

Antioxidant and antidiabetic activities of *Zingiber officinale* var. *Rubrum* extracted with natural deep eutectic solvents

^{1,3}Kartini, S., ^{1,*}Abu Bakar, M.F., ¹Abu Bakar, F.I., ³Hendrika, Y., ³Juariah, S. and ²Endrini, S.

¹Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM), KM 1, Jalan Panchor, Hub Pendidikan Tinggi Pagoh, 84600 Muar, Johor, Malaysia

²Faculty of Medicine, Abdurrah University, Jalan Riau Ujung no 73, Air Hitam, Payung Sekaki, Pekanbaru, Riau 2829, Indonesia

³Faculty of Pharmacy and Health Sciences, Abdurrah University, Jalan Riau Ujung no 73, Air Hitam, Payung Sekaki, Pekanbaru, Riau 2829, Indonesia

Article history:

Received: 5 December 2023

Received in revised form: 17

July 2024

Accepted: 25 July 2024

Available Online: 23

September 2024

Keywords:

NADES,

DPPH,

FRAP,

α -amylase inhibition,

α -glucosidase inhibition,

Zingiber officinale var.

Rubrum

DOI:

[https://doi.org/10.26656/fr.2017.8\(S5\).1](https://doi.org/10.26656/fr.2017.8(S5).1)

Abstract

Polyphenols and flavonoid compounds are known to have antioxidant activity and can reduce blood glucose levels. *Zingiber officinale* var. *Rubrum* is one of the plants with high polyphenol content. This study aimed to extract phenolic and flavonoid compounds from this plant with various solvents primarily natural deep eutectic solvents (NADES), namely 70% ethanol, NADES1 [citric acid: sucrose (1:1)], NADES2 [sucrose: glucose: fructose (1:1:1)], NADES3 [choline chloride: glycerol (1:2)], and NADES4 [glycerol: urea (1:1)]. The phenolic and flavonoid levels were determined using spectrophotometer, the antioxidant activity was measured using DPPH and Ferric Reducing Antioxidant Power (FRAP) methods, while the antidiabetic activity was evaluated using enzymatic assay of α -amylase and α -glucosidase enzymes. The findings indicated that all NADES outperformed 70% ethanol in extracting phenols, with NADES1 showing the highest phenolic content. Meanwhile, the highest flavonoid content was observed in extracts with 70% ethanol and NADES1. The results of antioxidant activity analysis using DPPH revealed that extracts with 70% ethanol, NADES1, and NADES4 exhibited considerable strength (IC₅₀: 45-49 μ g/mL), with the average values of the three solvents being not significantly different. From the results of the FRAP assay, NADES3 demonstrated the highest strength Antioxidants Activity Index (AAI: 29.24 \pm 3.08 mmol/g), whereas 70% ethanol showed the lowest strength (AAI: 22.76 \pm 5.23 mmol/g). The average AAI values for all solvents indicated no significant differences. Among the solvents, NADES1 exhibited very strong inhibitory activity against α -amylase (19.62 \pm 0.20 μ g/mL), while for α -glucosidase, NADES1 was stronger (IC₅₀: 57.36 \pm 6.08 μ g/mL) than other solvents. In conclusion, NADES can be a promising tool for extracting secondary metabolites from *Zingiber officinale* var. *Rubrum* as it shows both antioxidant and antidiabetic activities.

1. Introduction

Diabetes mellitus (DM), whose worldwide prevalence is expected to increase from 1.2% to 4.4% worldwide by 2030, is a fat and protein metabolic disorder caused by abnormalities in insulin secretion and/or action as well as a metabolic disease characterized by hyperglycemia (Teixeira *et al.*, 2000). Hyperglycemia produces free radicals, particularly reactive oxygen species (ROS) which lead to oxidative damage. Therefore, antioxidant compounds that also control blood glucose and prevent diabetes complications are absolutely essential (Bonfont-Rousselot *et al.*, 2000).

Active compounds in the polyphenol group in plants have antioxidant activity (Ghasemzadeh *et al.*, 2016; Munadi, 2018; Supu *et al.*, 2018; Tiwari and Rao, 2002) and can reduce blood glucose levels (Ridwan *et al.*, 2012) by inhibiting the α -amylase and α -glucosidase enzymes as carbohydrate catabolism so that glucose absorption does not occur.

Red ginger (*Zingiber officinale* var. *Rubrum*) is among the plants with high polyphenols. Several studies have examined the polyphenol content, antioxidant activity, and ability of this plant to inhibit the alpha-amylase enzyme. This rhizome contains about 493

*Corresponding author.

Email: fadzelly@uthm.edu.my

mg/100 g of flavonoids and 1077 mg/100 g of phenols in powdered samples (Ghasemzadeh *et al.*, 2016), has strong to very strong antioxidant activity with IC₅₀ values of 10.35-42.5 µg/mL (Munadi, 2018; Supu *et al.*, 2018), and exhibits stronger DPPH antioxidant activity than other varieties (Fajrin *et al.*, 2019; Wiendarlina and Sukaesih, 2019). Measurements using CUPRAC and DPPH methods reveal that extracts from several solvent fractions have an antioxidant capacity of 2143±0.9 µmol/g and 4256±3 µmol/g, respectively (Pratoko *et al.*, 2018). Dried capsules of red ginger are able to reduce fasting blood sugar levels in T2DM patients and have α-amylase inhibitory activity of 3.14mg/mL (Almasdy *et al.*, 2013; Arman *et al.*, 2016; Putra *et al.*, 2020). Furthermore, the functional drink formula containing this rhizome has α-glucosidase inhibitory activity at a concentration of 873.2 µg/mL (Safitri *et al.*, 2016).

Efforts to discover safe solvents to extract bioactive compounds are extremely important, taking into account not only their polarity levels (Firdayani and Winarni Agustini, 2015) but also their safety for health. The polarity of a solvent can be increased by adding a certain amount of water. In terms of toxicity, ethanol is considered the safest among existing organic solvents. Dewi *et al.* (2022) reported that ethanol mixed with water can extract the highest levels of phenolic compounds in cocoa shells compared to other organic solvents. Another study by Widarta and Arnata (2017) also showed that 70% ethanol is able to extract avocado leaves with the highest antioxidant activity. Meanwhile, alternative solvents that are also safe for health and the environment are the Natural Deep Eutectic Solvents (NADES).

NADES is a mixture of two or more compounds that have a lower melting point than one of the components (Abbott *et al.*, 2004; Hayyan *et al.*, 2010). DES can be classified as derivatives of organic acids, choline chloride, alcohols, and mixtures of other sugars (Espino *et al.*, 2016). This solvent is easy to synthesise because the salt components and the hydrogen bond donor (HBD) or complexing agent can be effortlessly mixed and converted into NADES without the need for further purification (Jhong *et al.*, 2009; Singh *et al.*, 2012). Additionally, this solvent has the ability to extract phenolic and flavonoid compounds (Dai *et al.*, 2013; Dheyab *et al.*, 2021). To the best of the authors' knowledge, no studies have been carried out on the extraction of bioactive compounds using this solvent for red ginger. Therefore, this study aims to determine the phenolic and flavonoid levels, antioxidant activity (DPPH and FRAP), and antidiabetic activity (inhibition of α-amylase and α-glucosidase enzymes) of *Zingiber officinale* var. *Rubrum* extracts with various NADES

and 70% ethanol solvent.

2. Materials and methods

2.1 Research methods

The tools and materials used in this study were: analytical scales, glassware, hot plate, magnetic stirrer, thermometer, water bath, UV-Vis spectrophotometer, centrifuge, a set of evaporation tools, 1,1-Diphenyl-2-picrylhydrazyl or DPPH (Merck), methanol, 70% ethanol, raw vitamin C, acetate buffer (pH 3.6), 2,4,6-tripyridyl-striazine or TPTZ (Sigma), FeCl₃.6H₂O, potassium persulfate, 10% aluminum chloride, potassium acetate, α-amylase enzyme, NADES (citric acid, sucrose, choline chloride, glycerol and urea) (Sigma), FeSO₄.7H₂O, DNS reagent, acarbose, phosphate buffer (pH 6.8), sodium hydroxide (Merck), gallic acid, quercetin, Folin-Ciocalteu's phenol reagent, Na₂CO₃ (Merck), sodium nitrite, aluminum nitrate, P-Nitrophenyl-α-D-glucopyranoside or PNPG (Sigma), α-glucosidase enzyme (Sigma), and *Zingiber officinale* var. *Rubrum* rhizomes.

2.2 *Zingiber officinale* var. *Rubrum* extraction

The sample was extracted as described previously by Alam *et al.* (2014). Approximately, 4 g of sample powder was mixed with 40 mL of 70% ethanol in a closed flask, then macerated for 24 hrs and repeated twice. The macerated mixture was concentrated into a thick extract. Meanwhile, bioactive compounds were extracted with NADES following the methods adopted from previous studies (Choi *et al.*, 2011; Dheyab *et al.*, 2021; Mansinhos *et al.*, 2021), by mixing 1 mL of each NADES (NADES1, NADES2, NADES3 and NADES4) with 20±0.1 mg of each powdered sample in a glass container. The sample mixture was stirred at 60-70°C using a hot plate and magnetic stirrer for 60 mins. After the sample was cooled and centrifuged, the supernatant was taken for further experiments.

2.3 Total phenolic content

The total phenolic content of the extract was tested using the method by Taga *et al.* (1984) with slight modifications. A total of 0.5 mL of Folin-Ciocalteu's phenol reagent and 7.5 mL of distilled water were added to 0.2 mL of the sample and shaken then left for 10 mins. After that, 1.5 mL of 20% sodium carbonate solution was added to the solution and let sit for 10 mins. Then, 10 mL of distilled water was added to the mixture and diluted 10 times. The absorption was measured at 760 nm. The standard curve for the total phenol equivalent of gallic acid was also prepared using Folin-Ciocalteu's phenol reagent.

2.4 Total flavonoid content

The flavonoid content of the sample was determined using a method by Abu Bakar *et al.* (2020) with slight modifications. Quercetin was used as a standard. A total of 0.3 mL of 5% sodium nitrite was added to 0.5 mL of the sample and left for 5 mins. Then, 0.6 mL of 10% aluminum nitrate was added to the solution and let sit for 5 mins. Next, 2 mL of 1M sodium hydroxide was added to the mixture and diluted 10 times. The absorption was read at a wavelength of 510 nm. Quercetin standard curve was created for a concentration range of 0.5-100 ppm.

2.5 Antioxidant activity test using 1,1-diphenyl-2-picrylhydrazyl assay

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay with a 96-well microplate was carried out using the method employed in previous studies (Zhang *et al.*, 2006; Endrini, 2011). The 1.5×10^{-4} M solution of DPPH in methanol was added to each extract with 70% ethanol and NADES solvents at various concentrations (31, 25, 62.5, 125, 250 and 500 ppm) then the reaction mixture was shaken vigorously. The amount of the remaining DPPH was determined at 490 nm. The radical scavenging activity was measured by the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{[\text{Abs control} - \text{Abs extract}]}{(\text{Abs control})} \times 100$$

The antioxidant activity of plant extracts is partially expressed as IC_{50} , which is defined as the concentration (in ppm) of the extract required to inhibit the formation of DPPH radicals by 50%.

2.6 Antioxidant activity test using ferric reducing antioxidant power assay

The FRAP assay used the method by Abu Bakar *et al.* (2009). FRAP reagent was prepared by mixing 300 mM of acetate buffer solution (pH 3.6), 10 mM of 2,4,6-tripyridyl-striazine (TPTZ), and 20 mM of $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1, then heated to 37°C in a water bath. A total of 100 μ L of extract sample and 300 μ L of distilled water were added to the cuvette. After adding the sample mixture to the FRAP reagent, a second reading at 593 nm was taken after 4 mins. The change in absorbance after 4 mins from the initial blank reading was compared to the $FeSO_4$ standard curve, from which the FRAP value of the sample was determined. To determine the concentration of antioxidants in the sample, the absorbance of the standard solution was plotted against its concentration to create a standard curve and compare the sample's absorbance to this curve.

2.7 α -amylase inhibitory activity assay

The α -amylase inhibitory activity assay followed the method from previous studies (Nair *et al.*, 2013; Ali *et al.*, 2020). The mixture containing 200 μ L of sodium phosphate buffer (pH 5.9), 200 μ L of α -amylase enzyme, and 70% ethanol extract in the concentration range of 20 -100 μ g/mL was incubated for 10 mins at room temperature, then 200 g/mL was added to the solution. The reaction was stopped by adding 400 μ L of DNS reagent and boiling for 5 mins. After cooling, the mixture was diluted with water, and the absorbance was measured at 540 nm. Acarbose was used to control the inhibition calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{[\text{Abs control} - \text{Abs extract}]}{(\text{Abs control})} \times 100$$

The IC_{50} value was determined from a plot of percentage inhibition versus log inhibitor concentration and calculated by non-linear regression analysis of the mean inhibition value.

2.8 α -glucosidase inhibitory activity assay

The α -glucosidase inhibitory activity assay was performed using a method modified from that proposed by Nair *et al.* (2013). First, α -glucosidase enzyme was dissolved in 100 mM of phosphate buffer (pH 6.8) for use as an enzyme extract. Meanwhile, PNPG was used as a substrate. *Zingiber officinale* var. Rubrum extracts were made in varying concentrations of 10-100 g/mL. Each of these extracts was mixed with phosphate buffer (pH 6.8) at 30°C for 5 mins. After that, 3 mL of 50 mM sodium hydroxide was added to the mixture, then the absorbance was read at 540 nm. Percent inhibition was calculated using the formula below:

$$\text{Inhibition (\%)} = \frac{[\text{Abs control} - \text{Abs extract}]}{(\text{Abs control})} \times 100$$

The IC_{50} value was determined from a plot of percentage inhibition versus log inhibitor concentration and calculated by non-linear regression analysis of the mean inhibition value. Acarbose was used as a reference α -glucosidase inhibitor.

2.8 Data analysis

The data obtained were analyzed using Analysis of Variance (One-way ANOVA) with post-hoc Tukey Honestly Significant Difference (HSD) for comparison.

3. Results and discussion

3.1 Total phenolic and flavonoid contents

As seen in Figure 1 and Table 1, all NADESs can extract more phenolics (35.32 ± 0.7 to 69.6 ± 1.2 mg GAE/g), whereas 70% ethanol can extract more flavonoid

compounds (105.1 ± 0.01 mg GAE/g). The average levels of phenols and flavonoids in the five solvents were significantly different, with the flavonoids in 70% ethanol and NADES1 having the highest levels. Meanwhile, those in NADES2, NADES3, and NADES4 were not significantly different.

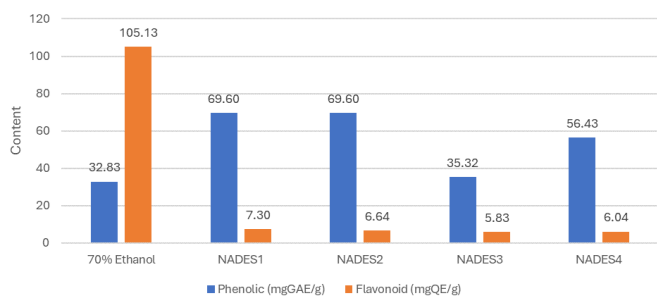


Figure 1. Phenolic and flavonoid contents in various solvents.

Table 1. Total phenolic and flavonoid contents of *Zingiber officinale* var. Rubrum.

Samples	Phenolic (mg GAE/g)	Flavonoid (mg QE/g)
Ethanol 70%	32.8 ± 0.01^a	105.1 ± 0.01^a
NADES 1	69.603 ± 1.198^b	7.305 ± 0.65^b
NADES 2	40.397 ± 0.991^c	6.638 ± 0.11^c
NADES 3	35.317 ± 0.727^d	5.829 ± 0.26^c
NADES 4	56.429 ± 0.952^c	6.043 ± 0.31^c

Values are presented as mean \pm SD. Values with different superscript within the same column are statistically significantly different ($p < 0.05$) based on the ANOVA test.

The extraction of phenolic compounds from natural materials with NADES is a solid-liquid extraction process. As mentioned previously, NADES is a mixture of two or more components, making it capable of forming a liquid mixture with a melting point far below that of its individual constituents due to hydrogen bonding interactions between the hydroxyl groups on its components, which is also assisted by water. The heating process during the preparation of NADES helps the formation of more hydrogen bonds (Datu *et al.*, 2019). In addition, the solubility of phenols and flavonoids in NADES is also possible due to hydrogen bonds formed between the compounds and NADES, as demonstrated in quercetin with xylitol-choline chloride-water (1:2:3) (Dai *et al.*, 2013). Furthermore, Oscar Zannou (2022) also found that various NADESs can extract more phenols in blackberry fruit than ethanol, with the phenolic levels ranging from 1.20 ± 0.17 to 9.35 ± 0.39 mg GAE/g.

Among the variety of NADES used in this study, the highest phenolic compound was obtained from the acidic NADES1 (citric acid and sucrose in a ratio of 1:1). The citric acid in this NADES acts as HBA, whereas the sucrose acts as HBD. This finding confirms a previous study by Datu *et al.* (2019) which found that acidic NADES (lactic acid-sucrose) can extract 573,443 mg

GAE/g of phenols. Meanwhile, as a group of sugar derivatives, NADES2 is not much different from NADES1, with the pH value of NADES2 (pH 1.5) being higher than NADES1 (pH 2.8).

In NADES4, glycerol acts as a hydrogen bond acceptor (HBA) and urea acts as a hydrogen bond donor (HBD), resulting in a covalent hydrogen bond interaction with the phenols. Phenols are also soluble in choline chloride and glycerol-based NADES3 (1:2) because of the mass transfer from solid to liquid due to the same polarity between NADES and phenolic compounds (Lu and Liu, 2022). The same NADES used in the study of Dheyab *et al.* (2022) also extracted more phenols than other compounds in the *Eucalyptus camaldulensis* (eucalyptus) plant. Similarly, in another study by Razborsk *et al.* (2020), five types of DES with choline chloride as the basic ingredient were all able to extract more phenols from Chokeberry (*Aronia melanocarpa*).

The ability of 70% ethanol to extract compounds depends on its polarity, and the addition of 30% water can increase the polarity of ethanol. The results of this study revealed the highest total flavonoid content in *Zingiber officinale* var. Rubrum extract with 70% ethanol (105.1 ± 0.01 mg GAE/g). This is in line with the finding of a study by Suhendra *et al.* (2019) that 70% ethanol can extract the highest amount of flavonoids (90.91 ± 0.78 mg GAE/g) from *Imperata cylindrica* (L) Beauv extract among other ethanol solvents with varying concentrations of 40% to 90%. Flavonoids contain glycoside compounds that are more easily soluble in water (Arifin and Ibrahim, 2018).

3.2 Antioxidant activity

Antioxidants are essential chemicals in suppressing oxidative stress which plays a key role in the development of degenerative diseases such as diabetes mellitus. In this regard, antioxidants act as free radical inhibitors to help cell repair. In this study, the testing of the antioxidant activity of *Zingiber officinale* var. Rubrum extracts with 70% ethanol and four types of NADES adopted the DPPH radical method with a 96-well microplate which is more common and easier to do (Kusumadewi *et al.*, 2022). The mechanism of this test is as follows: antioxidant reacts with DPPH to form stable diphenyl picryl hydrazine, and the antioxidant effect is comparable to the loss of the purple color (Biswas *et al.*, 2022). The uptake is measured as the radical scavenging activity (Pokorný *et al.*, 2001; Putkaradze *et al.*, 2021), and the inhibitory activity is expressed in IC_{50} , where $IC_{50} < 50$ μ g/L is considered very strong, 50-100 μ g/mL is strong, 100-150 μ g/mL is moderate, and 151-200 μ g/mL is weak (Zuhra *et al.*, 2008; Santoso *et al.*, 2022). Testing antioxidant activity using the FRAP method is

determining antioxidants based on the ability of antioxidant compounds to reduce Fe^{3+} ions to Fe^{2+} , where the antioxidant activity is analogous to the reducing ability of these compounds (Maryam *et al.*, 2016). The antioxidant activity is expressed by the AAI (Antioxidant Activity Index) value; the greater the AAI value, the stronger the activity. The results of this test can be seen in Figure 2 and Table 2.

Table 2. Results of antioxidant activity test with different methods (DPPH and FRAP).

Samples	Antioxidant Activity	
	(DPPH) IC_{50} ($\mu\text{g}/\text{mL}$)	(FRAP) AAI (mmol/g)
Et-OH	45.07±0.28 ^a	22.76±5.23 ^a
NADES 1	49.14±4.01 ^a	27.01±4.41 ^a
NADES 2	132.46±0.11 ^b	27.12±4.20 ^a
NADES 3	182.61±4.9 ^c	29.24±3.08 ^a
NADES 4	47.26±0.3 ^a	25.97±14.69 ^a
Ascorbic Acid	7.93±4.4 ^d	53.22±2.56 ^b

Values are presented as mean±SD. Values with different superscript within the same column are statistically significantly different ($p < 0.05$) based on the ANOVA test.

Pearson Correlation of phenols with DPPH is moderate and negative ($r = -0.519$).

Pearson Correlation of flavonoids with DPPH is moderate and negative ($r = -0.416$).

Pearson Correlation of flavonoids with FRAP is very weak and negative (-0.275), while that of phenols with FRAP is very weak and positive ($r = 0.041$).

Among the four NADESs, the IC_{50} values of NADES1 and NADES4 were categorized as very strong (IC_{50} : 47.26±0.3 to 49.14±4.01 $\mu\text{g}/\text{mL}$) and were not significantly different ($p < 0.05$) compared to the other two NADESs. The antioxidant power of NADES1 and NADES4 is closely related to their higher phenolic content than the other NADESs. Furthermore, the Pearson correlation of phenols with DPPH shows a moderate and negative relationship ($r = -0.519$), meaning that the higher the phenolic content, the smaller the IC_{50} value and the stronger the antioxidant activity. Phenols capture DPPH radicals so that they are oxidized to produce a more stable radical form. Radical scavenging activity is determined by the amount of hydroxy (OH) bonded to the aromatic ring of the benzoyl or cinnamic acid molecule. The methoxy group bonded to positions 3 and 5 of the aromatic ring can increase the radical scavenging activity of phenolic acid compounds (Nenadis *et al.*, 2003). This finding supports those of previous studies (Abu Bakar *et al.*, 2015; Wardani *et al.*, 2020; Kartini *et al.*, 2023) that there is a relationship between phenolic content and antioxidant activity. The extract with 70% ethanol has far greater flavonoid content than the other extracts. Flavonoid captures DPPH radicals so that they are oxidized to produce more stable radicals with low reactivity. In addition, flavonoids donate hydrogen radicals (H^{\bullet}) from their aromatic rings to reduce toxic free radicals and produce flavonoid radicals whose resonance is stabilized, thus making them

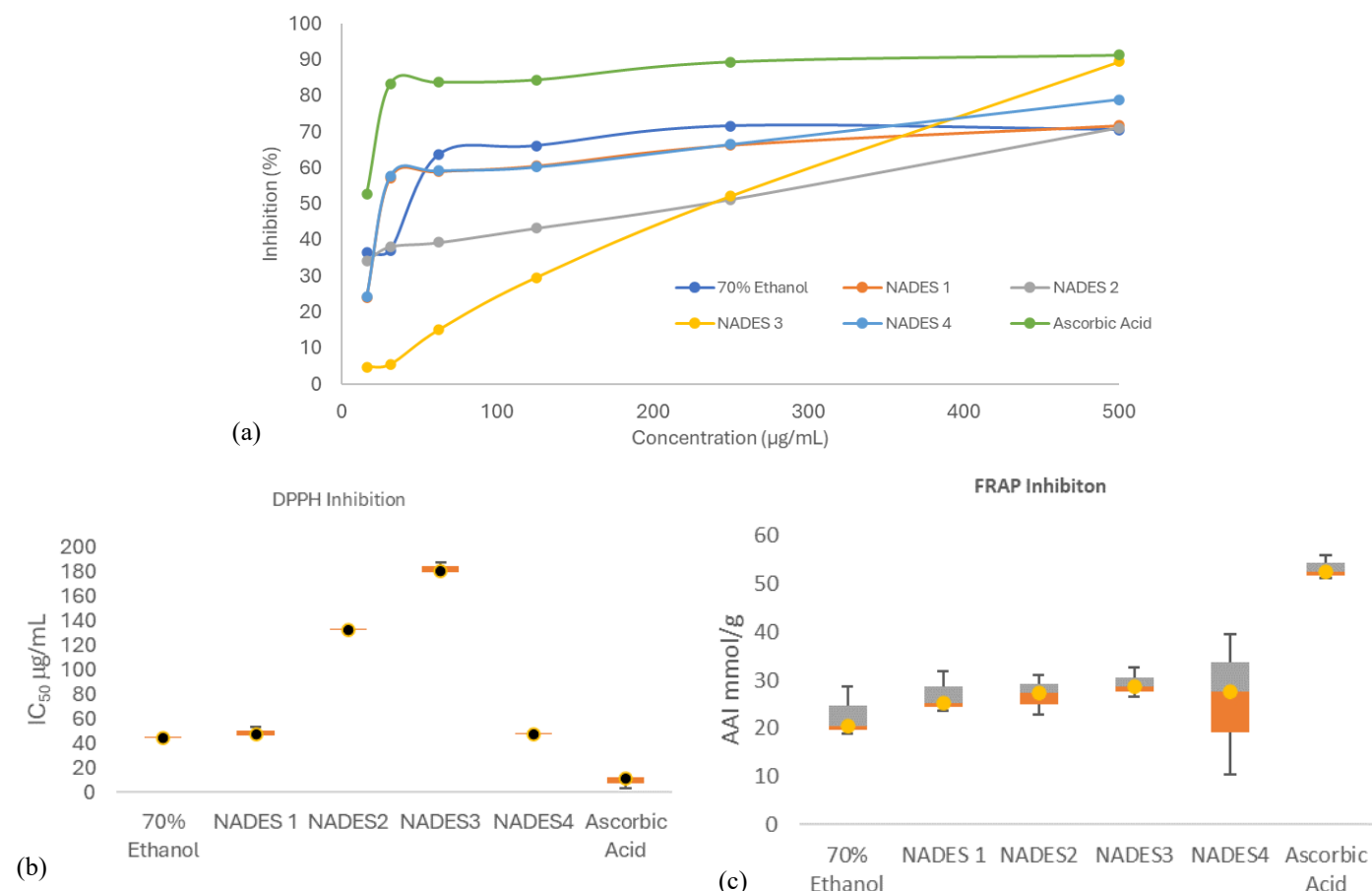


Figure 2. (a). Percentage of inhibition, (b) Box plot of IC_{50} , (c) Box plot of AAI of different solvents.

non-toxic (Amić *et al.*, 2003). Pearson correlation of flavonoids with DPPH has a moderate and negative relationship ($r = -0.416$), which means that the higher the flavonoid content, the smaller the IC_{50} and the stronger the antioxidant activity.

The FRAP method was applied in this study to evaluate the potential of *Zingiber officinale* var. Rubrum extracts with NADES to act as a reducing agent. The highest potential for reducing Fe^{3+} to Fe^{2+} is found in extract with NADES3 (29.24 ± 3.08 mmol/g). Meanwhile, the lowest potential is observed in extract with 70% ethanol (22.76 ± 5.23 mmol/g), indicating the lowest ability to transfer electrons. Although all solvents show no significant differences, the extracts of *Zingiber officinale* var. Rubrum with NADES and 70% ethanol exhibit the ability to transfer electrons. This is in line with a study by Jurić *et al.* (2021) which found that there is no significant difference in all peppermint extracts with sugar-based choline chloride NADES. Pearson correlation of phenols with FRAP is very weak and positive, with AAI ($r = 0.041$). This means that the greater the phenolic content, the greater the AAI value but the strength is very weak. On the other hand, the

Pearson correlation of flavonoids with FRAP is very weak and negative, with AAI ($r = -0.275$), meaning that the greater the flavonoid content, the smaller the AAI value and the strength is also very weak.

3.3 Inhibitory activity of α -amylase and α -glucosidase enzymes

The α -amylase and α -glucosidase enzymes are key enzymes in carbohydrate digestion; α -amylase breaks down starch into dextrin and is further hydrolysed into glucose by the α -glucosidase enzyme. Inhibition of these enzymes will delay glucose absorption in the intestine to reduce postprandial blood glucose levels. Phenolic compounds and flavonoids in plants which have hydroxyl (OH) functional groups exhibit an inhibitory effect on the α -amylase enzyme through hydroxylation and substitution bonds in the β ring whose principle is similar to that of acarbose (Feng *et al.*, 2011; Pawestri *et al.*, 2021). The results of the analysis of α -amylase and α -glucosidase inhibitory activities in *Zingiber officinale* var. Rubrum extracted with various solvents can be seen in Figure 3 and Table 3.

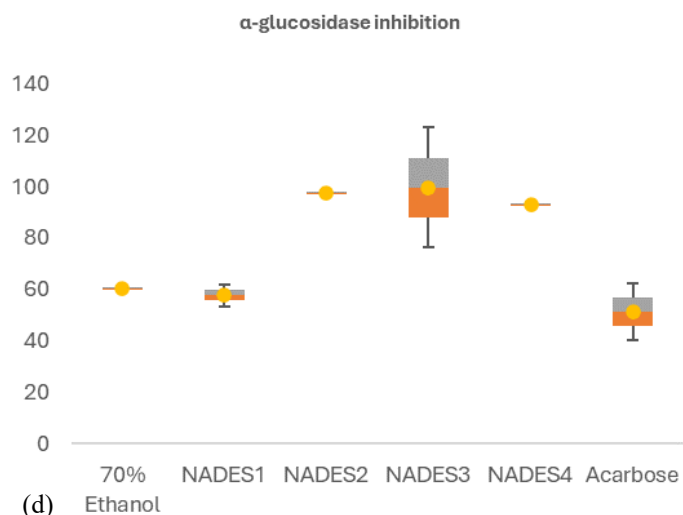
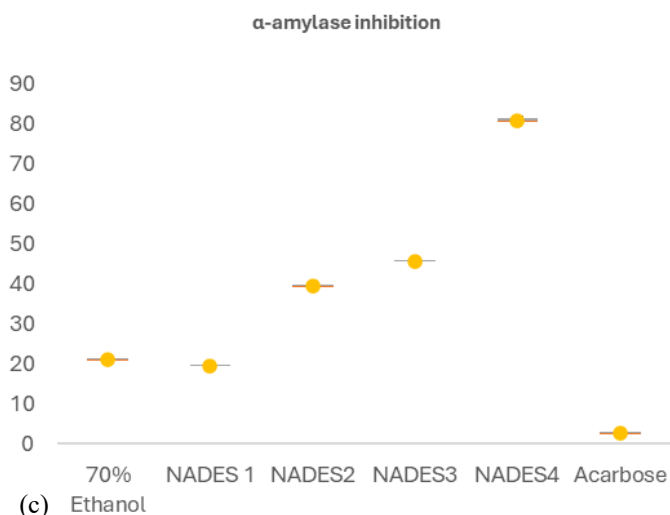
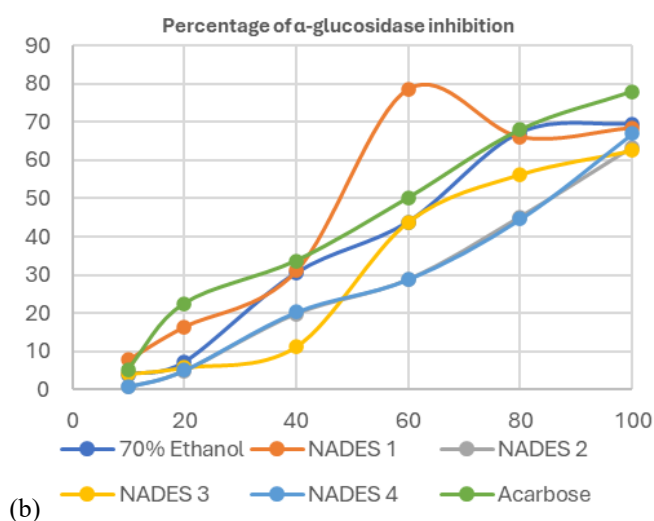
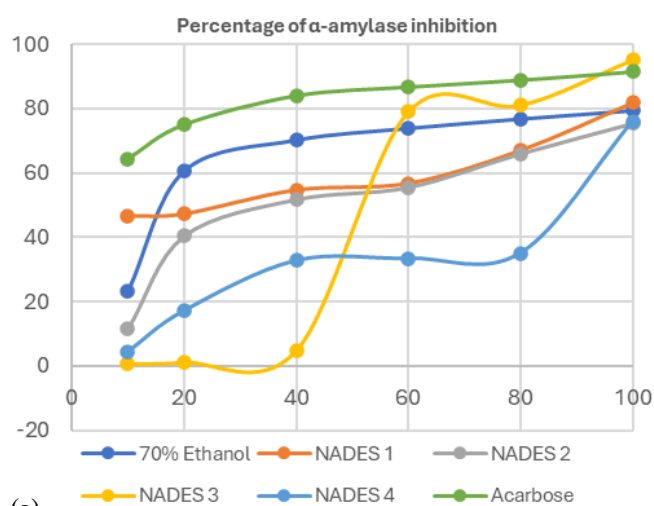


Figure 3. (a) Percentages of α -amylase inhibition, (b) percentages of α -glucosidase inhibition, (c) box plots of α -amylase inhibition, (d) box plots of α -glucosidase inhibition.

Table 3 displays the antioxidant activities of α -amylase and α -glucosidase inhibitions. The inhibitory activity of the α -amylase enzyme in all solvents except NADES4 has IC_{50} value varying from strong to very strong (IC_{50} : 21.29 \pm 0.30 to 80.8 \pm 0.81 μ g/mL). The Pearson correlation of phenolic content with α -amylase inhibition is very weak and positive ($r = 0.090$), while that of flavonoid content with α -amylase is moderate and negative (-0.468). As for the α -glucosidase enzyme, the inhibitory activity in various solvents falls into the strong category (IC_{50} : 60.29 \pm 0.37 to 97.35 \pm 0.74 μ g/mL), except for NADES1 which shows very strong activity (IC_{50} : 57.36 \pm 6.08 μ g/mL) and has the highest phenolic and flavonoid content among all solvents. The Pearson correlation of phenols with the α -glucosidase enzyme is weak and negative ($r = -0.271$), which means that the greater the phenolic content, the smaller the IC_{50} value. Meanwhile, the Pearson correlation of total flavonoids with the α -glucosidase enzyme is negative and moderate ($r = -0.487$). Overall, flavonoids contribute strongly to the inhibition of the α -amylase and α -glucosidase enzymes in *Zingiber officinale* var Rubrum extracts; the higher the flavonoid content, the smaller the IC_{50} value. A similar study by Xia *et al.* (2023) on α -glucosidase inhibition in the flavonoid group Astilbin also found α -glucosidase inhibitory activity in *Engelhardia roxburghiana* Wall. and *Smilax glabra* Roxb extracts with choline chloride: lactic acid NADES (IC_{50} : 0.64 g/L). Flavonoids act as non-competitive inhibitors of enzymes, whereas substrates can bind enzymes simultaneously on different bond sides so that the enzyme cannot be active. On the other hand, competitive

inhibition of enzymes by mimicking substrates and binding to the active site of the enzyme can cause the enzyme to experience reduced activity, or even make it carry out no activity at all (Abu Bakar *et al.*, 2018).

4. Conclusion

From the results of this study, it can be concluded that NADES is able to extract *Zingiber officinale* var. Rubrum with higher phenols than flavonoids, while 70% ethanol can extract higher flavonoids than NADES. Thus, NADES can be a great tool for the extraction of secondary metabolites in *Zingiber officinale* var. Rubrum which have antioxidant and antidiabetic activities.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study is funded by Abdurrah University through the 2021 Internal Grant Program for Basic Research Excellence for Collaborative Higher Education (*Penelitian Dasar Unggulan Perguruan Tinggi Kerjasama/PDUPT-K*) scheme in collaboration with Universiti Tun Hussein Onn Malaysia.

References

- Abbott, A.P., Boothby, D., Capper, G., Davies, D.L. and Rasheed, R.K. (2004). Deep eutectic solvents formed between choline chloride and carboxylic acids: Versatile alternatives to ionic liquids. *Journal of the American Chemical Society*, 126(29), 9142–9147. <https://doi.org/10.1021/ja048266j>
- Abu Bakar, F.I., Abu Bakar, M.F., Abdullah, N., Endrini, S. and Fatmawati, S. (2020). Optimization of extraction conditions of phytochemical compounds and anti-gout activity of *Euphorbia hirta* L. (ara tanah) using response surface methodology and liquid chromatography-mass spectrometry (LC-MS) Analysis. *Evidence-Based Complementary and Alternative Medicine*, 2020, 4501261. <https://doi.org/10.1155/2020/4501261>
- Abu Bakar, F.I., Abu Bakar, M.F., Abdullah, N., Endrini, S. and Rahmat, A. (2018). A review of Malaysian medicinal plants with potential antidiabetic. *Advances in Pharmacological Sciences*, 2018(1), 8603602. <https://doi.org/10.1155/2018/8603602>
- Abu Bakar, M.F., Ahmad, N.E., Suleiman, M., Rahmat, A. and Isha, A. (2015). *Garcinia dulcis* Fruit Extract Induced Cytotoxicity and Apoptosis in HepG2 Liver cancer cell line. *BioMed Research International*, 201, 916902. <https://doi.org/10.1155/2015/916902>

Table 3. Antidiabetic activity of *Zingiber officinale* var. Rubrum in different solvents.

Samples	Antidiabetic Activity	
	α -amylase Inhibition	α -glucosidase Inhibition
	IC_{50} (μ g/mL)	IC_{50} (μ g/mL)
Et-OH	21.29 \pm 0.30 ^a	60.29 \pm 0.37 ^a
NADES 1	19.78 \pm 0.41 ^a	57.36 \pm 6.08 ^a
NADES 2	39.39 \pm 0.59 ^b	97.35 \pm 0.74 ^a
NADES 3	45.57 \pm 0.10 ^c	80.25 \pm 33 ^a
NADES 4	80.8 \pm 0.81 ^d	92.85 \pm 0.22 ^a
Acarbose	2.53 \pm 0.18 ^e	49.94 \pm 15.3 ^a

Values are presented as mean \pm SD. Values with different superscript within the same column are statistically significantly different ($p < 0.05$) based on the ANOVA test.

Pearson Correlation of phenols with α -amylase is very weak and positive ($r = 0.090$).

Pearson Correlation of flavonoids with α -amylase is moderate and negative ($r = -0.468$).

Pearson Correlation of phenols with α -glucosidase ($r = -0.271$).

Pearson Correlation of flavonoids with α -glucosidase ($r = -0.487$).

- Abu Bakar, M.F., Mohamed, M., Rahmat, A. and Fry, J. (2009). Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). *Food Chemistry*, 113(2), 479–483. <https://doi.org/10.1016/j.foodchem.2008.07.081>
- Alam, M.A., Juraimi, A.S., Rafii, M.Y., Abdul Hamid, A., Aslani, F., Hasan, M.M., Mohd Zainudin, M.A. and Uddin, M.K. (2014). Evaluation of antioxidant compounds, antioxidant activities, and mineral composition of 13 collected purslane (*Portulaca oleracea* L.) accessions. *BioMed Research International*, 2014, 296063. <https://doi.org/10.1155/2014/296063>
- Ali, H., Abu Bakar, M.F., Majid, M., Muhammad, N. and Lim, S.Y. (2020). In vitro anti-diabetic activity of stingless bee honey from different botanical origins. *Food Research*, 4(5), 1421–1426. [https://doi.org/10.26656/fr.2017.4\(5\).411](https://doi.org/10.26656/fr.2017.4(5).411)
- Almasdy, D., Martini, R.D. and Arman, E. (2013). The effect of dried red ginger powder (*Zingiber officinale*) on patients with type 2 diabetes mellitus. Proceeding of "The International Conference on Herbal Medicine Industrialization as Complementary Therapy in Natural Disasters", p. 87–94. Yogyakarta, Indonesia.
- Amić, D., Davidović-Amić, D., Bešlo, D. and Trinajstić, N. (2003). Structure-radical scavenging activity relationships of flavonoids. *Croatica Chemica Acta*, 76(1), 55–61.
- Arifin, B. and Ibrahim, S. (2018). Struktur, bioaktivitas dan antioksidan flavonoid. *Jurnal Zarah*, 6(1), 21–29. <https://doi.org/10.31629/zarah.v6i1.313> [In Bahasa Indonesia].
- Arman, E., Almasdy, D. and Martini, D. (2016). Pengaruh pemberian serbuk kering jahe merah terhadap pasien diabetes melitus tipe 2 *Jurnal Ipteks Terapan*, 10(3), 161–169. <https://doi.org/10.22216/jit.2016.v10i3.523> [In Bahasa Indonesia].
- Biswas, S.K., Dana, S. and Pathak, P.S. (2022). In vitro anti-diabetic and anti-oxidant activities of *Oroxylum indicum* (Kurtz): a potent wild medicinal plant north-eastern region in India. *Pharmacognosy and Phytochemistry*, 11(5), 38-44.
- Bonnefont-Rousselot, D., Bastard, J.P., Jaudon, M.C. and Delattre, J. (2000). Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes and Metabolism*, 26(3), 163–176.
- Choi, Y.H., van Spronsen, J., Dai, Y., Verberne, M., Hollmann, F., Arends, I.W.C.E., Witkamp, G.J. and Verpoorte, R. (2011). Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology? *Plant Physiology*, 156(4), 1701–1705. <https://doi.org/10.1104/pp.111.178426>
- Dai, Y., Witkamp, G.J., Verpoorte, R. and Choi, Y.H. (2013). Natural deep eutectic solvents as a new extraction media for phenolic metabolites in *Carthamus tinctorius* L. *Analytical Chemistry*, 85(13), 6272–6278. <https://doi.org/10.1021/ac400432p>
- Datu, K.A.T., Fitriani, N. and Ahmad, I. (2019). Pengaruh Penggunaan Metode Lactic Acid-Sucrose dengan Microwave Assisted Extraction (MAE) terhadap Polifenol Total dari Herba Suruhan (*Peperomia pellucida* (L.) Kunth). *Proceeding of Mulawarman Pharmaceuticals Conferences*, 10, 114–117. <https://doi.org/10.25026/mpc.v10i1.373> [In Bahasa Indonesia].
- Dewi, S.R., Stevens, L.A., Pearson, A.E., Ferrari, R., Irvine, D.J. and Binner, E.R. (2022). Investigating the role of solvent type and microwave selective heating on the extraction of phenolic compounds from cacao (*Theobroma cacao* L.) pod husk. *Food and Bioproducts Processing*, 134, 210–222. <https://doi.org/10.1016/j.fbp.2022.05.011>
- Dheyab, A.S., Abu Bakar, M.F., AlOmar, M., Sabran, S.F., Muhamad Hanafi, A.F. and Mohamad, A. (2021). Deep eutectic solvents (DESs) as green extraction media of beneficial bioactive phytochemicals. *Separations*, 8(10), 176. <https://doi.org/10.3390/separations8100176>
- Dheyab, A.S., Ibrahim, A.J.K., Aljumily, E.K., AlOmar, M.K., Bakar, M.F.A. and Sabran, S.F. (2022). Antimycobacterial activity and phytochemical properties of *Eucalyptus camaldulensis* (eucalyptus) extracted by deep eutectic solvents. *Materials Today: Proceedings*, 65(5), 2738–2742. <https://doi.org/10.1016/j.matpr.2022.06.017>
- Endrini, S. (2011). Antioxidant activity and anticarcinogenic properties of "rumput mutiara" (*Hedyotis corymbosa* (L.) Lam.) and "pohpohan" (*Pilea trinervia* (Roxb.) Wight). *Journal of Medicinal Plants Research*, 5(16), 3715–3718.
- Espino, M., Fernández, M.D.L.A., Gomez, F.J.V. and Silva, M.F. (2016). Natural designer solvents for greening analytical chemistry. *TrAC - Trends in Analytical Chemistry*, 76, 126–136. <https://doi.org/10.1016/j.trac.2015.11.006>
- Fajrin, F.A., Imandasari, N., Barki, T., Sulistyningrum, G., Afifah, Kristiningrum, N., Puspitasari, E. and Holiday, D. (2019). The activity of red ginger oil in antioxidant study in vitro and antihyperalgesia effect in alloxan-induced painful diabetic neuropathy in mice. *Thai Journal of Pharmaceutical Sciences*, 43(2), 69–75.
- Feng, J., Yang, X.W. and Wang, R.F. (2011). Bio-assay guided isolation and identification of α -glucosidase

- inhibitors from the leaves of *Aquilaria sinensis*. *Phytochemistry*, 72(2–3), 242–247. <https://doi.org/10.1016/j.phytochem.2010.11.025>
- Firdayani, F. and Winarni Agustini, T. (2015). Ekstraksi senyawa bioaktif sebagai antioksidan alami *Spirulina platensis* segar dengan pelarut yang berbeda. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 18(1), 28–37. <https://doi.org/10.17844/jphpi.2015.18.1.28> [In Bahasa Indonesia].
- Ghasemzadeh, A., Jaafar, H.Z.E. and Rahmat, A. (2016). Variation of the phytochemical constituents and antioxidant activities of *Zingiber officinale* var. *rubrum* Theilade associated with different drying methods and polyphenol oxidase activity. *Molecules*, 21(6), 780. <https://doi.org/10.3390/molecules21060780>
- Hayyan, M., Mjalli, F.S., Hashim, M.A. and AlNashef, I.M. (2010). A novel technique for separating glycerine from palm oil-based biodiesel using ionic liquids. *Fuel Processing Technology*, 91(1), 116–120. <https://doi.org/10.1016/j.fuproc.2009.09.002>
- Jhong, H.R., Wong, D.S.H., Wan, C.C., Wang, Y.Y. and Wei, T.C. (2009). A novel deep eutectic solvent-based ionic liquid used as electrolyte for dye-sensitized solar cells. *Electrochemistry Communications*, 11(1), 209–211. <https://doi.org/10.1016/j.elecom.2008.11.001>
- Jurić, T., Mičić, N., Potkonjak, A., Milanov, D., Dodić, J., Trivunović, Z. and Popović, B.M. (2021). The evaluation of phenolic content, in vitro antioxidant and antibacterial activity of *Mentha piperita* extracts obtained by natural deep eutectic solvents. *Food Chemistry*, 362, 130226. <https://doi.org/10.1016/j.foodchem.2021.130226>
- Kartini, S., Juariah, S., Mardhiyani, D., Abu Bakar, M.F., Abu Bakar, F.I. and Endrini, S. (2023). Phytochemical properties, antioxidant activity and α -amilase inhibitory of *Curcuma Caesia*. *Journal of Advanced Research in Applied Sciences and Engineering Technology*, 30(1), 255–263. <https://doi.org/10.37934/araset.30.1.255263>
- Kusumadewi, A.P., Martien, R., Pramono, S., Setyawan, A.A., Windarsih, A. and Rohman, A. (2022). Application of FTIR spectroscopy and chemometrics for correlation of antioxidant activities, phenolics and flavonoid contents of Indonesian *Curcuma xanthorrhiza*. *International Journal of Food Properties*, 25(1), 2364–2372. <https://doi.org/10.1080/10942912.2022.2134418>
- Lu, W. and Liu, S. (2022). Choline chloride-based deep eutectic solvents (Ch-DESs) as promising green solvents for phenolic compounds extraction from bioresources: state-of-the-art, prospects, and challenges. *Biomass Conversion and Biorefinery*, 12(7), 2949–2962. <https://doi.org/10.1007/s13399-020-00753-7>
- Mansinhos, I., Gonçalves, S., Rodríguez-Solana, R., Ordóñez-Díaz, J.L., Moreno-Rojas, J.M. and Romano, A. (2021). Ultrasonic-assisted extraction and natural deep eutectic solvents combination: a green strategy to improve the recovery of phenolic compounds from *Lavandula pedunculata* subsp. *lusitanica* (Chaytor) Franco. *Antioxidants*, 10(4), 582. <https://doi.org/10.3390/antiox10040582>
- Maryam, S., Baits, M. and Nadia, A. (2016). Pengukuran aktivitas antioksidan ekstrak etanol daun kelor (*moringa oleifera lam.*) menggunakan metode frap (Ferric Reducing Antioxidant Power). *Jurnal Fitofarmaka Indonesia*, 2(2), 115–118. <https://doi.org/10.33096/jffi.v2i2.181> [In Bahasa Indonesia].
- Munadi, R. (2018). Analisis komponen kimia dan uji antioksidan ekstrak rimpang merah (*Zingiber officinale* Rosc. Var. *rubrum*). *Cokroaminoto Journal of Chemical Science*, 2, 1–6. [In Bahasa Indonesia].
- Nair, S.S., Kavrekar, V. and Mishra, A. (2013). In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. *European Journal of Experimental Biology*, 3(1), 128–132.
- Nenadis, N., Zhang, H.-Y. and Tsimidou, M.Z. (2003). Structure–antioxidant activity relationship of ferulic acid derivatives: effect of carbon side chain characteristic groups. *Journal of Agricultural and Food Chemistry*, 51(7), 187–1879. <https://doi.org/10.1021/jf0261452>
- Oscar Zannou, I.K. (2022). Greener extraction of anthocyanins and antioxidant activity from blackberry (*Rubus* spp) using natural deep eutectic solvents. *LWT Food Science and Technology*, 158, 113184. <https://doi.org/113184>
- Pawestri, S., Wijayanti, R. and Kurniantio, D. (2021). Kajian Pustaka : Potensi kandungan polifenol pada *Sargassum sp* . sebagai alternatif penanganan diabetes mellitus tipe 2. *Jurnal Ilmu Pangan Dan Hasil Pertanian*, 6(2), 13–34. <https://doi.org/10.26877/jiphp.v5i2.8988> [In Bahasa Indonesia].
- Pokorný, J., Yanishlieva, N. and Gordon, M. (2001). *Antioxidants in Food: Practical Applications*. Cambridge, UK: Woodhead Publishing.
- Pratoko, D.K., Firdha Aprillia Wardhani, Nia Kristiningrum, Fifteen Aprilia Fajrin, D. and Pangaribowo, A. (2018). Kadar fenolat dan flavonoid total serta kapasitas antioksidan ekstrak etanol dan fraksi jahe merah (*Zingiber officinale* var. *Rubrum*). *Al-Kimia*, 6(2), 171-183. [In Bahasa Indonesia].
- Putkaradze, J., Diasamidze, M., Vanidze, M. and Kalandia, A. (2021). Antioxidant activity of *Prunus*

- cerasifera* products. *International Journal of Life Sciences*, 10(3), 52–54.
- Putra, I.M.W.A., Sukei, K.A. and Sulistyadewi, N.P.E. (2020). Antioxidant capacity and α -amylase inhibition by avocado (*Persea americana mill*) peel and red ginger (*Zingiber officinale* var. Rubrum) based Functional Drink. *Acta Chimica Asiana*, 3(1), 135–142. <https://doi.org/10.29303/aca.v3i1.22>
- Razborssek, M., Ivanovic, M., Krajnc, P. and Kolar, M. (2020). Choline chloride based natural deep eutectic solvents as extraction media for extracting phenolic. *Molecules*, 25(7), 1619. <https://doi.org/10.3390/molecules25071619>
- Ridwan, A., Astrian, R.T., Anggraini, D., Kelompok, B., Fisiologi, K., Hewan, P., Sains, D., Sekolah, B., Dan, I. and Hayati, T. (2012). Pengukuran efek antidiabetes polifenol (polyphenon 60) berdasarkan kadar glukosa darah dan histologi pankreas mencit (mus musculus l.) s.w. jantan yang dikondisikan diabetes mellitus. *Jurnal Matematika and Sains*, 17(2), 78-82. [In Bahasa Indonesia].
- Safithri, M., Kurniawati, A. and Syaefudin (2016). Formula of *Piper crocatum*, *Cinnamomum burmanii*, and *Zingiber officinale* extracts as a functional beverage for diabetics. *International Food Research Journal*, 23(3), 1123–1130.
- Santoso, U.T., Rohman, T., Hidayah, N., Setiani, D.L., Dwi, I. and Risqa, E. (2022). Senyawa Baru Turunan N,O-Karboksimetil Kitin sebagai Antioksidan Sangat Kuat Berdasarkan Hasil Analisis Hubungan Kuantitatif Struktur-Aktivitas. *Prosiding Seminar Nasional Lingkungan Lahan Basah*, 7(2), 17–21. [In Bahasa Indonesia].
- Singh, B.S., Lobo, H.R. and Shankarling, G.S. (2012). Choline chloride based eutectic solvents: Magical catalytic system for carbon-carbon bond formation in the rapid synthesis of β -hydroxy functionalized derivatives. *Catalysis Communications*, 24, 70–74. <https://doi.org/10.1016/j.catcom.2012.03.021>
- Suhendra, C.P., Widarta, I.W.R. and Wiadnyani, A.A.I.S. (2019). Pengaruh konsentrasi etanol terhadap aktivitas antioksidan ekstrak rimpang ilalang (*Imperata cylindrica* (L) Beauv.) pada ekstraksi menggunakan gelombang ultrasonik. *Jurnal Ilmu Dan Teknologi Pangan*, 8(1), 27-35. <https://doi.org/10.24843/itepa.2019.v08.i01.p04> [In Bahasa Indonesia].
- Supu, R.D., Diantini, A., Levita, J., Padjadjaran, U., Java, W., Timur, U.I. and Java, E. (2018). Red ginger (*Zingiber officinale* var. rubrum): its chemical constituents, pharmacological activities and safety. *Fitofarmaka Jurnal Ilmiah Farmasi*. 8(1), 25–31. <https://doi.org/10.33751/jf.v8i1.1168>
- Taga, M.S., Miller, E.E. and Pratt, D.E. (1984). Chia seeds as a source of natural lipid antioxidants. *Journal of the American Oil Chemists' Society*, 61(5), 928–931. <https://doi.org/10.1007/BF02542169>
- Teixeira, C.C., Rava, C.A., Mallman Da Silva, P., Melchior, R., Argenta, R., Anselmi, F., Almeida, C.R.C. and Fuchs, F.D. (2000). Absence of antihyperglycemic effect of jambolan in experimental and clinical models. *Journal of Ethnopharmacology*, 71(1–2), 343–347. [https://doi.org/10.1016/S0378-8741\(00\)00185-9](https://doi.org/10.1016/S0378-8741(00)00185-9)
- Tiwari, A.K. and Rao, J.M. (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Science*, 83(1), 30–38.
- Wardani, Y.K., Kristiani, E.B.E. and Suchahyo. (2020). Korelasi Antara Aktivitas Antioksidan dengan Kandungan Senyawa Fenolik dan Lokasi Tumbuh Tanaman *Celosia argentea* Linn. *Bioma*, 22(2), 136–142. [In Bahasa Indonesia].
- Widarta, I.W.R. and Arnata, I.W. (2017). Ekstraksi komponen bioaktif daun alpukat dengan bantuan ultrasonik pada berbagai jenis dan konsentrasi pelarut. *Agritech*, 37(2), 148. <https://doi.org/10.22146/agritech.10397> [In Bahasa Indonesia].
- Wiendarlina, I.Y. and Sukaesih, R. (2019). Perbandingan Aktivitas antioksidan jahe emprit. *Jurnal Fitofarmaka Indonesia*, 6(1), 315–324. <https://doi.org/10.33096/jffi.v6i1.464> [In Bahasa Indonesia].
- Xia, H., Lv, C., Lu, Y., Zeng, C., Qing, S. and Shi, M. (2023). Natural deep eutectic ready to use extract of astilbin: Super high in vitro bioaccessibility, α -amylase and α -glucosidase enzyme inhibition kinetics. *Food Research International*, 173(Part 2), 113368. <https://doi.org/10.1016/j.foodres.2023.113368>
- Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D.A. and Barrow, C.J. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *Journal of Applied Phycology*, 18(3–5), 445–450. <https://doi.org/10.1007/s10811-006-9048-4>
- Zuhra, C.F., Tarigan, J.B. and Sihotang, H. (2007). Aktivitas senyawa flavonoid dari daun katuk (*Sauropus androgynus* (L) Merr.). Medan, Indonesia: Lembaga Penelitian Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Sumatera Utara. [In Bahasa Indonesia].