



Antidiabetic activity screening and nmr profile of vegetable and spices commonly consumed in Indonesia

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Abstract

Apart from their nutritional value, vegetables and spices were empirically known to have functional properties such as antioxidant and antidiabetic activity. However, scientific evidence of these health effects is limited. In our effort to find potent raw material for antidiabetic functional food development, α -glucosidase inhibition and antioxidant activity of 15 Indonesian vegetables and spices methanolic extracts were screened. Their phytochemical profiles were assessed by determining total phenolic content (TPC) and ¹H NMR fingerprinting. The results showed that the *Syzygium polyanthum*, *Pluchea indica*, *Etlingera elaitor*, and *Cosmos caudatus* had the highest GIA (IC_{50} 11.76 \pm 0.32, 12.17 \pm 0.18, 53.13 \pm 2.87, and 61.33 \pm 1.21 μ g/ml, respectively). The AA and TPC of the four samples were also higher than the others. The ¹H NMR profiles of the active samples were different from the non-active samples mainly in the aromatic region. Further observation of the spectra revealed that caffeoylquinic derivatives and esculetin were identified in *P. indica*; while gallic acid, syringic acid, and myricetin were identified in *S. polyanthum*. These compounds were known to have antidiabetic activity through different mechanisms.

Keywords: antidiabetic activity; α -glucosidase; vegetable and spices; NMR.

Practical Application: α -glucosidase inhibitor and DPPH-antioxidant activity of vegetables and spices.

1 Introduction

Vegetables and spices have been widely used for the prevention and treatment of many diseases since 500 b.c.e. by the ancient Greeks and later by the Chinese (Kelly, 2009). The use of vegetables and spices as medicine is mostly based on empirical experiences and supported by scientific-based research in the laboratory.

Bioactive compounds of the plant are often presented as minor secondary metabolites and are very diverse between species. The process for identifying bioactive compounds from the plant is not easy because of the limited availability, complex structures, low stability, mixture forms with different boiling points and polarity, and the large cost requirements for the selection of bioactive compounds (Mishra et al., 2008; Zhang et al., 2018). A lot of research has been carried out to find out the antidiabetic activity from plant species, and more than 800 plant species are known to have antidiabetic activity (Saad et al., 2017). Nevertheless, the research of plant species that have antidiabetic activity remains attractive, especially to find species that are effective and safe for diabetes prevention and treatment, given complaints of side effects and toxicities from consumption of the hypoglycemic drug used for long-term therapy (Derosa & Maffioli, 2012). The exploration of vegetables and spices for the prevention and treatment of degenerative diseases, including diabetes mellitus, is becoming an important research topic recently.

The studies on antidiabetic, anti-hyperglycemic, and hypoglycemic potential of 30 commonly consumed fruits, vegetables, oils and spices were comprehensively reviewed (Beidokhti & Jäger, 2017). These botanicals exhibited their antidiabetic activities through several different mechanisms, especially by inducing insulin secretion in β -cells. Groups of compounds such as anthocyanins, flavonoids, and alkaloids were mentioned to be associated with the reported antidiabetic activity.

Delaying glucose absorption by inhibiting the associated enzymes, such as α -glucosidase, could be one of the therapeutic methods in diabetes mellitus treatment. Plant extracts were reported as important sources of α -glucosidase inhibitor compounds as recently reviewed elsewhere. Flavonoids and alkaloids were the two major groups of compounds associated with α -glucosidase inhibition activity of the reported plants (Kumar et al., 2011). In more recent studies, flavonoids rutin and astragaloside isolated from mulberry leaves, were reported to have α -glucosidase inhibitory activity (IC_{50} of 8.05 and 7.09 μ g/ml, respectively) (Hong et al., 2013). Similarly, two flavonoids isolated from *Desmos cochinchinensis*, desmoscochinflavone A and B, both showed α -glucosidase inhibitory activity with IC_{50} of 0.9 μ M (Meesakul et al., 2019).

Indonesia has various types of vegetables and spices. Some of them are commonly served in the daily meals of Indonesian families, but some are still underutilized and only consumed in

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a small rural area. Examples of the first group are *Amaranthus Tricolor* L., *Sauropus androgynus*, *Ocimum xcitriodorum*, *Solanum nigrum* L., and *Talinum triangulare*. In contrary, vegetables and spices such as *Pluchea indica*, *Cosmos caudatus*, *Pilea trinervia* Wight, *Etlingera elaitor*, and *Solanum torvum* Swartz are not so well-known as previous, although they might have health-benefit properties such as antioxidants or antidiabetes.

In this study, 15 common Indonesian vegetables were evaluated for antidiabetic and antioxidant activity through in vitro α -glucosidase inhibition and DPPH methods. The chemical profile of the samples was evaluated by measuring the total phenolics content. NMR analysis was also conducted to obtain the more comprehensive phytochemical profile of the samples. NMR was chosen since it has several advantages such as high reproducibility, simple sample preparation, fast analysis time, and wide detection window from non-polar to polar compounds. With 2D NMR measurement, better information for structural elucidation was provided (Verpoorte et al., 2007). NMR spectral profiles between active and non-active samples can be compared to estimate possible active compounds. Up to now, the spectral data of Indonesian vegetables and spices are still very rare. This study was expected to provide initial important information on the antidiabetic and antioxidant potential of common Indonesian vegetables to be used as a reference when one intends to develop antidiabetic functional food.

2 Materials and methods

2.1 Samples preparation

Fifteen edible plants were used in this research which include eleven leafy vegetables, two flowery spices/vegetables and two fruits. Their local names, family, the edible parts, and the way that they are traditionally consumed are presented in Table 1.

Several samples were harvested from the experimental garden of the Tropical Biopharmaca Research Center, IPB University, these are: CC, CB, NS, OC, PA, PT, PI, SA, SP, TT, SN and ST, and the others were purchased from the local market near IPB University, Bogor, Indonesia. The samples were taken freshly and

immediately kept in -20°C . After 48 hours, they were put in the freeze dryer for another 48 hours. The dried samples were stored in the freezer until the extraction process. The moisture content of the dried samples was measured based on the Association of Official Analytical Chemists (2012).

2.2 Samples Extraction

The dried samples were powdered, sieved (20 mesh), and then subjected to the extraction process. The extraction process was carried out based on the method of Yuliana et al. (2011) by adding 80% methanol (Merck, USA) two times of the sample volume into 20 g samples, followed by ultrasonication (Bransonic Ultrasonic Cleaner 8510E MTH, USA) for 30 min at room temperature. The filtrate was taken and dried using a rotary evaporator (Buchi Rotavapor R-210, Buchi Labortechnik Switzerland) at a temperature of 40°C until dry extract was obtained.

2.3 Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using procedure described by Ainsworth & Gillespie (2007). Ten mg of the extract was dissolved with methanol 50% and then mixed with 100 μL of 1 N Folin Ciocalteu reagent 10% (v/v). The mixture was added with 800 μL of 700 mM sodium carbonate and incubated for 2 hours at room temperature. Then 200 μL mixture was transferred to 96 well microplates, and the absorbance was measured at 765 nm. TPC was expressed as μg gallic acid equivalent (GAE)/mg sample.

2.4 Determination of Antioxidant Activity (AA)

Antioxidant activity was determined using the DPPH method described by Lee et al. (2015) with a slight modification. Ten mg of the sample was dissolved in DMSO and then diluted with ethanol to obtain a concentration of 1000 ppm. One hundred μL of the extract solution was taken and added with 100 μL DPPH 125 μM . The solution was incubated for 30 minutes at room temperature and dark conditions. The absorbance was

Table 1. General information on vegetables and spices used in the study.

| No | Species | Abbr. | Family | Local name | Consumed/ used as | The part used in this study |
|----|-------------------------------------|-------|----------------|--------------|-------------------|-----------------------------|
| 1 | <i>Apium graveolens</i> L | AG | Apiaceae | Seledri | Condiment | Leaves |
| 2 | <i>Cosmos caudatus</i> | CC | Asteraceae | Kenikir | Raw | Leaves |
| 3 | <i>Cyclea barbata</i> | CB | Menispermaceae | Cincau | Cooked | Leaves |
| 4 | <i>Etlingera elaitor</i> | EE | Zingiberaceae | Kecombrang | Cooked | Flower |
| 5 | <i>Nothopanax Scutellarium</i> Merr | NS | Araliaceae | Mangkokan | Cooked | Leaves |
| 6 | <i>Ocimum xcitriodorum</i> | OC | Lamiaceae | Kemangi | Raw and condiment | Leaves |
| 7 | <i>Pandanus amaryllifolius</i> Roxb | PA | Pandanaceae | Pandan | Condiment | Leaves |
| 8 | <i>Pilea trinervia</i> Wight | PT | Urticaceae | Pohpohan | Raw | Leaves |
| 9 | <i>Pluchea indica</i> | PI | Asteraceae | Beluntas | Cooked | Leaves |
| 10 | <i>Sauropus androgynus</i> | SA | Phyllanthaceae | Katuk | Cooked | Leaves |
| 11 | <i>Sesbania grandiflora</i> | SG | Fabaceae | Turi | Cooked | Flower |
| 12 | <i>Solanum nigrum</i> L. | SN | Solanaceae | Leunca | Raw or cooked | Fruit |
| 13 | <i>Solanum torvum</i> Swartz | ST | Solanaceae | Takokak | Raw or cooked | Fruit |
| 14 | <i>Syzygium polyanthum</i> | SP | Myrtaceae | Salam | Condiment | Leaves |
| 15 | <i>Talinum triangulare</i> | TT | Portulacaceae | Ginseng jawa | Cooked | Leaves |

measured using a microplate reader at 517 nm. The AA was expressed as % inhibition. This result was used to determine the concentration series of the sample to calculate IC_{50} value, different concentration series were made for each sample. The most diluted concentration series were prepared for *S. polyanthum* extract (0.781 to 12.5 $\mu\text{g/ml}$), while the most concentrated one was for *S. grandiflora* extract (15.625 to 1000 $\mu\text{g/ml}$). Ascorbic acid was used as a reference compound.

2.5 Determination of α -Glucosidase Inhibitory Activity (GIA)

The assay was performed using a method described by Sancheti et al. (2007). Acarbose was used as a standard. Ten mg of the sample was dissolved in DMSO and then diluted with phosphate buffer 0.1 M (pH 6.9) at several concentrations. The enzyme solution was prepared by mixing 25 μL α -glucosidase 0.04 U/mL with phosphate buffer solution of 0.1 M (pH 6.9). The mixture for the analysis consisting of 50 μL phosphate buffer 0.1 M (pH 6.9), 25 μL solution p-nitrophenyl- α -D-glucopyranoside (dissolved in 0.1 M phosphate buffer solution pH 6.9), and 10 μL samples or acarbose (for positive control). The mixture was incubated at 37 °C for 30 minutes. The reaction was stopped by adding 100 μL sodium carbonate 0.2 M solution, and the enzymatic hydrolysis reaction was measured at 410 nm using a microplate reader.

The IC_{50} was determined using linear regression equation obtained from the dose response curve. The curve was made by plotting % GIA (y-axis) and sample concentration (x-axis.) The concentration series for *S. polyanthum* and *P. indica* were between 18.18 to 2.27 $\mu\text{g/ml}$, while for *C. caudatus*, and *E. elaitor* were between 90.91 to 9.09 $\mu\text{g/ml}$.

2.6 NMR Analysis

The NMR analysis of the sample was conducted according to Wijaya et al. (2017) with modification. Fifty mg of the sample was diluted with CD_3OD , mixed by vortex for 2 min at room temperature and ultrasonicated at 1000 g for 15 minutes. Eight hundred μL of samples were transferred into a 5 mm NMR tube and analysed using 500 MHz NMR (JEOL NMR Spectrometer, USA) with deuterated methanol as the internal lock. The NMR spectra was recorded at a frequency of 500.16 MHz. The temperature was maintained at 25 °C, Each ^1H NMR spectra consisted of 128 scans requiring 10 min, 26 s acquisition, and relaxation delay time of 1.5 s. The TSP was used as reference at δ 0.00.

Phasing, baseline, and reference corrections of NMR Spectra were performed manually using MNOVA version 13.0. The metabolites were identified by comparing the ^1H -NMR spectra of the sample and the published literature.

2.7 Statistical analysis

The quantitative data was reported as a mean \pm SD (standard deviation), and the significant differences were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. P-value < 0.05 was considered to be significant. Pearson correlations were performed to evaluate the correlation between various parameters. The following r

criteria was used: $r < 0.3$ = poor; $0.3 \leq r < 0.6$ = fair/moderate; $0.6 \leq r < 0.8$ = moderately strong; and $r \geq 0.8$ = very strong correlation (Schober et al., 2018).

3 Results and Discussion

3.1 The moisture content of the dry samples and yields of extract.

Vegetables are considered as an important vitamin and minerals sources. (Chotimah et al., 2013). Additionally, it possesses various health benefit activities. Several underutilized Indonesian vegetables were reported to contain health-beneficial phytochemicals such as phenolics, carotenoids, and ascorbic acids (Andarwulan et al., 2012). Despite the high diabetes occurrence in Indonesia, the antidiabetic potential of Indonesian local vegetables is not yet fully explored, particularly those with α -glucosidase inhibitor activity. The 15 samples used in this study consisted of vegetables and spices which are commonly served in Indonesian daily meals. They might be consumed as raw vegetables, especially in the West part of Java, or as condiments to increase the taste and the aroma of other dishes. Other samples are usually cooked before consumed, e.g. as stewed vegetables, stir-fry, or as ingredients in different types of Indonesian soups. The parts of plants that are eaten are also different, but leaves are the most common ones (Table 1).

The moisture content of the dry samples slightly varied from 4.89% (*S. torvum*) to 12.18% (*O. xcitriodorum*) (Figure 1) while the yield of extraction was more diverse among samples. *S. grandiflora* and *N. Scutellarium* Merr had the highest extraction yield (39.33% and 29.77%, respectively), while *S. polyanthum* and *O. xcitriodorum* extracts were the lowest (10.24% and 10.18%, respectively) (Figure 1). The highest yield of *S. grandiflora* flower is probably due to the high sugar content of the plant. Previous study reported that the extraction of *S. grandiflora* flowers with 70% acetone solvent resulted in a yield of 33.30% with sugar as one of the largest components (10.74%) (Gowri & Vasantha, 2010). Information on the chemical composition of *N. Scutellarium* Merr is still very rare. A study conducted almost five decades ago is the only literature that the plant was among the green leafy vegetables in Puerto Rico with significant protein content (Martin et al., 1977). In a more recent report, stigmasta-5, 22-dien-3-O- β -D-galactopyranoside was identified in this plant (Syafrial & Efdi, 2015).

S. polyanthum and *O. xcitriodorum*, both are aromatic plants, are rich in essential oils. *S. polyanthum* leaves contained higher volatiles compound but lower total phenolics content as compared to *S. polyanthum* bark (Ismail & Wan Ahmad, 2019). A comparison of essential oils content and composition between several species of *Ocimum* revealed that *O. citriodirum* had the least essential oil yield as compared to *O. basilicum*, *O. virride*, and *O. kilimandscharicum* (Rawat et al., 2017).

3.2 Total Phenolic Content (TPC)

The role of phenolic compounds in prevention and treatment of various diseases, such as cancer and diabetes, was highlighted in a number of reports. A recent review on the function of dietary phenolics compounds in diabetes mellitus prevention

and treatment summarized that this group of compounds demonstrated their activity through different mechanisms. One of the common pathways is by interfering carbohydrate metabolism and improving insulin secretion performance of the beta-cells (Dias-Soares et al., 2017).

Total phenolics content of the 15 samples quite varied, ranged from 1.07±0.36 to 37.99±1.39 µg GAE/mg extract (Table 2). The highest TPC was obtained from *P. indica* extract. The TPC of *P. indica* and *S. polyanthum* extracts were previously reported, and the results varied. TPC of methanolic extract of *P. indica* originated from Thailand was much lower than our results that was 1.13 µg GAE/mg (Srimoon & Ngiewthaisong, 2015). TPC of ethanolic extract of *P. indica* from Indonesia was 164.8 µg GAE/mg

(Indradi et al., 2017). Several phenolic compounds were identified in *P. indica* leaves, such as quercetin, kaempferol, myricetin, and several caffeoylquinic derivatives (Andarwulan et al., 2010; Arsiningtyas et al., 2014; Vongsak et al., 2018).

TPC of *S. polyanthum* obtained in this study was also different from other previous reports. It was lower than those of reported in ethanolic extract of *S. polyanthum* from Malaysia, which was 111.25 µg GAE/mg (Har & Ismail, 2012), but higher than water extract of *S. polyanthum* from Singapore, that was only 11 µg GAE/mg DW (Wong et al., 2006). The variation of TPC content even in one species may be attributed to various factors, such as extraction method, plant maturity, and agro-climate conditions (Imran et al., 2014).

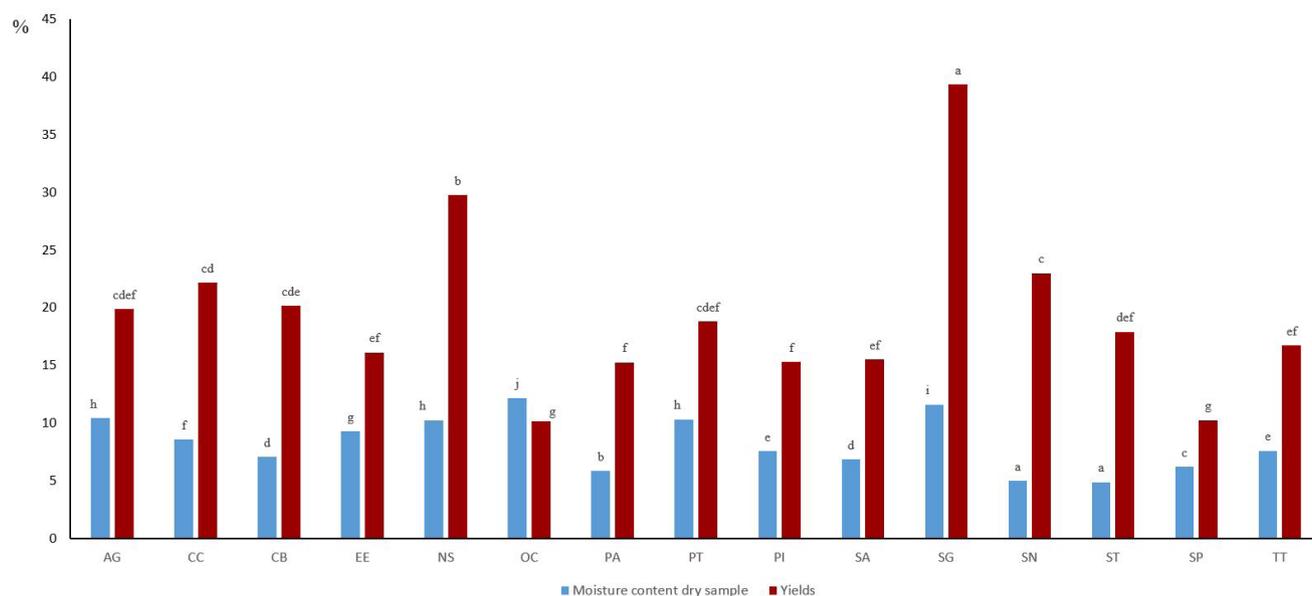


Figure 1. Moisture content and yields of vegetables and spices extracts. See Table 1 for abbreviations. The same letter at the same bar color representing not significant differences (Duncan Multiple Comparison tests, $p=0.05$).

Table 2. Total phenolic content and IC₅₀ value of the antioxidant activity and α-glucosidase inhibitory activity.

| No | Species | Phenolic content (µg GAE/mg extract) | IC ₅₀ DPPH (µg/mL) | α-glucosidase inhibition activity at 90.90 µg/ml (%) |
|----|-------------------------------------|--------------------------------------|-------------------------------|--|
| 1 | <i>Apium graveolens</i> L | 3.36 ± 0.22 g | 48.93 ± 0.74 f | 0.22 ± 0.13 g |
| 2 | <i>Cosmos caudatus</i> | 21.34 ± 1.48 c | 7.37 ± 0.27 b | 80.38 ± 0.93 c |
| 3 | <i>Cyclea barbata</i> | 2.52 ± 0.22 gh | 201.58 ± 2.12 j | 0.36 ± 0.13 fg |
| 4 | <i>Etlingera elaitor</i> | 14.43 ± 0.83 d | 12.41 ± 0.83 c | 78.78 ± 1.24 d |
| 5 | <i>Nothopanax Scutellarium</i> Merr | 2.95 ± 0.19 gh | 66.42 ± 1.55 g | 1.13 ± 0.23 f |
| 6 | <i>Ocimum xcitriodorum</i> | 6.08 ± 0.21 e | 31.25 ± 0.81 d | 0.22 ± 0.06 g |
| 7 | <i>Pandanus amaryllifolius</i> Roxb | 2.99 ± 0.14 gh | 283.38 ± 1.66 k | 0.07 ± 0.11 g |
| 8 | <i>Pilea trinervia</i> Wight | 3.07 ± 0.12 gh | 67.04 ± 1.76 g | 0.58 ± 0.17 fg |
| 9 | <i>Pluchea indica</i> | 37.99 ± 1.39 a | 4.34 ± 0.42 ab | 91.01 ± 0.64 b |
| 10 | <i>Sauropus androgynous</i> | 2.10 ± 0.12 h | 99.58 ± 1.53 h | 5.10 ± 0.39 e |
| 11 | <i>Sesbania grandiflora</i> | 1.07 ± 0.36 i | 588.48 ± 2.57 l | 0.36 ± 0.13 fg |
| 12 | <i>Solanum nigrum</i> L. | 2.46 ± 0.45 gh | 106.54 ± 4.55 i | 0.11 ± 0.06 g |
| 13 | <i>Solanum torvum</i> Swartz | 4.70 ± 0.32 f | 28.24 ± 3.85 d | 0.15 ± 0.13 g |
| 14 | <i>Syzygium polyanthum</i> | 25.37 ± 0.92 b | 2.46 ± 0.23 a | 99.42 ± 0.45 a |
| 15 | <i>Talinum triangulare</i> | 6.24 ± 0.23 e | 39.68 ± 0.48 e | 0.04 ± 0.27 g |
| 16 | Ascorbic acid | - | 4.48 ± 0.03 ab | - |

The same letter at same column are not significantly different according to Duncan multiple comparison tests at $p = 0.05$.

Reports on phenolic compounds which were identified in *S. polyanthum* are fewer than those of *P. indica*. Caffeic acid, gallic acid, vanillic acid, syringic acid, (1-(2,3,5-trihydroxy-4-methylphenyl)hexane-1-one; 1-(2,3,5-trihydroxy methyl phenyl) octane-1-one; and (4E)-1-(2,3,5-trihydroxy-4-methyl phenyl) decan-1-one) were compounds reported to be found in *S. polyanthum* (Har & Ismail, 2012; Lelono & Tachibana, 2013; Setyawati et al., 2018).

The lowest TPC was detected in *S. grandiflora* with the value of 1.07 µg GAE/mg. This value was somewhat close to previous TPC found in ethanolic extract of *S. grandiflora* from India (3.17 µg GAE/mg) (Siddhuraju et al., 2014). However, methanolic extract of *S. grandiflora* from Malaysia contained higher TPC, that was 208.80 µg GAE/mg (Mustafa et al., 2010). Several phenolic compounds that were identified from *S. grandiflora* were catechin, epicatechin, quercetin, myricetin, luteolin and naringenin (Mustafa et al., 2010).

3.3 Antioxidant Activity (AA)

The role of stress oxidative in the pathogenesis of type II diabetes mellitus (T2DM) and its vascular complications was well-known. A large prospective cohort study conducted by other researchers reported that subjects who consumed higher amount of high total antioxidant capacity diet had lower risk of T2DM (Mancini et al., 2017).

In this study, antioxidant activity of the 15 samples was determined using DPPH method. The results showed that *S. grandiflora* extract had the lowest AA (IC₅₀ 588.48 µg/ml), while *S. polyanthum* extract had the highest AA among others (IC₅₀ 2.46 µg/mL). *P. indica*, *C. caudatus*, and *E. elaitor* extracts also showed relatively high AA with IC₅₀ 4.34, 7.37, and 12.41 µg/mL, respectively (Table 2). Interestingly, *S. polyanthum* extract exhibited higher AA than ascorbic acid (IC₅₀ 4.38 µg/ml), indicating that the extract is potential as an excellent antioxidant, especially its ability to neutralise radical compounds by donating its proton.

Antioxidant activity of *S. polyanthum* extracted with different solvents was reported previously. Several studies showed that methanolic extract of this plant had the highest antioxidant activity (IC₅₀ 17.46 µg/mL) as compared to those extracted by other solvents (Widyawati et al., 2016; Hidayati et al., 2017; Ramadhania et al., 2017). These reports indicated that the responsible active compounds of *S. polyanthum*, might be relatively polar. Two antioxidant compounds that were isolated from the methanol-water extract of *S. polyanthum* were gallic acid and syringic acid (Lelono & Tachibana, 2013).

Based on its IC₅₀ value, antioxidant activity of certain sample can be classified into 5 groups; IC₅₀ < 10 µg/ml = very strongly active, 10-50 µg/ml = strongly active; 50-100 µg/mL = moderately active; 100-250 µg/ml = weakly active, > 250 µg/ml = inactive (Phongpaichit et al., 2007). In this case, *S. polyanthum*, *P. indica*, and *C. caudatus* extract were classified as very strong active AA; *E. elaitor*, *S. torvum* Swartz, *O. xcitriodorum*, *T. triangulare*, and *A. graveolens* L. extracts were strongly active AA; *N. Scutellarium* Merr, *P. trinervia* Wight, and *S. androgynus* as moderately active AA; while *S. ningrum* L and *C. barbata* were considered as weakly

active. *P. amaryllifolius* Roxb and *S. grandiflora* were classified as inactive antioxidants.

The Pearson correlation analysis showed a fair/moderate correlation between AA and TPC of the 15 samples ($r = -0.44$, $p < 0.05$). It indicated that phenolic was not the only group of compounds responsible for the AA activity, but other group of compounds, such as alkaloids and terpenoids, might contribute as well to AA (Gan et al., 2017). The phenolic compounds act as an antioxidant through several mechanisms: donating their hydrogen, chelating metal ions, or enzymes that are involved in the production of free radicals (Chen et al., 2015; Nimse & Pal, 2015). In this study we used DPPH method, thus, the suitable antioxidant mechanism of the phenolic compounds was through hydrogen donation to the radicals, which changed it into less or non-radicals.

3.4 α-Glucosidase Inhibitory Activity (GIA)

One of common antidiabetic drugs mechanism is by preventing or delaying complex carbohydrates digestion into simple saccharides (glucose) (Derosa & Maffioli, 2012). It can be done by disrupting the activity of enzymes important for carbohydrates digestion, such as α-glucosidase. α-glucosidase is found in the brush border of small intestine. The enzyme catalyses the hydrolysis of 1,4-α bonds of oligo- and disaccharides and converts them into monosaccharides (glucose) (Lebovitz, 1997).

α-glucosidase inhibitor activity of some vegetables was recently reported. Among the tested vegetables, *Anacardium occidentale*, *Polyscias fruticosa* and *Arcypteris irregularis* showed the highest inhibition with IC₅₀ of 108.04, 107.79 and 145.54 µg/mL, respectively (Yuliana et al., 2020). In another research, vegetables *Syzygium polyanthum*, *Anacardium occidentale*, *Zingiber officinale* Roxb showed the highest α-glucosidase inhibitor activity among 42 samples tested in the study (IC₅₀ 19.06, 107.79 and 145.54 µg/mL, respectively) (Elya et al., 2015).

In this study, four extracts showed the highest activity at tested concentration (90.90 µg/ml), those are *S. polyanthum* (99.42%), followed by *P. indica*, *C. caudatus* and *E. elaitor* (91.0, 80.38 and 78.78%, respectively) (Table 2). The IC₅₀ of these four potent samples was 11.76±0.32, 12.17±0.18, 61.33±1.21 and 53.13±2.87 µg/ml, respectively. The inhibition activity these samples were weaker than acarbose (IC₅₀ 0.39 µg/ml).

Two researches on antidiabetic activity of Indonesian *S. polyanthum* methanol and ethanol extracts showed lower GIA activity value than our result (IC₅₀ 92 and 19.06 ppm, respectively) (Lelono & Tachibana, 2013; Elya et al., 2015). In the first study, three benzoic acid derivatives, i.e. gallic acid, vanillic acid, and syringic acid, were isolated and identified from methanol-water extract of *S. polyanthum*. The compounds were reported to exhibit GIA by 20, 27 and 35% at the tested concentration. It was also reported that the activity of each benzoic acid derivatives was lower than the mixture of those three compounds (42.38%), and also even lower than methanol-water crude extract of *S. polyanthum* (62%) (Lelono & Tachibana, 2013). This result indicated that the bioactive compound in the extract might work synergistically to inhibit the enzyme but further study is required to confirm it.

The antidiabetic activity of *P. indica* through GIA was also previously reported. Water extract of the juvenile leaves of this plant had IC₅₀ of GIA 25-51 ppm, while the IC₅₀ of GIA at the other stages of maturity were 61-104 ppm (Vongsak et al., 2018). Guided fractionation by chromatography method was successfully identified the five caffeoylquinic acid derivatives. Compounds with the highest GIA was addressed to 3,4,5-Tri-O-caffeoylquinic acid methyl ester, 1,3,4,5-tetra-O-caffeoylquinic acid and 3,4,5-Tri-O-caffeoylquinic acid (IC₅₀ 0.452, 2.486 and 2.938 ppm, respectively) (Arsiningtyas et al., 2014).

Next, the GIA values of *S. polyanthum* and *P. indica* were compared with those of dairy-food product such as whey-dairy beverage, cheese, and dairy dessert. It was reported elsewhere that various dairy food products exhibited potential GIA, those are whey-raspberry flavored beverages, minas frescal cheese, and dairy-blueberry flavored dessert treated with ohmic heating (Ferreira et al., 2019; Kuriya et al., 2020; Rocha et al., 2020). Although the abovementioned studies did not mention the IC₅₀ of the samples, based on the protocol mentioned in the method section, approximately 28.57 µg/ml of whey-raspberry flavored beverages, minas frescal cheese, and dairy-blueberry flavored dessert showed GIA by 98.40-99.70, 59.10-69.50 and 75.20-90.10%, respectively. These activities were higher than GIA of *S. polyanthum* and *P. indica* which were used in our study. As previously discussed, phenolic compounds showed important role in GIA of *S. polyanthum* and *P. indica*. Several studies reported that the compounds responsible for GIA of animal-derived foods were globular protein and peptide such as β-lactoglobulin (whey-milk protein) and Val-Thr-Gly-Arg-Phe-Ala-Gly-His-Pro-Ala-Ala-Gln (egg yolk protein), respectively (Lacroix & Li-Chan, 2013; Zambrowicz et al., 2014). To the best of our knowledge, studies aiming at direct comparison between GIA of protein-peptide and phenolic compounds are not yet reported.

The Pearson analysis showed that GIA had strong positive correlation with TPC ($r = 0.92$, $p < 0.05$), indicating the TPC was responsible for the GIA. This finding was in agreement with Liu et al. (2015) who reported that there was a strong correlation between GIA and TPC of guava leaves water extract.

Virtual screening and docking studies showed that several polyphenols such as caffeic acid, curcumin, cyanidin, epicatechin, quercetin, and ferulic acid had high-affinity binding on the active site of α-glucosidase enzyme especially at arginin⁴⁰⁷ and arginin⁴¹¹ (Rasouli et al., 2017). The interaction between phenolic substances and the amino acid at the active site of the glucosidase enzyme will take place by hydrogen or hydrophobic bond (Proença et al., 2017). It seems that phenolic substances have a flexible backbone which makes it fits with the active site of the enzyme.

3.5 Metabolite profiling with NMR

Nuclear Magnetic Resonance (NMR) is widely applied to carry out metabolite profiling of natural resources. This technique produces hundreds or more of spectra that describe the profile of primary and secondary metabolites. The spectral intensity

is also relevant to the concentration of these metabolites (Verpoorte et al., 2007; Kim et al., 2010).

To get a comprehensive snapshot of the phytochemical content of the 15 samples, their ¹H NMR spectra were compared with previous reports and some spectral databases. ¹H NMR spectral of all samples showed high intensity in the region of δ 3.0-5.5 (Figure 2a), which could be attributed to saccharides/sugars present in all extracts especially α-glucose (δ 5.11-5.13 d; $J=3.8$ Hz) and β glucose (δ 4.46-4.5 d; $J=7.8$ Hz) (Verpoorte et al., 2007). Other high intensity signals could also be assigned as amino acid signals including alanine (δ 1.45-1.48, d, $J=7.2$ Hz), glutamate (δ 4.2.04-2.07, m), and threonine (δ 1.32, d, $J=6.6$ Hz) (Verpoorte et al., 2007).

In contrast, all spectra had lower intensity at 5.70 – 9.00 ppm regions, which varied between extracts (Figure 2b). The signals in this region are typically for phenolics compounds, including flavonoids (Kim et al., 2010). The active samples (particularly no. 1, 2 and 3) showed higher intensity in this area, even though it also appeared in the spectra of non-active samples (Spectra no. 10, 12, 13, 14 and 15 in Figure 2b). Apparently different samples contained different types of phenolic compound which associate with their GIA activity. Phenolic compounds are common metabolites which are very diverse in different plant species. Various researches reported that bioactive compounds responsible for α-glucosidase inhibitory activity belonged to the phenolic group, such as fuscaxanthone J and K which were extracted from the roots of *G. fusca* (Nguyen et al., 2017).

As previously mentioned, *S. polyanthum* and *P. indica* had the strongest α-glucosidase inhibitor activity among others. Based on the intensity of signals at 5.7-9.0 ppm region, apparently *P. indica* had more intense and more diverse phenolic compounds than *S. polyanthum* (Figure 3B).

The presence of caffeoylquinic derivatives with antidiabetic activity was previously reported by Arsiningtyas et al. (2014). After comparing the ¹H NMR profile with previous report by Gao et al. (2008) and Ge et al. (2018), typical signal of 4,5-di-O-caffeoylquinic acid methyl ester was identified in *P. indica* (Figure 3A). The methyl ester proton was marked by singlet at δ 3.8 (s, 3H, OCH₃), while the two caffeoyl substituents were assigned by signal at δ 7.52 (d, $J = 15.9$ Hz, 2H, H-7), 7.46 (d, $J = 15.9$ Hz, 2H, H-7'), 7.10 (d, $J = 2.1$ Hz, 2H, H-2), 7.09 (d, $J = 2.0$ Hz, 2H, H-2'), 7.00 (dd, $J = 4.6, 2.0$ Hz, 2H, H-6), 6.98 (dd, $J = 4.1, 2.0$ Hz, 2H, H-6'), 6.49 (d, $J = 8.2$ Hz, 2H, H-5/H-5') 6.31 (d, $J = 15.9$ Hz, 2H, H-8), 6.24 (d, $J = 15.8$ Hz, 2H, H-8'). The quinic structure was observed by the presence of singlets at δ 5.34 (s, 1H, H-5), 5.12 (s, 1H, H-4), 3.79 (s, 1H, H-3), 2.33 (s, 1H, H-6), 2.31 (s, 1H, H-2). 5-O-caffeoylquinic acid was also identified by the signal at δ 7.56 (d, $J = 16.5$ Hz, 1H, H-7), 7.02 (s, 1H, H-2), 6.90 (d, $J = 7.0$ Hz, 1H, H-6), 6.49 (d, $J = 8.2$ Hz, 1H, H-'), 6.28 (d, $J = 18.6$ Hz, 1H, H-8) (Ge et al., 2018).

Typical NMR signals of esculetin was identified in *P. indica* spectra after comparing the spectra with NMR data from Xia et al. (2015). This compound was assigned based on the existence of doublet at δ 6.22 (d, $J = 9.6$ Hz, 1H, H-3), singlets at δ 6.68 (s, 1H, H-8) and δ 6.80 (s, 1H, H-5) and doublet at δ 7.59 (d, $J = 9.9$ Hz, 1H, H-4) (Figure 3A). The antidiabetic

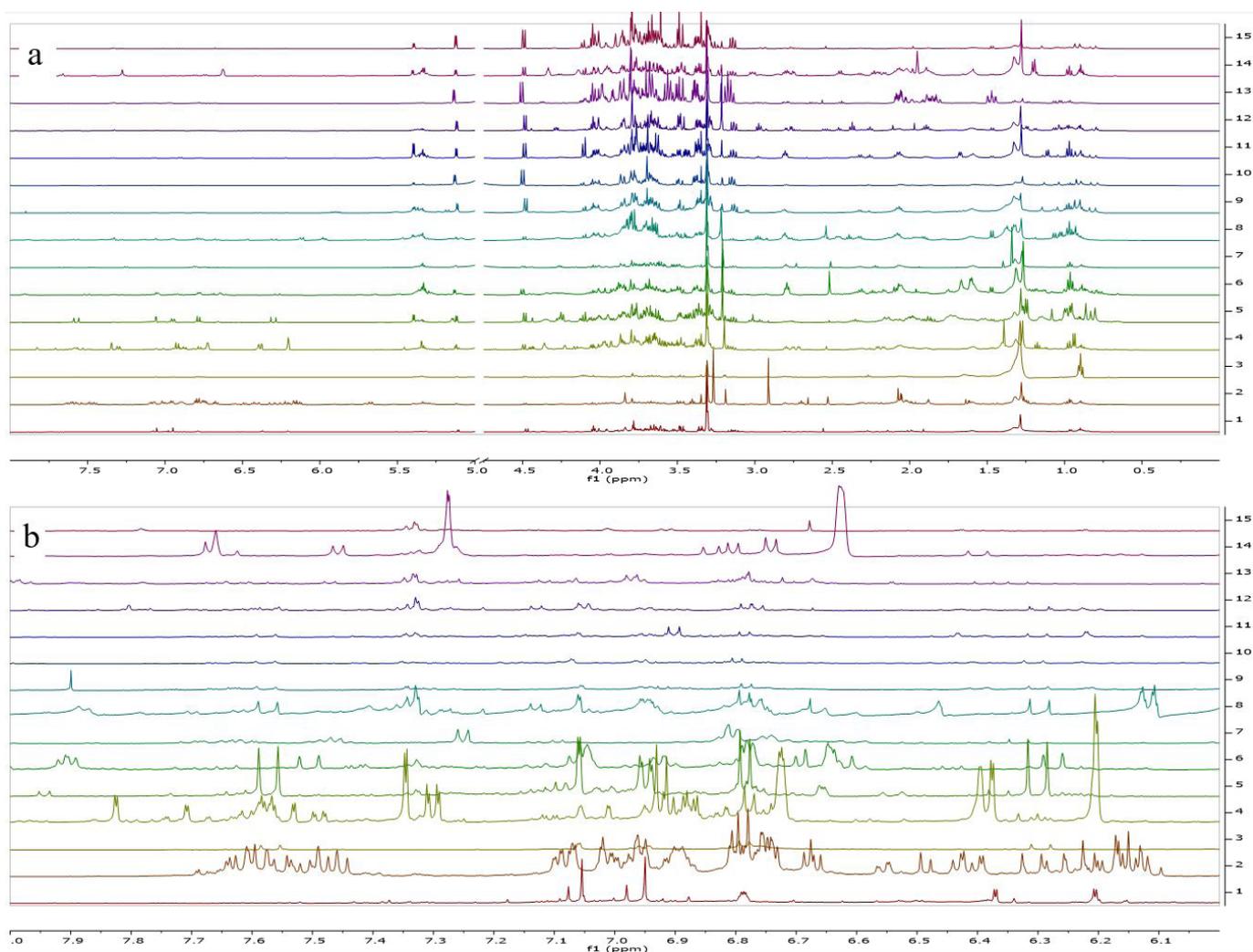


Figure 2. NMR spectra of the samples (a) full spectra; (b) aromatic region. (1. *S. polyanthum*; 2. *P. indica*; 3. *E. elaitor*; 4. *C. caudatus*; 5. *S. torvum* Swartz; 6. *O. xcitriodorum*; 7. *T. triangulare*; 8. *A. graveolens* L; 9. *N. scutellarium* Merr; 10. *P. trinervia* Wight; 11. *S. androgynus*; 12. *S. nigrum* L.; 13. *C. barbata*; 14. *P. amaryllifolius* Roxb; 15. *S. grandiflora*).

activity of esculetin was exerted by their ability to decrease the levels of plasma glucose, triglycerides, and cholesterol of adult wistar rats fed by high-fat diet. The level of rats plasma insulin was also found to significantly increased (Kadakol et al., 2017).

The ^1H NMR spectra of *S. polyanthum* showed few peaks at the aromatic region, indicating less phenolic compounds variation. Typical signals of syringic acid and gallic acid was identified in its spectra after comparing with previous study as a reference (Zhao et al., 2012). Methoxy residue was observed as a singlet at δ 3.75 (s, 3H, 3-5- OCH_3), while another proton was pointed out by signal at δ 7.12 (s, 1H, H-2/H-6) (Figure 3B). This finding was supported by HMBC spectra which showing interaction between H-2/H-6 and C3 and C4 (Figure 4a). Gallic acid was identified with the presence of singlet at δ 7.06 (s, H-2/H-6) (Figure 3B), supported by HMBC spectra which showing correlation between H-2/H-6 and C1, C2/6, C3/5, C4, and C7 (Figure 4b). The GIA of these two compounds was reported previously (Lelono & Tachibana, 2013).

Typical signals of flavonoid myricetin was also observed on the ^1H NMR of *S. polyanthum* extract after matching it with

reported spectra (Phan et al., 2015). Two proton at A ring of myricetin was attributed by signal at δ 6.21 (d, $J = 2.1$ Hz, 1H, H-6) and 6.3 (d, $J = 2.1$ Hz, 1H, H-8), while the proton at C ring was assigned by signal at δ 7.08 (s, 2H, H-2'/H-6') (Figure 3B). The HMBC analysis revealed the correlation between H-6 and C5, C8 and C10; H-8 and C6, C9 and C10, and also between H-2'/H-6' and C1', C2'/C6', C3'/C5' and C4' (Figure 4c). The existence of myricetin in *S. polyanthum* leaves was the first time reported in this study. This compound was previously reported to inhibit α -glucosidase activity with IC_{50} 9.4 μM (Meng et al., 2016).

P. amaryllifolius Roxb was one of our sample with low GIA. Its ^1H NMR spectra showed less signals variations. The presence of coumaric acid can be detected by the signals at δ 6.40 (d, $J = 15.9$ Hz, 1H, H-2), δ 6.80 (d, $J = 8.6$ Hz, 2H, H-9) and δ 7.46 (d, $J = 8.6$ Hz, 2H, H-6/H-8) (Silva et al., 2016) (Figure 3C). The trans-p-coumaric acid isolated from *Muehlenbeckia tamnifolia* was reported to weakly inhibit the activity of α -glucosidase by 27% at maximal tested concentration (625 $\mu\text{g}/\text{ml}$) (Torres-Naranjo et al., 2016).

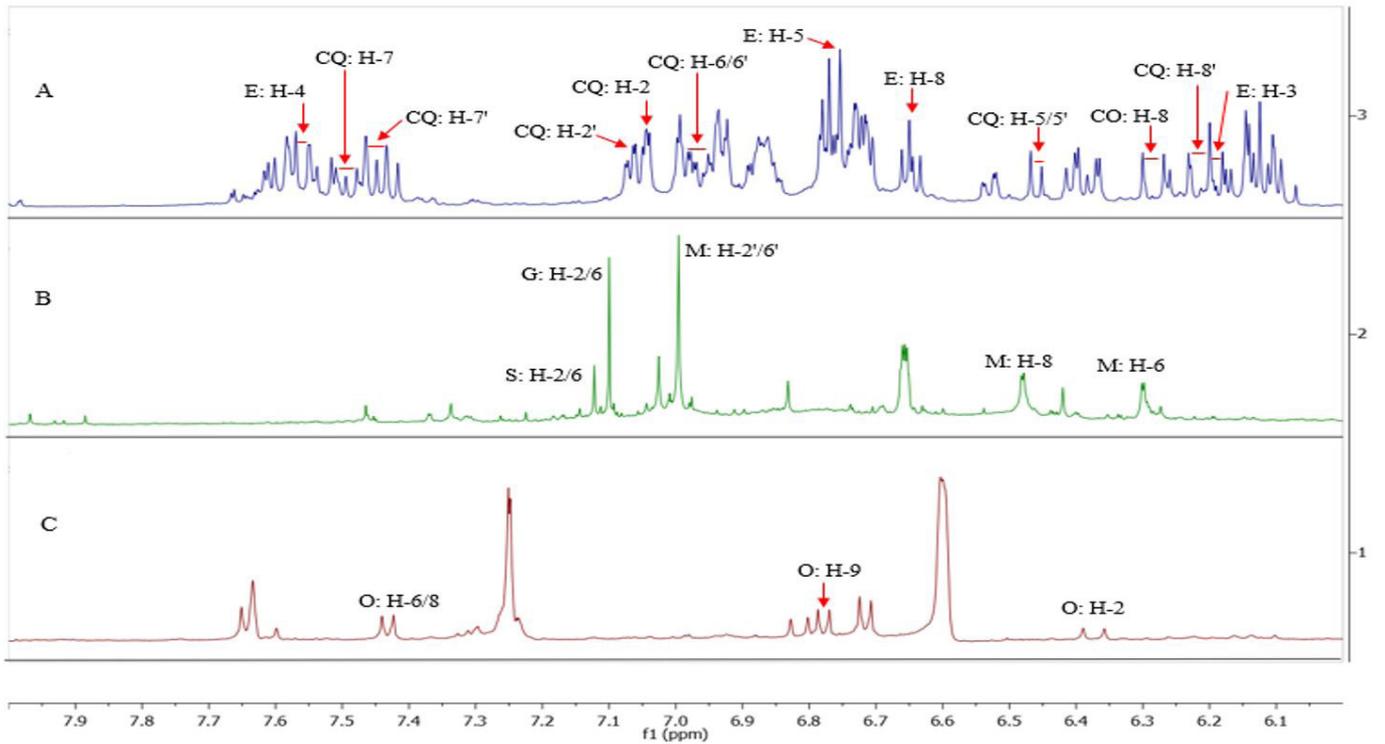


Figure 3. Aromatic area of ¹H NMR spectra (δ 6.0-8.0 ppm) of samples with high (A. *P. Indica*; B. *S. polyanthum*) and low (C. *P. amaryllifolius* Roxb) GIA. The identified component was indicated with a symbol as follows: CQ: 4,5-di-O-caffeoylquinic acid methyl ester; E: Esculetin; M: Myricetin; G: Gallic acid; S: Syringic acid; and O: Coumaric acid.

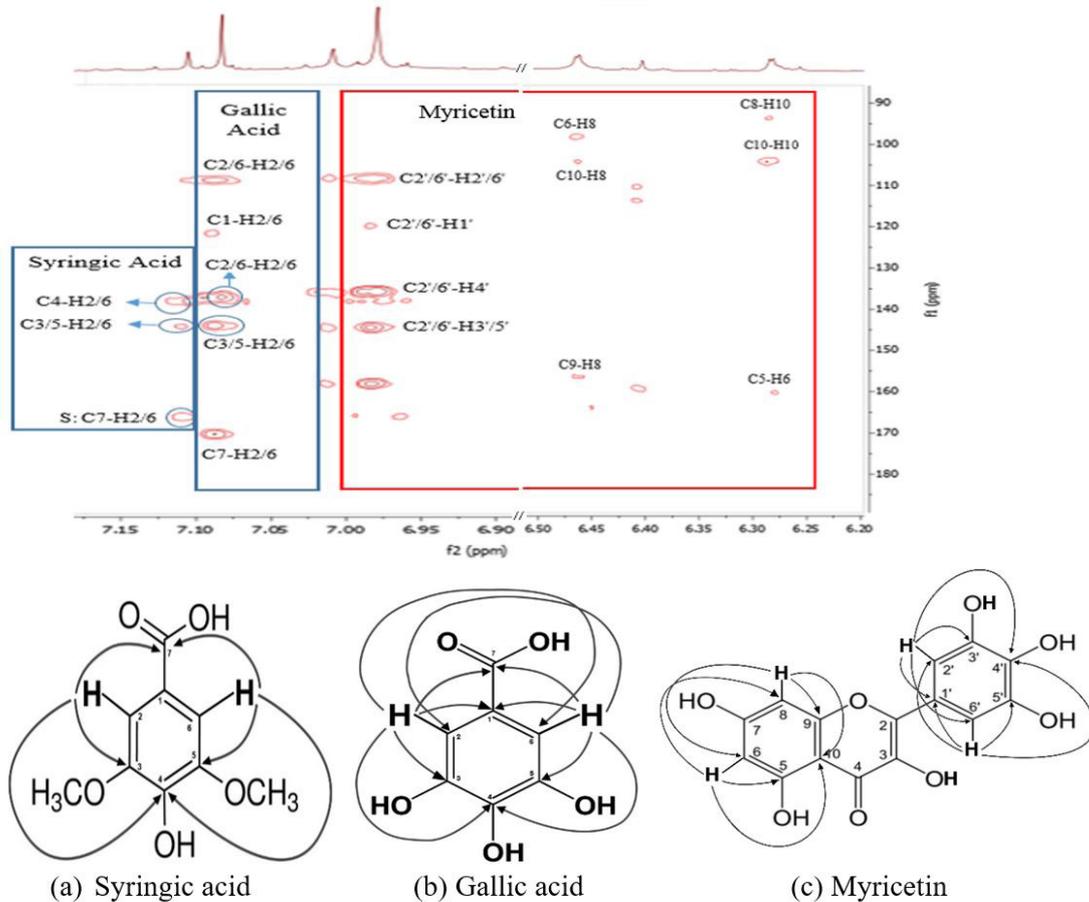


Figure 4. HMBC spectra of *S. polyanthum* extract showing typical proton-carbon correlation of (a) syringic acid, (b) gallic acid, and (c) myricetin.

4 Conclusions

The result of this study showed that *S. polyanthum* and *P. indica* extracts were the most potent antioxidants and α -glucosidase inhibitors. The TPC of both extracts were also the highest among other samples, in which *P. indica* had higher TPC than *S. polyanthum*. Phenolic regions of ^1H NMR spectra varied between samples. *P. indica* had more intense peaks at this region than *S. polyanthum* but both showed more intense typical aromatic signals than other samples. Pearson's correlation analysis showed that antioxidant activity had moderate correlation with TPC, but GIA showed strong positive correlation with TPC. Typical signals of phenolic compounds previously reported to have antidiabetic activity, such as caffeoylquinic derivatives and esculetin, were identified in ^1H NMR of *P. indica*. Typical signals of some common phenolics such as gallic acid, syringic acid, and myricetin were identified from *S. polyanthum*. Further research to identify compounds responsible for antioxidant and α -glucosidase inhibitor from these two potent plants are required.

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