpha-glucosidase inhibitory properties of *Bawang Dayak* bulb extracts. *Jurnal Teknologi Dan Industri Pangan*, **24**(2), 161–167.

Fernandes, R.P.P., Trindade, M.A., Tonin, F.G., Lima, C.G., Pugine, S.M.P., Munekata, P.E.S., Lorenzo, J.M., de Melo, M.P. 2016. Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. J*ournal of Food Science and Technology*, **53**(1), 451–460.

Gupta, A.D., Rajpurohit, D. 2011. Antioxidant and antimicrobial activity of nutmeg (*Myristica fragrans*). In *Nuts & Seeds in Health and Disease Prevention* (pp. 831–839). Academic Press.

Ha, M.T., Vu, N.K., Tran, T.H., Kim, J.A., Woo, M.H., Min, B.S. 2020. Phytochemical and pharmacological properties of *Myristica fragrans* Houtt.: an updated review. *Archives of Pharmacal Research*, **43**(11), 1067–1092.

Han, K.L., Choi, J.S., Lee, J.Y., Song, J., Joe, M.K., Jung, M.H. 2008. Therapeutic potential of peroxisome proliferators–activated receptor-alpha/gamma dual agonist with alleviation of endoplasmic reticulum stress for the treatment of diabetes. *Diabetes*, **57**(3), 737–745.

Han, K.L., Choi, J.S., Lee, J.Y., Song, J., Joe, M.K., Jung, M.H., Hwang, J.K. 2008. Therapeutic potential of peroxisome proliferators-activated receptor-α/γ dual agonist with alleviation of endoplasmic reticulum stress for the treatment of diabetes. *Diabetes*, **57**(3), 737–745.

Hanhineva, K., Törrönen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkänen, H., Poutanen, K. 2010. Impact of dietary polyphenols on carbohydrate metabolism. I*nternational Journal of Molecular Sciences*, **11**(4), 1365–1402.

Hasbullah, Faridah, D.N., Dewi, F.N.A., Indrasti, D., Andarwulan, N. 2023. Effect of nutmeg on glycemic status in rat and mice: a systematic review. *Food Science and Technology*, **43**(e130122), 1–10.

Hasim, Andrianto, D., Lestari, E.D., Faridah, D.N. 2017. Antioxidant activity of dragon fruit vine extract (*Hylocereus undatus*) by DPPH and Rancimat method. *Journal Gizi dan Pangan*, **12**(3), 203–210.

Ismiyarto, N., Mustika, R. 2009. Isolation, identification of essential oil from nutmeg mace (*Myristica fragrans*) and Activity test as a larvicidal. *Jurnal Kimia Sains Dan Aplikasi*, **12**(1), 23–30.

Katare, C., Saxena, S., Agrawal, S., Prasad, G., Bisen, P.S. 2012. Flax seed : a potential medicinal food. *Journal of Nutrition & Food Sciences*, **2**(1), 1–8.

KC, Y., Parajuli, A., Khatri, B.B., Shiwakoti, L.D. 2020. Phytochemicals and quality of green and black teas from different clones of tea plant. *Journal of Food Quality*, **2020**, 1–13.

Lee, Y.G., Rodriguez, I., Nam, Y.H., Gwag, J.E., Woo, S.H., Kim, H.G., Ko, J., Hong, B.N., Kang, T.H., Baek, N. 2019. Recovery effect of lignans and fermented extracts from Forsythia koreana flowers on pancreatic islets damaged by alloxan in zebrafish (*Danio rerio*). *Applied Biological Chemistry*, **62**(7), 1–9.

Livesey, G., Taylor, R., Hulshof, T., Howlett, J. 2008. Glycemic response and health - A systematic review and meta-analysis: Relations between dietary glycemic properties and health outcomes. A*merican Journal of Clinical Nutrition*, **87**(1), 258S-268S.

Maesaroh, K., Kurnia, D., Al Anshori, J. 2018. Comparison of DPPH, FRAP and FIC antioxidant activity test methods against ascorbic acid, gallic acid and quercetin. *Chimica et Natura Acta*, **6**(2), 93–100.

Malik, A. 2016. Antidiabetic Activity and isolation of bioactive compounds from ethanol extract of piper betle l. leaf. In *Seminar Nasional Kimia dan Pendidikan Kimia* Purwandari, V., Pulungan, A.N., Simatupang, L., Sihobing J.L. (Eds.), (pp. 1–6). UNIMED Press.

Manzano, S., Williamson, G. 2010. Polyphenols and phenolic acids from strawberry and apple decrease glucose uptake and transport by human intestinal Caco-2 cells. *Molecular Nutrition and Food Research*, **54**(12), 1773–1780.

Mendhekar, S.Y., Balsaraf, C.D., Bangar, M.S., Jadhav, S.L., Gaikwad, D.D. 2017. TLC profile study Mace (*Aril*) of Myristica malabarica Lamk . (*Myristicaceae*). *The Journal of Phytopharmacology*, **6**(6), 329–334.

Meutia. 2013. Effect of green tea extract addition in extruded rice process to decrease glycemic index. In *Departemen Ilmu Dan Teknologi Pangan Fakultas Teknologi Pertanian Institut Pertanian Bogor Bogor*.

Nagja, T., Vimal, K., Sanjeev, A. 2016. Myristica fragrans : A comprehensive review. *International Journal of Pharmacy and Pharmaceutical Sciences*, **8**(2), 9–12.

Oboh, G., Isaac, A.T., Akinyemi, A.J., Ajani, R.A. 2014. Inhibition of key enzymes linked to type 2 diabetes and sodium nitroprusside induced lipid peroxidation in rats' pancreas by phenolic extracts of avocado pear leaves and fruit. I*nternational Journal of Biomedical Science*, **10**(3), 210–218.

Pan, A., Sun, J., Chen, Y., Ye, X., Li, H., Yu, Z., Wang, Y., Gu, W., Zhang, X., Chen, X., Demark-Wahnefried, W., Liu, Y., Lin, X. 2007. Effects of a flaxseed-derived lignan supplement in type 2 diabetic patients: A Randomized, double-blind, cross-over trial. *PLoS ONE*, **2**(11), 1–7.

Poovitha, S., Parani, M. 2016. In vitro and in vivo α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). *BMC Complementary and Alternative Medicine*, **16**(1), 1–8.

Rema, J., Krishnamoorthy, B. 2012. Nutmeg and mace. In H*andbook of Herbs and Spices*: *Second Edition*. Woodhead Publishing Limited.

Sahreen, S., Khan, M.R., Khan, R.A. 2017. Evaluation of antioxidant profile of various solvent extracts of Carissa opaca leaves: An edible plant. *Chemistry Central Journal*, **11**(1), 1–7.

Santoso, A.W., Simamora, A., Timotius, K.H. 2018. Bioactivities of Water Extract and Essential Oil from the Mace of Myristica fragrans Houtt. *Jurnal Kedokteran Yarsi*, **26**(2), 99–106.

Scheepers, A., Joost, H.G., Schürmann, A. 2004. The glucose transporter families SGLT and GLUT: Molecular basis of normal and aberrant function. *Journal of Parenteral and Enteral Nutrition*, **28**(5), 364–371.

Schober, P., Schwarte, L.A. 2018. Correlation coefficients: Appropriate use and interpretation. *Anesthesia and Analgesia*, **126**(5), 1763–1768.

Shan, B., Cai, Y.Z., Sun, M., Corke, H. 2005. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry*, **53**(20), 7749–7759.

Shekhar, T.C., Anju, G. 2014. Antioxidant activity by DPPH radical scavenging method of ageratum conyzoides linn leaves. *American Journal of Ethnomedicine*, **1**(4), 244–249.

Simamora, A., Santoso, A.W., Timotius, K.H. 2018. Bioactivities of methanol and ethyl acetate mace extracts of myristica fragrans houtt. *Pharmacognosy Communications*, **8**(3), 103–107.

Soehendro, A.W., Manuhara, G.J., Nurhartadi, E. 2015. Effects of temperatures on antioxidant and antimicrobia activity of melinjo seed (*Gnetum gnemon* L.) with ethanol and water as solvent. *Jurnal Teknosains Pangan*, **4**(4), 15–24.

Sulaiman, S.F., Ooi, K.L. 2012. Antioxidant and anti food-borne bacterial activities of extracts from leaf and different fruit parts of Myristica fragrans Houtt. *Food Control*, **25**(2), 533–536.

Sultana, A., Najeeya, A.G.F., Anjum, A. 2018. Traditional Unani uses with multiple pharmacological activities of aril of Myristica fragrans (Mace). *Humanitas Medicine*, **8**(2), 1–7.

Tan, K.P., Khoo, H.A., Azrina, A. 2013. Comparison of antioxidant components and antioxidant capacity in different parts of nutmeg (*Myristica fragrans*). *International Food Research Journal*, **20**(3), 1049–1052.

Topal, F. 2018. Antidiabetic potential: Effect of resorcinol on α-glycosidase and α- amylase enzymes. *Cumhuriyet Science Journal,* **39**(4), 828–832.

Tran, N., Pham, B., Le, L. 2020. Bioactive compounds in anti-diabetic plants: from herbal medicine to modern drug discovery. *Biology*, **9**(252), 1–31.

Wijayanti, P.A., Kunarto, B., Pratiwi, E., Rohadi. 2018. Total phenolic, flavonoids, anthocyanins and antioxidant activity of nutmeg fuli oleoresin (*Myristica fragrans* Houtt) extracted using solid liquid microwave assisted extraction method. *JTPHP*, **13**(1), 1–9.

Williamson, G. 2013. Possible effects of dietary polyphenols on sugar absorption and digestion. *Molecular Nutrition and Food Research*, **57**(1), 48–57.

Worawalai, W., Phuwapraisirisan, P. 2019. Samin-derived flavonolignans , a new series of antidiabetic agents having dual inhibition against α -glucosidase and free radicals. *Natural Product Research*, **34**(22), 3169–3175.

Xu, Z., Wang, X., Zhou, M., Ma, L., Deng, Y., Zhang, H., Zhao, A., Zhang, Y., Jia, W. 2008. The antidiabetic activity of total lignan from fructus arctii against alloxan-induced diabetes in mice and rats. *Phytotherapy Research*, **22**, 97–101.

Yao, Y., Sang, W., Zhou, M., Ren, G. 2010. Phenolic composition and antioxidant activities of 11 celery cultivars. *Journal of Food Science*, **75**(1), 9–13.

Zhang, C., Jayashree, E., Kumar, P.S., Nair, M.G. 2015. Antioxidant and anti-inflammatory compounds in nutmeg (*Myristica fragrans*) pericarp as determined by in vitro assays. *Natural Product Communications*, **10**(8), 8–11.

Zhao, C.C., Shao, J.H., Chen, J., Zhang, X.H., Gu, W.Y., Shen, J., Liu, Y. 2020. Lignan constituents from the fruits of viburnum macrocephalum f. keteleeri and their α-amylase, α-glucosidase, and protein tyrosine phosphatase 1b inhibitory activities. *Journal of Agricultural and Food Chemistry*, **68**(40), 11151-11160.

Zhou, S.H., Fang, Z.X., Lü, Y., Chen, J.C., Liu, D.H., Ye, X.Q. 2009. Phenolics and antioxidant properties of bayberry (*Myrica rubra* Sieb. et Zucc.) pomace. *Food Chemistry*, **112**(2), 394-399.