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CONTEMPORARY RESEARCH IN NATURAL PRODUCTS

Article

Valorization of Malaysian agro-industrial by-products: A comparative study of antioxidant properties in standardized NaDES-polyphenol enriched extracts

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NO

ABSTRACT

Oxidative stress is an imbalance between free radical generation and endogenous antioxidant defences is a key contributor to chronic diseases such as cancer, metabolic disorders, and cardiovascular conditions. Polyphenolic compounds from agro-industrial by-products are recognized for their strong antioxidant properties; however, their wider application remains limited by variability in polyphenol content, inefficient extraction methods, and the lack of standardized analytical approaches. This study aims to address these challenges by comparing and correlating the antioxidant potential of standardized NaDES–polyphenol enriched extracts derived from selected underexplored Malaysian commodity crop by-products. Polyols derived natural deep eutectic solvents (NaDES) combined with ultrasound-assisted extraction were employed to obtain polyphenol-rich extracts, which were quantified for total phenolic content (TPC) and total flavonoid content (TFC). Antioxidant activities were evaluated using rapid colorimetric assays, including 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), nitric oxide (NO) radical scavenging, and ferric reducing antioxidant power (FRAP). Chemometric analysis using partial least squares–discriminant analysis (PLS-DA) was applied to determine correlations between polyphenolic profiles, extraction solvents, and antioxidant responses. NaDES-extracted samples consistently exhibited higher TPC (1.14 mg GAE/g) and TFC (33.6 mg QCE/g) compared to aqueous extracts, demonstrating the superior solubilization and extraction efficiency of NaDES. Strong positive correlations were observed between TPC/TFC and all antioxidant assays, confirming the reliability of these rapid methods for screening antioxidant potency. PLS-DA revealed distinct clustering based on extraction solvents, indicating their influence on polyphenolic composition and bioactivity. Overall, this study highlights the untapped potential of Malaysian commodity crop by-products as sustainable sources of natural antioxidants and underscores the effectiveness of NaDES as a green and efficient extraction strategy for polyphenol recovery.

GRAPHICAL ABSTRACT

1. INTRODUCTION

Oxidative stress has long been recognized as a major factor influencing human health and disease, arising from an imbalance between the generation of reactive oxygen and nitrogen species (ROS/RNS) and the capacity of endogenous antioxidant defences to neutralize them (Wu et al., 2013). Under physiological conditions, ROS such as superoxide anion, hydrogen peroxide, and hydroxyl radicals contribute to essential cellular processes including signal transduction, immune defense, and regulation of gene expression (Pizzino et al., 2017). However, excessive or uncontrolled ROS production disrupts cellular homeostasis, leading to oxidative modifications of proteins, lipids, and nucleic acids. This oxidative damage is strongly implicated in the onset and progression of chronic diseases such as diabetes, cardiovascular disorders, atherosclerosis, neurodegenerative diseases, inflammatory conditions, and various cancers (Pooja et al., 2025). Although the human body possesses robust enzymatic antioxidant systems including superoxide dismutase, catalase, and glutathione peroxidase, these protective mechanisms may be overwhelmed during aging, infection, metabolic stress, and exposure to environmental pollutants (Frei & Ph, 1994). This has driven considerable interest in exogenous antioxidants, particularly those originating from plant-based foods and medicinal botanicals.

Polyphenols represent one of the most diverse and potent groups of plant-derived antioxidants. These secondary metabolites, which include phenolic acids, flavonoids, tannins, stilbenes, and lignans, are synthesized through the phenylpropanoid pathway and play key roles in plant defence against environmental stressors and pathogens (Dai & Mumper, 2010). Their antioxidant capacity arises from multiple mechanisms, including free radical scavenging, metal ion chelation, inhibition of pro-oxidant enzymes, and modulation of endogenous antioxidant pathways through Nrf2 activation (Rzepecka-stojko et al., 2014). Numerous studies have demonstrated strong correlations between total phenolic content and antioxidant capacity measured through assays such as DPPH, ABTS, and FRAP, confirming polyphenols as major contributors to bioactivity in plant extracts (Che Zain, Jakariah, et al., 2020; Joey et al., 2025). Beyond antioxidation, polyphenols exhibit anti-inflammatory, anti-proliferative, cardioprotective, and neuroprotective effects, supporting their potential applications in functional foods, nutraceuticals, and therapeutic formulations (Tangney & Rasmussen, 2013).

Malaysia's agricultural sector produces a wide variety of polyphenol-rich materials, including both edible plants and agro-industrial by-products. Commodity crops such as oil palm (*Elaeis guineensis* Jacq.), mango (*Mangifera indica)*, cocoa (*Theobroma cacao*), kenaf (*Hibiscus cannabinus*), and Tenggek Burung (*Melicope pteleifolia*)

generate substantial biomass annually, much of which remains underutilized (**Figure 1**). These materials contain phenolic acids, flavonoids, tannins, and other bioactive compounds that offer substantial antioxidant potential. For example, oil palm tissues including mesocarp, leaflets, and empty fruit bunches are rich in caffeic acid, ferulic acid, gallic acid, and tocotrienols, with demonstrated radical-scavenging activity comparable to commercial antioxidants (Che Zain, Lee, Mad Nasir, et al., 2020; Sulistiarini et al., 2022). Metabolomics studies further highlight the diverse polyphenolic profile of oil palm leaf extracts and their strong correlations with antioxidant and wound-healing effects (Che Zain, Lee, Sarian, et al., 2020). Similarly, *Mangifera indica* (particularly the Malaysian "Harum Manis" cultivar) contains mangiferin, quercetin, kaempferol, and chlorogenic acid, bioactive constituents concentrated in leaves, peel, and seeds which exhibit high phenolic content and strong activity in DPPH and FRAP assays (Mukhlis et al., 2024; Peron et al., 2024). Traditional herbs such as *Melicope pteleifolia*, widely used in Southeast Asian ethnomedicine, possess high flavonoid content particularly kaempferol and quercetin derivatives that contribute to antioxidant and anti-inflammatory effects validated through DPPH, ABTS, and NO-inhibition assays (Binh et al., 2025; Kabir et al., 2017). Likewise, *Hibiscus cannabinus* (kenaf), a valuable industrial and medicinal plant, displays considerable variability in phenolic content across genotypes, with several exhibiting antioxidant capacities comparable to or exceeding commonly consumed vegetables (Binh et al., 2025). Cocoa (*Theobroma cacao*), another important Malaysian crop, is globally recognized for its rich catechin, proanthocyanidin, and anthocyanin composition, which strongly correlates with its high antioxidant potential across DPPH, ABTS, ORAC, and FRAP assays (Joey et al., 2025).

Figure 1. Taxonomy classification of oil palm, harum manis mango, Tenggek Burung, cocoa tree, and kenaf.

Despite their promising phytochemical profiles, the efficient extraction and standardization of polyphenol-rich extracts remain significant challenges. Conventional solvents such as ethanol, methanol, or acetone often exhibit limited selectivity, may degrade thermolabile compounds, and raise environmental and safety concerns. Recent advances in green extraction technologies, particularly natural deep eutectic solvents (NaDES), offer a sustainable

and highly efficient alternative. NaDES comprise hydrogen-bond donor and acceptor components commonly choline chloride, betaine, sugars, polyols, or organic acids capable of forming tunable supramolecular solvents with superior solubilizing capacities for diverse polyphenols (Che Zain, Yeoh, Lee, & Shaari, 2021; García-Roldán et al., 2023). Studies show that NaDES enhance extraction efficiency through strong hydrogen bonding interactions, improved stabilization of phenolics, and reduced degradation of thermally sensitive compounds. For instance, NaDES-based extraction has yielded substantially higher phenolic content and antioxidant activity in *Moringa oleifera*, spent coffee grounds, and tropical herbs compared to ethanol or water (García-Roldán et al., 2023; Peng et al., 2025). Combining NaDES with ultrasound-assisted extraction further enhances mass transfer, disrupts plant cell walls, and increases recovery of flavonoid glycosides and phenolic acids (Che Zain, Yeoh, Lee, Afzan, et al., 2021).

Given Malaysia's rich botanical resources and the growing need for sustainable extraction technologies, there is significant potential to valorize agro-industrial by-products as sources of natural antioxidants. Establishing standardized extraction protocols and correlating phytochemical profiles with antioxidant performance is essential to support future industrial applications. Therefore, this study aims to evaluate the antioxidant properties of polyphenol-enriched extracts from selected Malaysian crops and by-products, compare the efficiency of NaDES and aqueous extraction methods, and determine correlations between total phenolic content, total flavonoid content, and antioxidant activity using DPPH, ABTS, nitric oxide scavenging, and ferric-reducing antioxidant power assays. The findings are expected to contribute toward the development of sustainable, high-value natural antioxidants aligned with circular economy principles and green bioprocessing strategies.

In this study, "standardized NaDES–polyphenol enriched extracts" refer to extracts produced using a defined and reproducible protocol, incorporating controlled raw material processing, fixed NaDES extraction parameters, and analytical normalization based on quantified polyphenol markers.

2 METHODOLOGY

2.1 Materials and chemicals

Commodity crop byproducts were obtained from various plantations across Malaysia: Oil palm (Serdang, Kedah), kenaf (LKTN, Kelantan), cocoa (Kodiang, Kedah), Tenggek Burung (Jeram, Selangor) and mango (Kangar, Perlis). Choline chloride, 1,2-propanediol, 1,4-butanediol, N-(2-naphthyl)ethlenediamine dihydrochloride were provided by Macklin (Shanghai, China). Sodium carbonate, sodium acetate trihydrate, organic solvents (methanol, and ethanol), ortho-phosphoric acid, potassium persulfate, aluminum chloride hexahydrate and iron (III) chloride anhydrous were supplied by Systerm (Selangor, Malaysia). Sodium nitroprusside, sulphanilamide, phosphate buffer saline (PBS) tablets and ABTS diammonium salt were supplied by SolarBio (Beijing, China). 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) were provided by TCI (Tokyo, Japan). Acetic acid and Folin–Ciocalteu reagent were provided by R&M Chemicals (Essex, United Kingdom). Ascorbic acid was provided by Chemiz (Selangor, Malaysia). Hydrochloric acid was provided by Fisher Scientific (Massachusetts, USA). Deionized water and distilled water were used in this study.

2.2 Preparation of powdered samples

The samples were prepared into fine powder using a standardized method established by (Che Zain, Lee, et al., 2021). The samples were meticulously dissected into small pieces measuring approximately 2.54 cm, or an inch, in length, and thereafter subjected to oven-drying at a temperature of 40 °C until a state of constant weight was attained. The dried samples were subsequently pulverized into a fine powder. To achieve a homogeneous powder with small particle size, the sample were grounded utilizing a Philips mechanical grinder (HR2056, Eindhoven, The Netherlands) and subsequently sieved through a sieve with a pore size of 105 µm (150 mesh). The sample powders were then stored at a temperature of 4 °C prior to utilization.

2.3 Synthesis of natural deep eutectic solvents

The synthesis of NaDES was performed utilizing two best combinations based on previous study (Che Zain, Yeoh, Lee, Afzan, et al., 2021), wherein choline chloride will be incorporated with each of 1,4-butanediol and 1,2-propanediol. The constituents comprising the NaDES were carefully quantified in alignment with the designated 1:4 ratio, representing HBA:HBD molar ratios with 33% water content, and the resulting blends were stored in screw-capped glass vials. Consequently, the mixtures were agitated using a magnetic hot plate stirrer (C-MAG HS7, IKA®, Wilmington, NC, USA) set to a rotational speed of 800 rpm for a duration of 30 minutes at 60 °C, with the systematic incorporation of the necessary volume of water to facilitate solvation until a homogeneous liquid phase is achieved. The resultant NaDES was preserved in screw-capped vials at ambient temperature.

2.4 Ultrasonic-NaDES assisted microextraction

The ultrasonic assisted microextraction was performed according to published method by (Che Zain, Yeoh, Lee, Afzan, et al., 2021). 0.26 g of sample powder of each interested plant was mixed with 6.5 mL of NaDES in a 10 mL centrifuge tube, where the solid:liquid ratio is 1:25, and vortexed at 3000 rpm for 30 seconds. The resulting mixture then underwent sonication in an ultrasonic water bath (Branson 2510 MT Ultrasonic Cleaner, Darmstadt, Germany) set to a frequency of 40 Hz at 25 °C for a period of 30 minutes. The extraction procedure was executed in triplicate. Following this, the mixture was centrifuged at 4000 rpm for 15 minutes to facilitate the separation of the supernatant from the precipitate. Deionized water was used as a control for polyphenol extraction for this research. A portion of the NaDES sample extract subsequently underwent a total phenolic content (TPC) assay and total flavonoid content (TFC) assay before undergoing rapid colorimetric assays.

2.5 Total phenolic content

The TPC assay was executed following methodologies previously established by (Che Zain, Lee, Mad Nasir, et al., 2020). Total phenolic content was measured by utilizing the Folin–Ciocalteu (F–C) reagent. A total volume of 120 µL of the mixture, which consists of 20 µL of the plant extract and 100 µL of the F–C reagent, was added to each well of a 96-well microtiter plate, after which the mixture was incubated for 5 minutes to allow for adequate reaction time. Upon completion of the incubation, an additional 80 µL of a 7.5% sodium carbonate solution was added to each well and incubated in the dark for 30 minutes. The absorbance of the resulting solution was measured at a 750 nm wavelength using a microtiter plate spectrophotometer to quantify the phenolic content. Every sample analyzed throughout this study underwent evaluation in three replicates to ensure the reliability and reproducibility of the results obtained. The values derived from these analyses were expressed in terms of milligrams of gallic acid equivalents per gram of dried powder (mg GAE/g dried powder), providing a standardized measure for comparative purposes across different samples.

2.6 Total flavonoid content

The TFC assay was performed following the method established by (Che Zain, Lee, Mad Nasir, et al., 2020), using an aluminum chloride complex-forming method. An aliquot of 125 µL of plant extract was mixed with 25 µL of 10% aluminum chloride solution, 375 µL of 95% ethanol, 25 µL of 1M sodium acetate solution, and 700 µL of distilled water in a 2 mL Eppendorf tube. Following this, 200 µL of the mixture was transferred to a 96-well microtiter plate and incubated for 40 min at 25 °C, at room temperature. Absorbance measurement was taken using a microplate reader at 415 nm. The TFC assay was performed in three replicates for each extract, and the flavonoid contents were calculated from a calibration curve constructed for a series of quercetin concentrations. The TFC values were expressed in milligrams of quercetin equivalents per gram of dried powder (mg QCE/g dried powder).

2.7 Ferric reducing antioxidant power (FRAP)

The FRAP assay was executed in accordance with the methodologies previously established by (Che Zain, Lee, Mad Nasir, et al., 2020). A 25 mL FRAP reagent was prepared where 2.5 mL 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) solution (10 mM) and 2.5 mL ferric chloride solution (3 mM) were mixed, and the rest of the volume was topped up with 30 mM acetate buffer and the pH adjusted to 3.6. The reagent was incubated for 15 min at 37 °C before being used. The FRAP assay involves the mixing of 20 µL of the extract with 180 µL of prepared reagent, and the absorbance measurement was read at 593 nm after incubation for 30 min at 37 °C. The FRAP assay of each sample was conducted in three replicates, and quantifications were based on the calibration curve constructed for a series of ascorbic acid concentrations. The results were reported as micrograms of ascorbic acid equivalents per milligram of dried powder (µg AAE/mg dried powder).

2.8 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH)

The DPPH assay to evaluate the free radical scavenging capabilities of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) was executed in accordance with the methodologies previously articulated by (Che Zain, Lee, Mad Nasir, et al., 2020). Various test concentrations of the sample extract were established, covering values from a minimum of 0.7 to a maximum of 100 µg mL^{-1} by conducting a sequence of serial dilutions derived from a concentrated stock solution with a concentration of 40 mg/mL. In the subsequent steps, a precisely measured 50-µL aliquot of the extract was combined with 100 µL of DPPH solution, which had a concentration of 1.77 mg dissolved in 3blue- of 100% methanol, and thoroughly mixed to ensure homogeneity; then the mixture was incubated in a dark environment to prevent any light-induced reactions from interfering with the assay results. Following a 30-minute incubation period, the absorbance of the resulting mixture was quantified at a wavelength of 515 nm utilizing an ELISA microtiter plate reader. The scavenging activity (SA) of the test samples was subsequently calculated using the following formula: SA % = $((A_0 - A_s)/A) \times 100\%$, where A_o is the absorbance of the blank, and A_s is the absorbance of the test sample. The entire assay procedure was executed in three replicates to ensure the reliability and reproducibility of the results, with the final reading expressed as the IC_{50} value, measured in µg mL^{-1} of dried powder. In this experimental setup, quercetin was used as a positive control to benchmark the antioxidant activity of the samples under investigation.

2.9 Nitric oxide radical scavenging activity (NO)

The NO assay was executed following the method established by (Che Zain, Lee, Mad Nasir, et al., 2020), with slight modification. Sodium nitroprusside (10 mM) in phosphate-buffered solution (pH = 7.4) was mixed with various concentrations of the extract prepared and incubated under light at room temperature for 15 min. The same reaction mixture without the tested extract, but with the equal amount of the solvent, serves as the control. After the incubation, 50 µL of Griess reagent, consisting of 1% sulfanilamide, 2.5% H_3PO_4, and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride, was added. Ascorbic acid was used as a positive control. The absorbance was measured at 550 nm, and the percentage of NO radical inhibition by the extract was calculated from the formula equation below: NO scavenging effect (%) = $((A_0–A_1) /A_0) \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard.

2.10 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity (ABTS)

The ABTS assay was executed following the method established by (Wang et al., 2024), with slight modification. 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) solution was synthesized through the reaction of 10 mL of a 7 mM aqueous solution of ABTS with 10 mL of a 2.45 mM potassium persulfate ($K_2S_2O_8$) solution. Following a 16-hour incubation in darkness, the concentration of the blue green ABTS radical solution was calibrated with ethanol to achieve an absorbance of 0.700 ± 0.020 (mean ± SD) at a wavelength of 734 nm. 10 µL of sample was added to the 195 µL ABTS solution within a 96-well plate, and the resultant mixture was incubated for 30 minutes. Absorbance was quantified at 734 nm using a microplate reader. Proper solvent control was

implemented for each assay conducted. The percentage of absorbance inhibitions at 734 nm was determined by employing the equation: ABTS scavenging effect (%) = $((A_B–A_A) / A_B) \times 100$, where A_B represents the absorbance of ABTS radical plus ethanol, and A_A denotes the absorbance of ABTS radical plus the sample extract or standard. Trolox will serve as reference material.

2.11 Statistical analysis

The data retrieved from polyphenolic contents (TPC and TFC), antioxidant (FRAP, DPPH, ABTS, and NO scavenging activities) assays were analyzed using GraphPad statistical software (San Diego, CA, USA) were applied. The values are shown as a mean ± standard deviation. To determine the significant differences of the values obtained, a one-way analysis of variance (ANOVA) was performed, followed by Tukey's test, where $p < 0.05$ was set as the significant level. This study also employs chemometrics analysis of the TPC, TFC, and antioxidant (FRAP, DPPH, ABTS, and NO) assays of the 5 groups, wherein they were analyzed using MetaboAnalyst (Chong et al., 2019). Prior to multivariate analysis, data preprocessing was carried out to enhance the interpretability and quality of the dataset used in Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA). The full dataset consisted of 144 extracts derived from a total of 16 various parts of the five plant species, each subjected to three different extraction solvents (including a control) and three biological replicates. Each extract was evaluated based on six quantitative features: TPC, TFC, FRAP, and three radical scavenging assays (DPPH IC_{50}, ABTS IC_{50}, and NO percentage of inhibition).

A filtering threshold was applied to eliminate irrelevant data points, noise or samples with low biological significance based on established literature values and assay specifics. Notably, samples were excluded if they had these condition: TPC < 0.05 mg GAE/g, TFC < 0.1 mg QCE/g, FRAP < 1.0 mg AAE/g, DPPH or ABTS IC_{50} > 1.0 mg/mL, or NO inhibition < 30%. These criteria were chosen to ensure that only those extracts with measurable antioxidant relevance and potential were retained for chemometric models. Thereafter, the filtered dataset underwent normalization and scaled (autoscaling), to prepare it for unsupervised analysis (PCA) and subsequent supervised analyses (PLS). This preprocessing helped in visualizing sample grouping patterns and establishing stronger relationships between polyphenol content and antioxidant activities.

3 RESULTS AND DISCUSSION

3.1 Polyphenolic content of extracts derived from various parts of crops byproducts

The total phenolic content of various plant parts was evaluated at an extract concentration of 40 mg/mL using three solvents: two natural deep eutectic solvents (NaDES), choline chloride–1,2-propanediol (CC:P) and choline chloride–1,4-butanediol (CC:B) and deionized water (diH_2O) as the aqueous control. The TPC profiles of kenaf, oil palm, mango, Tenggek Burung, and cocoa extracts obtained using these solvents are summarized in **Figure 2A**. As shown in Figure 2A (i), kenaf exhibited notably high TPC in its flowers, bulbs/buds, and leaves when extracted with NaDES, whereas stems demonstrated moderate levels and branches, roots, and seeds showed comparatively low phenolic content. In Figure 2A (ii), mango and oil palm mature leaves extracted with diH_2O displayed elevated TPC values, approaching 1.2 mg GAE/g dried powder. The TPC of NaDES-extracted mango, oil palm, and Tenggek Burung samples were comparable to active kenaf extracts, ranging from 0.4–0.5 mg GAE/g. Figure 2A (iii) illustrates that cocoa extracts consistently displayed moderate TPC across all solvents, although CC:P produced the highest yields. Overall, TPC exhibited a positive relationship with solvent polarity, with CC:P emerging as the most efficient extraction solvent. The highest TPC values were recorded for kenaf flower (KFL-CC:P) and mango leaf (MGL-CC:P), both exceeding 1.2 mg GAE/g. This enhanced extraction efficiency is attributed to the higher polarity of CC:P, which improves solvation of hydrophilic phenolics such as gallic and ellagic acids (Ratanasongtham et al., 2024). In contrast, diH_2O consistently yielded the lowest TPC, likely due to limited interaction with complex or bound phenolics and its inability to disrupt plant tissue matrices effectively. A

consistent trend was observed across all species: floral and leaf tissues contained higher TPC than roots, seeds, and pods. This is aligned with the biological roles of aerial organs, which synthesize abundant polyphenols in response to environmental stressors such as UV radiation and fluctuating temperatures (Di et al., 2014). Flowers typically accumulate pigments and UV-absorbing compounds for pollinator attraction, while leaves contain phenolics involved in photosynthesis and antioxidant defence (Ghasemzadeh & Ghasemzadeh, 2014). Thus, phenolic distribution is highly organ-specific and influenced by the metabolic activity and ecological function of each plant part. Previous studies corroborate these findings. Alam et al. (2021) reported that phenolic accumulation depends on both solvent characteristics and phenolic structure (Alam et al., 2021). Similarly, Feduraev et al. (2019) demonstrated that reproductive organs of Rumex spp. contained the highest phenolic levels during flowering and fruiting, with notable declines in later growth stages (Feduraev et al., 2019). Collectively, these results confirm that phenolic accumulation varies widely across plant organs and growth phases, reflecting the dynamic functional roles of polyphenols in plant development and stress adaptation.

The total flavonoid content displayed a clear positive relationship with solvent polarity, with higher TFC values obtained using more suitable solvents for flavonoid solubilization. **Figure 2B** presents the TFC profiles of kenaf, oil palm, mango, Tenggek Burung, and cocoa plant parts extracted using CC:P and CC:B NaDES, as well as deionized water. As shown in Figure 2B (i), kenaf flowers exhibited exceptionally high TFC when extracted with CC:B (33.587 ± 0.153 mg QCE/g dried powder), while stems, branches, roots, and seeds contained markedly lower flavonoid levels. Each mango leaf and Melicope sp. leaf showed high TFC with CC:B, reaching 16.211 ± 0.761 and 18.871 ± 1.730 mg QCE/g dried powder, respectively. Conversely, cocoa parts (Figure 2B (iii)) exhibited relatively low flavonoid content across all tissues (≤ 10 mg QCE/g), with aqueous extractions yielding slightly higher TFC values than NaDES-based extractions. The general extraction trend suggests that flavonoids typically more hydrophobic than other polyphenolic compounds are more efficiently extracted using CC:B, a less polar NaDES than CC:P. This indicates solvent selectivity based on polarity and hydrogen-bonding capacity, where CC:B is better suited for moderately polar flavonoids such as quercetin and kaempferol derivatives (Che Zain, Lee, et al., 2021). The higher TFC observed in aqueous cocoa extracts further supports the differential solubility of flavonoids depending on structural characteristics. Tissue distribution patterns reinforce that flavonoids predominantly accumulate in photosynthetic organs. Leaves and flowers, which are directly exposed to UV radiation and oxidative stress, showed substantially higher TFC than roots, stems, or seeds consistent with their roles in photoprotection, pigmentation, and defence (Agati et al., 2020). Kenaf flowers, in particular, displayed the highest flavonoid abundance, highlighting the tissue-specific nature of flavonoid biosynthesis. Overall, these findings demonstrate that both plant organ selection and solvent choice are critical determinants for maximizing flavonoid extraction efficiency.

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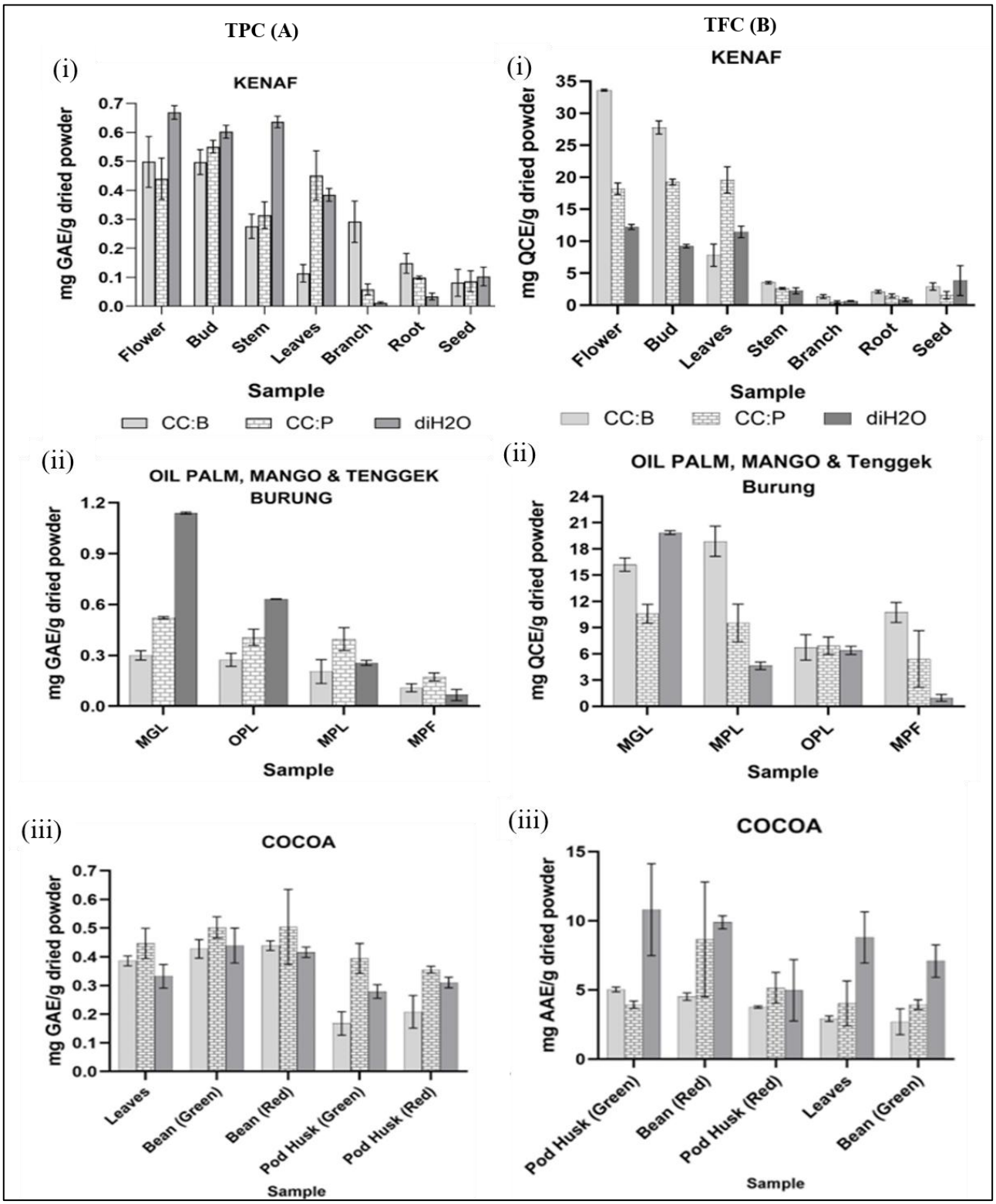

Figure 2. (A) Total phenolic content (TPC) in various parts of kenaf (i), oil palm, mango, Tenggek Burung (*Melicope* sp.) (ii) and cocoa (iii), extracted from two NaDES (CC:B and CC:P) and control (diH2O) solvent. **(B)** Total flavonoid content in various parts of kenaf (i), oil palm, mango, Tenggek Burung (Melicope sp.) (ii) and cocoa (iii), extracted from NaDES (CC:B and CC:P) and control (diH2O) solvent. Abbreviations: MPL: *Melicope* leaves, MPF: *Melicope* fruits, MGL: Mango leaves, OPL: Oil palm leaves.

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3.2 Antioxidant activities of extracts derived from various parts of commodity crops byproducts

3.2.1 Ferric-reducing antioxidant power

Ferric-reducing antioxidant power (FRAP) was assessed for all extracts at a concentration of 1000 ppm (1 mg/mL). Overall, **Figure 3A** shows that NaDES-based extracts particularly those obtained using CC:P exhibited markedly higher reducing capacities compared to aqueous extracts. As illustrated in Figure 3A (ii), matured mango leaves extracted with CC:P demonstrated the highest FRAP value among all samples, reaching 1465.214 ± 83.19 mg AAE/g dried powder. In Figure 3A (i), kenaf flowers, buds, leaves, and stems showed strong reducing activity for both NaDES solvents, with FRAP values exceeding 500 mg AAE/g. Kenaf leaves extracted with CC:B exhibited moderate FRAP activity, while branches, seeds, and roots displayed substantially lower values. Extracts from mango, oil palm, Tenggek Burung, and cocoa (Figures 3A (ii and iii)) generally showed moderate FRAP activity, again with CC:P consistently outperforming other solvents, except in Melicope sp. fruit, where CC:B produced slightly higher values. A consistent pattern emerged in which aerial organs: leaves and flowers exhibited higher FRAP activity than underground tissues such as roots or seeds. This trend aligns with the biochemical principle that tissues rich in phenolic hydroxyl groups exhibit greater electron-donating capacity, enabling more efficient reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions (Pan et al., 2022). The strong FRAP response strongly correlated with elevated TPC levels, indicating that phenolic constituents are major contributors to redox activity. Phenolic compounds containing ortho-dihydroxy (catechol) structures, such as quercetin derivatives, are particularly potent electron donors due to their ability to donate hydrogen atoms and stabilize resulting phenoxy radicals through resonance (Moazzen et al., 2022). This stabilization prevents propagation of oxidative chain reactions, further supporting their high FRAP reactivity. Hence, extracts rich in ortho-hydroxylated phenolics demonstrate superior reducing power compared to those lacking such structural features. The consistently higher FRAP values observed in CC:P extracts reflect the solvent's capacity to extract more polar, redox-active phenolics. Conversely, diH_2O extracts showed significantly lower FRAP activity, consistent with the limited solubilization of complex antioxidant phenolics in water (Windson et al., 2012). The clear alignment between FRAP trends and both TPC and TFC profiles further highlights the central role of phenolic constituents in redox-based antioxidant mechanisms.

3.2.2 NO radical scavenging activity

Figure 3B summarizes the nitric oxide (NO) radical scavenging activity of the extracts, measured at 40 mg/mL across various plant parts and three solvents: choline chloride–1,2-propanediol (CC:P), choline chloride–1,4-butanediol (CC:B), and deionized water (diH_2O). The performance of each extract was also compared with ascorbic acid as the positive control. Overall, extracts prepared using NaDES solvents (CC:P and CC:B) demonstrated markedly higher NO scavenging activity than those obtained using water. Among the two NaDES, CC:B consistently produced the highest inhibition percentages across most plant samples. The most potent activities were recorded for mango leaf-CC:B (91.12%), followed by kenaf flower-CC:B (89.84%), kenaf seed-CC:B (88.76%), and mango leaf-CC:P (87.30%). These four extracts displayed the strongest NO radical inhibition, with values comparable to or even exceeding that of ascorbic acid. Additional highly reactive samples included kenaf flower-CC:P (86.78%) and cocoa leaf-CC:P (74.58%). Moderate scavenging activity was observed in oil palm leaf-CC:P (72.61%) and Tenggek Burung leaf-CC:P (70.39%), while cocoa leaf-CC:B and Tenggek Burung leaf-CC:B displayed inhibition between 68–72%. These values place them within the mid-activity range. In contrast, the lowest NO inhibition was recorded in cocoa seed-diH_2O (47.13%), Tenggek Burung leaves-diH_2O (49.81%), and oil palm leaves-diH_2O (50.62%). Most diH_2O extracts remained below 55% inhibition, highlighting the limited efficiency of water in extracting NO-active compounds compared to NaDES.

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The results clearly show that the choice of extraction solvent critically influences the antioxidant performance of plant-based polyphenolic extracts. NaDES, particularly CC:P, produced extracts with significantly enhanced NO radical inhibition sometimes matching or surpassing the activity of ascorbic acid, a well-established antioxidant. This enhanced activity can be attributed to the unique physicochemical characteristics of NaDES, which improve the extraction efficiency of phenolics and flavonoids (Che Zain, Yeoh, Lee, & Shaari, 2021). CC:P, with its higher polarity and lower viscosity, allows better solubilization and diffusion of antioxidant molecules. Moreover, the extensive hydrogen-bonding interactions between NaDES components and polyphenolic hydroxyl groups improve extraction yield while stabilizing sensitive compounds, thus preserving their bioactivity (Joey et al., 2025). The strong performance of CC:P-extracted mango leaves and kenaf flowers underscores their potential as potent natural antioxidant sources. Nitric oxide scavenging primarily occurs through electron or hydrogen atom donation by phenolics and flavonoids. The high inhibition values of NaDES-based extracts indicate that these solvents not only retain but may even enhance the accessibility of active functional groups during the assay. Conversely, aqueous extracts showed much weaker activity due to the limited solubility of key phenolic compounds and the lack of stabilizing interactions in water (Windson et al., 2012). Consequently, NaDES especially CC:P emerge as superior extraction media for maximizing the antioxidant potential of plant-derived bioactive constituents. Their ability to match or surpass the NO radical scavenging efficiency of ascorbic acid positions these extracts as promising candidates for applications in nutraceuticals, functional foods, and biotechnological formulations aimed at mitigating oxidative stress.

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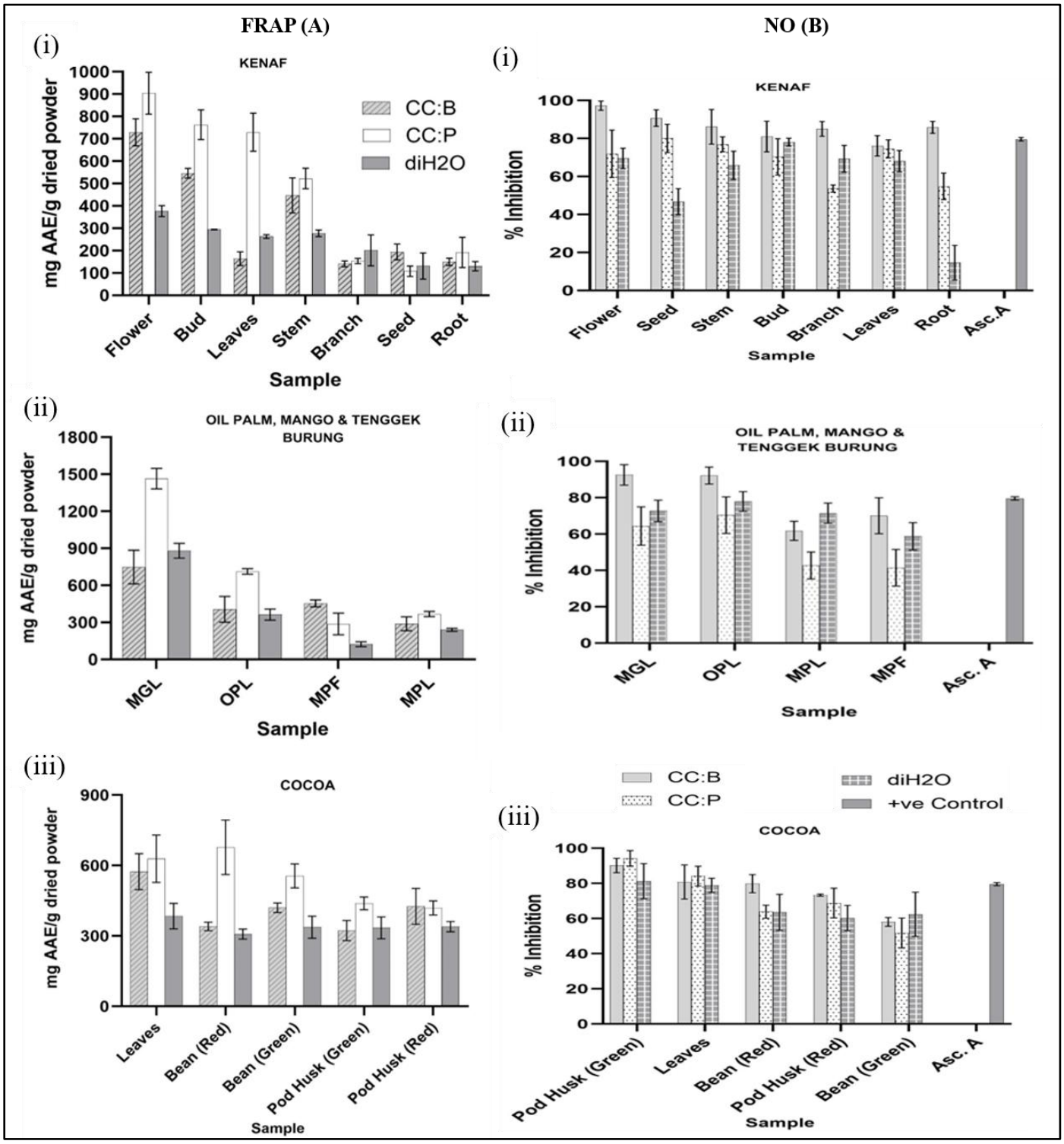

Figure 3. (A) Ferric-reducing antioxidant power in various parts of kenaf (i), oil palm, mango, Tenggek Burung (*Melicope* sp.) (ii) and cocoa (iii), extracted from NADES and control solvent. **(B)** Percentage inhibition of nitric oxide radical scavenging activity in various parts of kenaf (i), oil palm, mango, Tenggek Burung (*Melicope* sp.) (ii) and cocoa (iii). Abbreviations: MPL: *Melicope* leaves, MPF: *Melicope* fruits, MGL: Mango leaves, OPL: Oil palm leaves. Abbreviations: MPL: *Melicope* leaves, MPF: *Melicope* fruits, MGL: Mango leaves, OPL: Oil palm leaves.

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3.2.3 DPPH radical scavenging activity

The DPPH assay was conducted on 15 plant extracts at concentrations ranging from 5 mg/mL to 40 mg/mL. **Figures 4** illustrates the scavenging performance of each extract, grouped according to extraction solvent. Across all samples, a clear concentration-dependent increase in DPPH inhibition was observed. In Figure 4A (i and ii), kenaf flower extracts (KF-CC:B and KF-CC:P) consistently demonstrated the highest scavenging activity, increasing from 52.49% and 51.02% at 10 mg/mL to 87.32% and 86.15% at 40 mg/mL, respectively. Moderately reactive extracts included kenaf seed-CC:P, which rose from 39.91% to 78.35%. By contrast, stem and seed extracts prepared with deionized water (diH_2O) showed the lowest DPPH inhibition values. Figure 4B (ii) highlights that mango leaf-CC:P exhibited strong activity, increasing from 48.20% at 10 mg/mL to 84.90% at 40 mg/mL, closely following the performance of kenaf flowers and buds. Tenggek burung leaf-CC:P showed steady improvement from 38.40% to 76.91%. Oil palm leaf-CC:P increased from 33.20% to 69.84%, while Tenggek Burung fruit-CC:B rose from 30.45% to 66.03%, both reflecting moderate scavenging activity. In contrast, Tenggek Burung leaf-diH_2O and oil palm leaf-diH_2O extracts displayed weak activity, reaching only 45.78% and 46.12% at 40 mg/mL. As shown in Figure 4C, cocoa leaf-CC:B also demonstrated strong DPPH scavenging ability, reaching 82.75% at the highest concentration. Conversely, cocoa seed-diH_2O showed only minimal improvement, increasing from 30.24% at 10 mg/mL to 43.27% at 40 mg/mL.

Overall, the DPPH assay results reflect the varying radical-scavenging capacities of different plant parts. Kenaf flower and mango leaf extracts consistently exhibited the highest reactivity, whereas extracts from cocoa tissues and kenaf seed displayed weaker inhibition. These differences can be attributed to variations in hydrogen-donating capacity, which is enhanced in tissues rich in hydroxylated polyphenols, compounds abundant in leaves and flowers (Ilhami & Alwasel, 2023). High concentrations of these molecules contribute to stronger DPPH reduction, while other co-extracted constituents may also influence reaction kinetics and radical stability (Charlton et al., 2023). The findings clearly indicate that NaDES-based extractions, particularly those employing CC:P, enhance the scavenging performance of polyphenolic compounds. The superior activity of KF and MGL extracts aligns with their high TPC and TFC values reported earlier, supporting the premise that DPPH antioxidant activity driven by hydrogen-atom transfer mechanisms is strongly flavonoid-dependent. Flavonoids efficiently donate hydrogen atoms and therefore neutralize DPPH radicals more effectively than many other phenolics. Furthermore, the slightly lower polarity of CC:B may help preserve flavonoids susceptible to degradation in highly polar solvents, contributing to improved antioxidant activity (Bu et al., 2023). These results collectively demonstrate that both solvent type and extract concentration significantly influence antioxidant potential, and that NaDES ensures efficient extraction while maintaining the chemical integrity of bioactive constituents.

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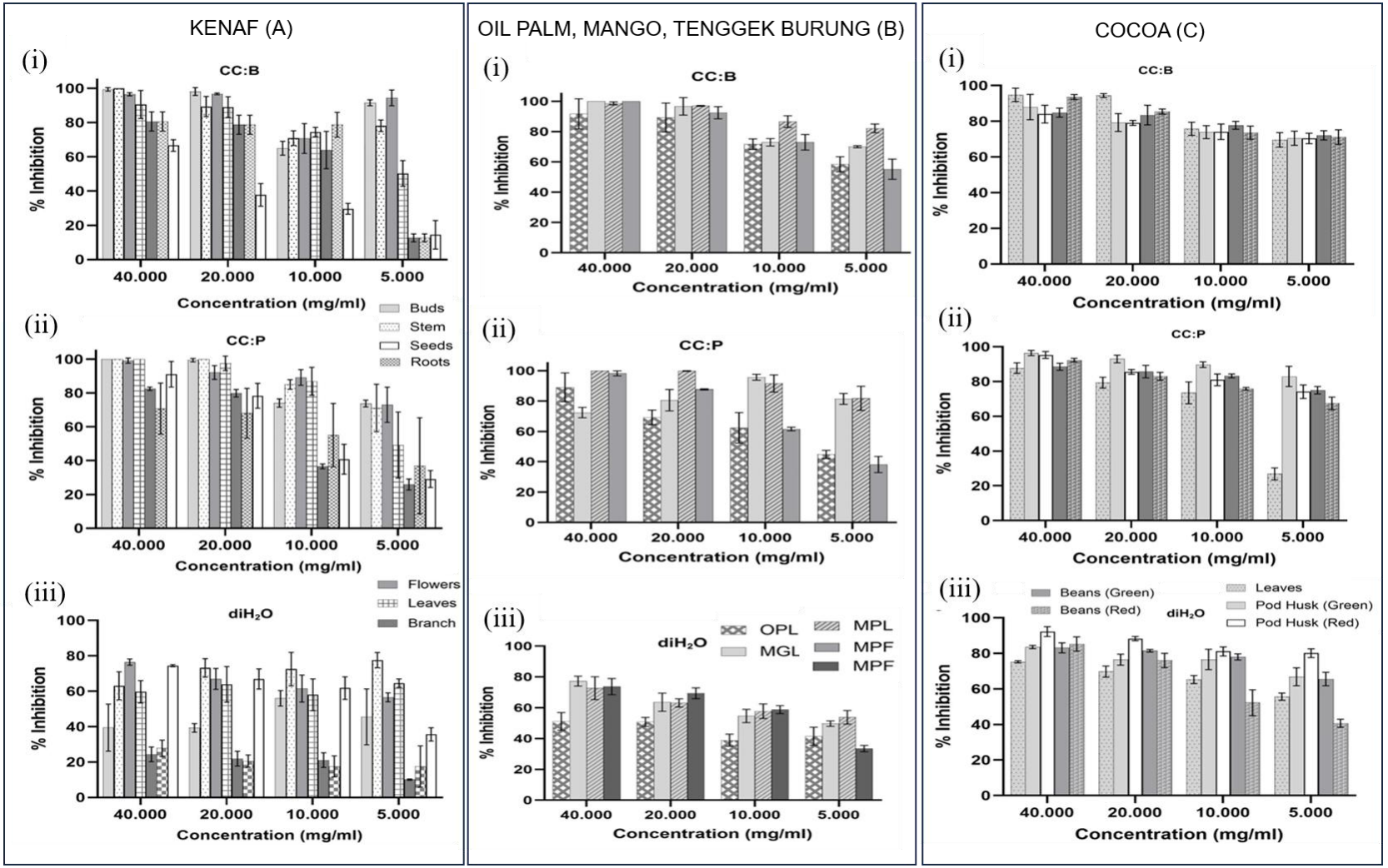

Figure 4. (A) Percentage inhibition of DPPH radical scavenging activity in various parts of kenaf; **(B)** Tenggek Burung, mango, and oil palm and **(C)** cocoa. Abbreviations: MPL: Melicope leaves, MPF: Melicope fruits, MGL: Mango leaves, OPL: Oil palm leaves.

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3.2.4 ABTS radical scavenging activity

The ABTS radical scavenging activity of all plant samples (5–40 mg/mL) is presented in **Figures 5.** Overall, the ABTS profiles closely resembled the concentration-dependent trends observed in the DPPH assay, but with even steeper increases among high-performing extracts. Several NaDES-based extracts achieved strong inhibition at intermediate doses, indicating high reactivity and efficient radical quenching. The highest ABTS inhibition was recorded in kenaf flower, cocoa leaf, and mango leaf extracts prepared using CC:P or CC:B. In these extracts, inhibition consistently exceeded 80% at the highest concentration, approaching levels comparable to standard antioxidants. For example, kenaf flower-CC:B increased from 60.92% at 20 mg/mL to 88.47% at 40 mg/mL, while cocoa leaf-CC:P and mango leaf-CC:P reached 87.16% and 85.29%, respectively, at 40 mg/mL. These extracts also demonstrated strong activity at earlier stages, often surpassing 75% inhibition by 30 mg/mL. Other NaDES-based extracts such as kenaf seed-CC:P and Tenggek Burung leaf-CC:P rose from 53.48% and 45.91% at 20 mg/mL to 79.56% and 77.48% at 40 mg/mL, reflecting steady concentration-dependent increases. Moderate ABTS activity was observed in oil palm leaf-CC:B (66.92%) and Tenggek Burung fruit-CC:B (64.38%) at 40 mg/mL. Among the lowest-performing samples were cocoa seed-diH_2O, which reached only 42.70%, and both Tenggek Burung stem-diH_2O and oil palm stem-diH_2O, which remained below 46%. These aqueous extracts showed minimal dose responsiveness, indicating weak ABTS-reactive compound extraction.

Collectively, these results confirm that NaDES-based extracts particularly those from leaf and flower tissues consistently exhibited superior ABTS radical scavenging across all concentration levels. Similar performance patterns were also reflected in the calculated IC_{50} values, where kenaf flower and mango leaf extracts prepared with CC:P again showed strong ABTS scavenging capabilities, while cocoa seed and pod extracts remained significantly less reactive. Unlike DPPH, which mainly detects hydrophobic antioxidants, the ABTS assay responds to both hydrophilic and lipophilic compounds. This wider solubility range explains the broader spread of activities among extracts and highlights ABTS as a more comprehensive indicator of total antioxidant capacity (Boligon et al., 2014). The strong performance of CC:P and CC:B extracts underscore the versatility of NaDES in solubilizing diverse antioxidant classes, particularly hydroxyl-rich and ring-conjugated structures found in flavonoids and tannins. Despite solvent effects, leaf and flower tissues still outperformed stems, pods, and seeds, confirming the combined influence of plant part and solvent system on antioxidant activity. Interestingly, while DPPH inhibition correlated more strongly with total flavonoid content (TFC), ABTS inhibition aligned better with total phenolic content (TPC) and reducing capacity (FRAP). This makes ABTS a valuable complementary assay for validating antioxidant activity across multiple compound classes and mechanisms.

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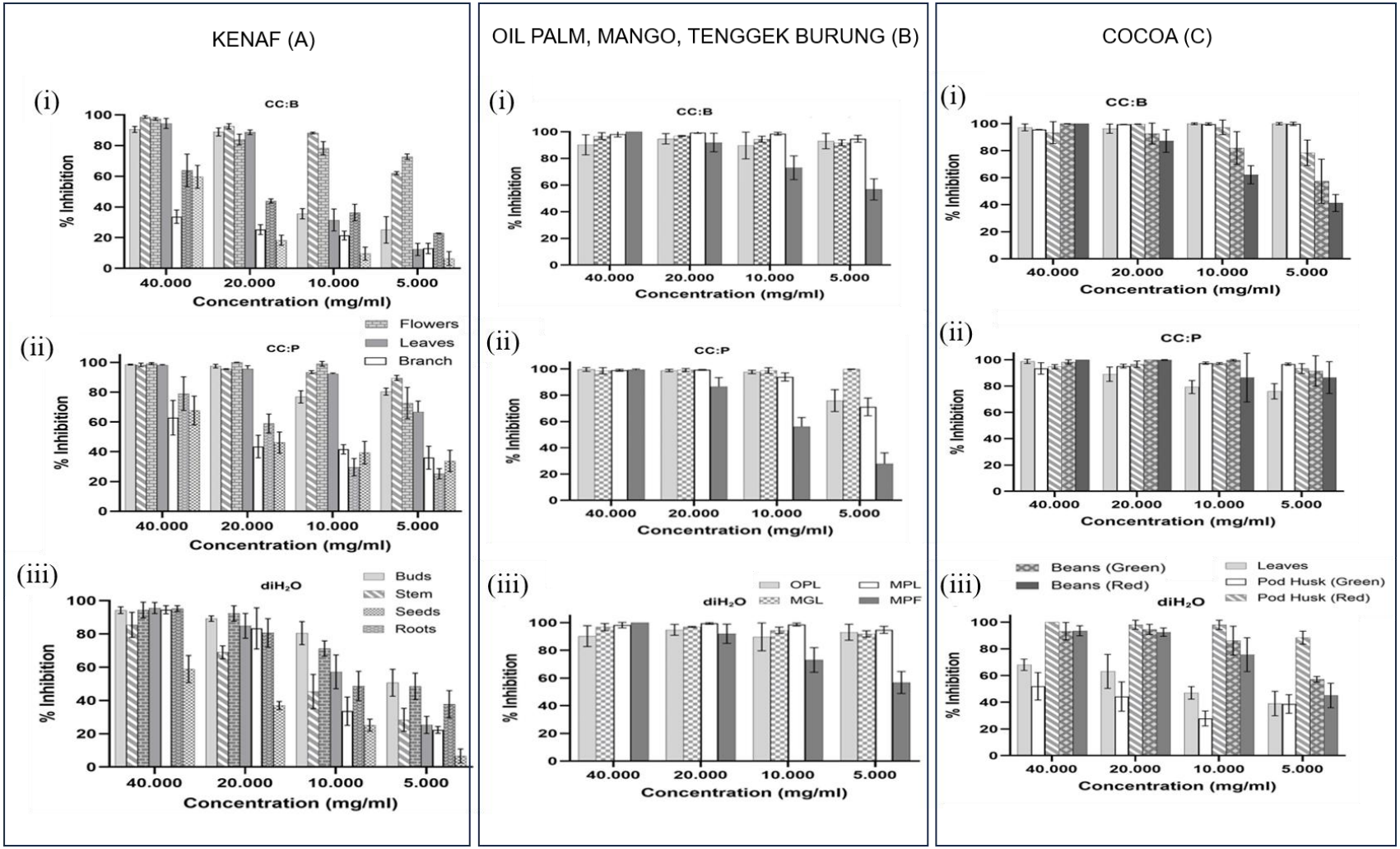

Figure 5. (A) Percentage inhibition of ABTS radical scavenging activity in various parts of kenaf; **(B)** Tenggek Burung, mango, and oil palm and **(C)** cocoa. Abbreviations: MPL: Melicope leaves, MPF: Melicope fruits, MGL: Mango leaves, OPL: Oil palm leaves.

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3.3 Influence of type of extraction solvent used on antioxidant activity

The type of solvent used for extraction, specifically NaDES (CC:B & CC:P), and deionized water, had a great impact on how well antioxidants could be extracted and their effectiveness. Polyphenolic compounds were extracted most efficiently by both CC:P and CC:B relative to the control, which was aqueous. On the other hand, the NaDES systems facilitated enhanced solubilization and stabilization of phenolic acids and flavonoids (Trivisiol et al., 2021), thereby improving assay responses across FRAP, DPPH, ABTS, and NO inhibition. CC:P, composed of choline chloride and 1,2-propanediol, provides a highly polar hydrogen bond-donating environment, making it ideal for extracting hydrophilic phenolics. Its high viscosity and zwitterionic nature promote solvation of phenolic hydroxyls (Tian et al., 2021), contributing to its strong performance, especially in FRAP and ABTS assays. Conversely, CC:B (choline chloride:1,4-butanediol) exhibits slightly lower polarity, which appears to favor the extraction of moderately polar flavonoids. Its enhanced efficacy in NO inhibition could be attributed to its ability to extract anti-inflammatory flavonoids more selectively. In comparison, diH2O, although biocompatible and environmentally friendly, exhibited poor extraction capacity for both phenolics and flavonoids due to metabolites limited solubility in aqueous solvent and its weak matrix penetration (Mitrović et al., 2024), leading to lower performance in all antioxidant assays. Thus, the structural properties of NaDES, including viscosity, polarity, and hydrogen bonding potential, are key determinants of extraction efficiency. Their tunable nature allows selective targeting of different phytochemical classes, offering an advantage over conventional solvents like water.

3.4 Chemometric analysis amongst antioxidant activities

Variation in metabolites across extracts from kenaf, oil palm, harum manis, Tenggek Burung, and cocoa was examined using multivariate data analysis (MVDA). Phenolic content and antioxidant activity data were first subjected to principal component analysis (PCA) to distinguish extract profiles and identify discriminating factors. The PCA model demonstrated good reliability, with an R^2X of 0.24 and a PERMANOVA F-value of 11.05, indicating statistically meaningful separation among groups. PC1 accounted for 37.5% of the variance and PC2 for 17.2%, explaining a cumulative 54.7% of total variation. The PCA score plot (**Figure 6A**) revealed four major clusters reflecting similarities in antioxidant and phenolic patterns, with high-TPC, high-FRAP, and low-IC_{50} extracts forming distinct groupings. Kenaf roots–diH_2O and green cocoa pod husk–CC:P appeared as outliers. Mango leaf extracts were clearly separated along PC1, whereas cocoa, kenaf, and Tenggek Burung extracts clustered more closely, suggesting comparable metabolite profiles. To further explore relationships between metabolites and bioactivity, supervised MVDA using partial least squares discriminant analysis (PLS-DA) was performed. The PLS-DA model showed moderate classification accuracy (Q^2 = 0.02), and permutation tests (n = 2000) confirmed model robustness, with permuted R^2 and Q^2 values consistently lower than the original. This indicated that the model was valid and not overfitted. The PLS-DA score plot (**Figure 6B**) showed clear species-based clustering similar to PCA. The biplot (**Figure 6D**) identified TPC, FRAP, and ABTS $1/IC_{50}$ as positively associated with several species' groups, while TFC, DPPH $1/IC_{50}$, and NO were oriented in the opposite direction, reflecting their inverse relationship with TPC. The biplot further showed that extracts with high TPC and TFC (e.g., KF-CC:P, MGL-CC:P, CL-CC:B) clustered close to vectors representing stronger antioxidant activity (high FRAP, low IC_{50}), illustrating their major influence on group separation. Pearson correlation analysis supported these findings, showing strong association between TPC and FRAP (r = 0.56), and moderate correlations between TPC with TFC (r = 0.47) and ABTS (r = 0.31). In contrast, DPPH $1/IC_{50}$ and NO showed weak correlations with TPC (r = 0.11 and r = 0.17), suggesting that non-phenolic constituents also contributed to these activities.

Collectively, these chemometric results emphasize that phenolic content is a principal determinant of antioxidant potential in the studied extracts. PCA confirmed that cocoa, kenaf, and Tenggek Burung share similar phytochemical signatures across solvents, likely due to extraction of similar metabolites in differing amounts. The

strong association of high-TPC/TFC extracts with high FRAP and low IC_{50} values aligns with extensive literature showing the strong antioxidant contributions of phenolics, particularly catechol-containing flavonoids, proanthocyanidins, and gallic acid derivatives (Bajpai et al., 2016; Pizzino et al., 2017). Meanwhile, weaker correlations with NO and DPPH indicate that additional structural or matrix-related factors modulate these activities. Although compound-specific identification was not performed, consistent relationships across PCA and PLS-DA models strongly indicate that polyphenols are primary contributors to antioxidant performance in these extracts. Future work incorporating chromatographic or spectrometric profiling is recommended to identify and quantify specific bioactive phenolics responsible for these activities.

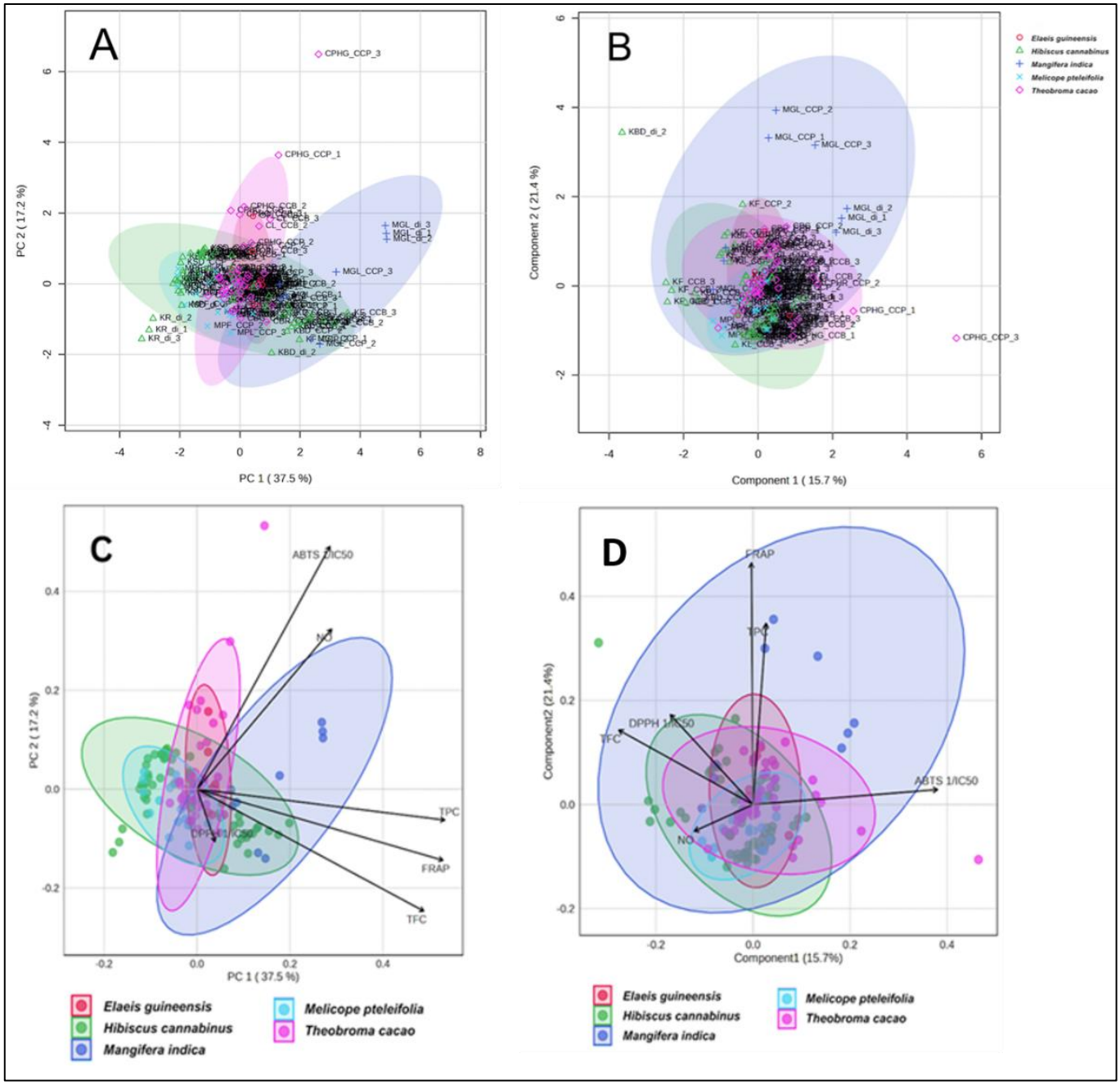

Figure 6. Principal component analysis (PCA) score plot **(A)** and biplot **(C)** constructed from six bioassays data of five groups of sample species. Partial-least square discriminant analysis (PLS-DA) score plot **(B)** and biplot **(D)**.

4 CONCLUSION

This study highlights the complex role of free radicals as both damaging agents and essential signalling molecules, underscoring the importance of a balanced redox state and the need for targeted rather than indiscriminate antioxidant use. NaDES solvents, particularly CC:P and CC:B, proved highly effective in extracting polyphenolic and flavonoid compounds, with kenaf flower and mango leaf showing the strongest antioxidant activities across multiple assays. Chemometric analyses confirmed that solvent polarity and tissue type significantly influence antioxidant profiles, and while phenolics and flavonoids were major contributors, the activity of low-TPC extracts suggests the involvement of other bioactive extracts. Although specific metabolites were not identified in this study, future research should employ targeted metabolomics, mechanistic investigations of solvent–solute interactions, and *in vivo* evaluations to validate bioavailability and pharmacological relevance. Advancing assay methods tailored for NaDES and integrating green extraction with chemometrics will further support the development of sustainable, high-value natural antioxidants and strengthen the role of NaDES as eco-friendly extraction systems for functional compounds.

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Consent for publication: Yes.

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Authors' contributions: Nur Aini Hadezir, Mohamad Shazeli Che Zain and Nurul Izza Ismail conceptualized and designed the research; Nur Aini Hadezir collected and analyzed the data, and Mohamad Shazeli Che Zain validated the data, wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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