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Short Note

DPPH Radical Scavenging Activity of Crude Methanolic Extracts from *Diospyros maritima* Blume and *Ixora philippinensis* Merr. Fruits on Mantanani Besar Island, Sabah, Malaysia

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Over the past century, phytochemicals from plants have become well-known for their potential bioactive compounds with high antioxidant activity. Generally, these phytochemicals have been identified as vitamin C, vitamin E, polyphenols, anthocyanins, and carotenoids (Sikora et al., 2008). These antioxidant compounds which can be found naturally existing in various plants and fruits exhibit a wide range of biological effects, such as anti-inflammatory, antibacterial, and anticancer properties. In tropical regions, species from the genera *Diospyros* L. (Ebenaceae family) and *Ixora* L. (Rubiaceae family) are ethnobotanically used in traditional medicine to treat various diseases (Kharat et al., 2013; Maridass, 2008; Rauf et al., 2017, 2024). The genus *Diospyros* L. comprises over 700 species worldwide, most of which are rich in phenolic compounds, flavonoids, and tannins (Mallavadhani et al., 1998; Maridass, 2008; POWO, 2022). On the other hand, *Ixora* L., a genus of about 500-600 species, is known for its phenolic compounds, flavonoids, triterpenoids, and sterols (Chen et al., 2016; Nadeem et al., 2024; Sunitha et al., 2015). In tropical regions, the traditional medicinal uses of both genera have been supported by demonstrating their antioxidant activity (Isnindar et al., 2011; Veeramuthu et al., 2023). Among these species, *Diospyros maritima* Blume (*D.maritima*) and *Ixora philippinensis* Merr. (*I.philippinensis*) are common tree species that are widely distributed in Southeast Asia, yet their antioxidant properties remain unexplored. Notably, these species are common and widely distributed on Mantanani Besar Island (Yuta et al., 2025), where they are ethnobotanically used by the locals for food and medicinal purposes. Although antioxidant activity has been evaluated for several native plant species from the island (How et al., 2022), the antioxidant potential of the fruits of *D. maritima* and *I. philippinensis* has not been adequately investigated. Therefore, this study aims to evaluate the antioxidant activity of fruit extracts from *D. maritima* and *I. philippinensis* using the DPPH free radical scavenging assay.

Fruit samples of *D. maritima* (300 g) and *I. philippinensis* (250 g) were collected at Mantanani Besar Island, which is located on the west coast of Sabah, Malaysia (6°42' 47" N, 116°21'17" E), on the 26th of June 2023. On-site identification was performed by Mr. Gisil Johnny, a botanist from the Institute for Tropical Biology and Conservation (ITBC), Universiti Malaysia Sabah (UMS).

The collected fruit samples were naturally dried in an enclosed environment at a temperature below 40 °C for three days. After that, the dried fruits were coarsely powdered and stored in a refrigerator (-20°C) until further chemical analysis. All the chemicals and reagents used were of analytical and American Chemical Society (ACS) grade: Gallic Acid (Chemicals P Ltd., Missouri, USA), Methanol (Fisher Scientific Ltd., New Jersey, USA), 1,1-diphenyl-2-picrylhydrazyl, DPPH (Sigma-Aldrich Chemicals P Ltd., Missouri, USA). The powdered samples were soaked in 100% methanol (Analytical grade; Fisher Scientific Ltd., New Jersey, USA) at room temperature for seven days. This is a maceration technique used to extract bioactive compounds from plant tissues (Abubakar & Haque, 2020). After the soaking period, the mixtures were filtered using Whatman No. 1 paper and cotton, then concentrated by a rotary evaporator, and dried by a desiccator. The weight of the dried crude methanolic extract was measured and then stored in the dark at -40°C at ITBC, UMS. Antioxidant activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH; Sigma-Aldrich Chemicals P Ltd., Missouri, USA) free radical model, following modifications to the method described by Maungchanburee et al. (2020). To prepare the working concentrations (50, 100, 200, 400, and 800 µg/mL), 40 mg of the methanolic crude extracts of *D. maritima* and *I. philippinensis* fruit were dissolved by 50 mL methanol, respectively and a series of serial-dilutions were performed until the last concentration of 50 µg/mL was obtained. Subsequently, for each sample solution, 1 mL of 0.3 mM DPPH (1.182 mg/mL) and methanol were added to reach a final volume of 3 mL. After vortex mixing, the mixture was incubated in the dark for 30 minutes at room temperature. After 30 minutes, the absorbance of the resulting solution was measured at wavelength 517 nm using a UV-1800 spectrophotometer (SHIMADZU Ltd., Kyoto, Japan), with the methanolic DPPH solution serving as the negative control. Gallic acid was used as a positive control. DPPH is a stable free radical that appears violet and is known for its strong absorption at a maximum wavelength of 517 nm (Soares et al., 1997). Through its antioxidant action, the violet DPPH radical is reduced by hydrogen- or electron-donating compounds, forming a pale yellow colour. This colour change enables the spectrophotometric measurement of antioxidant activity. The percentage of inhibition was calculated according to Equation 1:

$$\text{Inhibition Percentage (\%)} = \frac{(A_{control} - A_{sample})}{A_{control}} * 100 \qquad \text{Equation 1}$$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample. All measurements were performed in triplicate (n = 3) for each sample concentration and the positive control. The inhibitory concentration at 50% (IC_{50}) value for each sample was calculated using a non-linear three-parameter logistic regression model given by Equation 2 (Sebaugh, 2011):

$$Y = \frac{Max}{1+(\frac{X}{IC_{50}})^{Hill\ coefficient}} \qquad \text{Equation 2}$$

where Y is the radical scavenging activity of sample (% RSA), X is the concentration of the sample (µg/mL), Max is the Maximum response value of % RSA, and Hill coefficient is the steepness of the dose–response curve. The analysis was performed using the R software version 4.4.2 (2021). The classification of antioxidants based on IC_{50} values was adapted from Kusumawati et al. (2021) as shown in Table 1.

Table 1: Classification of antioxidants based on IC_{50} values.

IC_{50} value (µg/mL)	Antioxidant activity
< 50	Very strong
50-100	Strong
101-250	Medium
251-500	Weak
> 500	Not active

The results of the DPPH radical scavenging activity of *D. maritima* and *I. philippinensis* fruits, along with the gallic acid as positive control, are presented in Fig. 1. The activity of each methanolic crude extract increased as the concentration was raised, and a visible colour change from violet to pale yellow was observed in the vial.

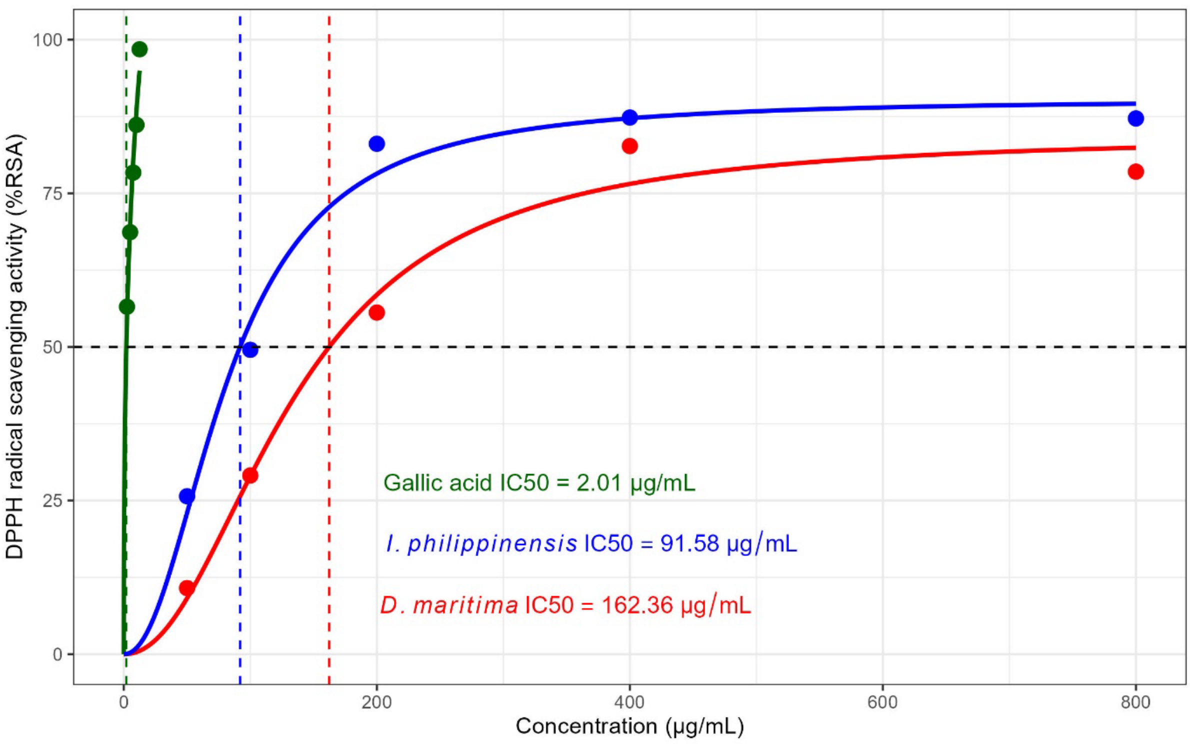

Figure 1: DPPH radical scavenging activity (% RSA vs Concentration graph for *Diospyros maritima* Blume (blue), *Ixora philippinensis* Merr. (red), and Gallic Acid control (green).

The IC_{50} values of both the sample and the control were calculated from a plotted graph of % RSA against the concentration of the extracts (Table 2). The IC_{50} values for the *D. maritima* were 162.36 ± 6.01 µg/mL, while that for *I. philippinensis* was 91.58 ± 1.81 µg/mL. A lower IC_{50} value indicates higher antioxidant capacity, suggesting that *D. maritima* exhibited moderate antioxidant activity, whereas *I. philippinensis* showed strong antioxidant activity (Table 1). For the positive control, more than 50 % inhibition was observed at the lowest tested concentration; therefore, the IC_{50} value was estimated by extrapolation of the fitted dose–response curve.

Table 2: Description of antioxidant test and IC_{50} results.

Concentration (µg/mL)	Radical scavenging activity of Sample (% RSA)		Concentration (µg/mL)	Radical scavenging activity of Positive Control (% RSA)
	D. maritima	*I. philippinensis*		Gallic acid
50.00	10.76 ± 2.17	25.70 ± 4.58	2.50	56.54 ± 2.58
100.00	29.08 ± 1.68	49.53 ± 2.50	5.00	68.67 ± 2.12
200.00	55.59 ± 3.24	83.06 ± 1.80	7.50	78.37 ± 1.47
400.00	82.69 ± 2.28	87.33 ± 1.59	10.00	86.13 ± 2.03
800.00	78.52 ± 1.95	87.18 ± 1.80	12.50	98.44 ± 1.44
IC_{50} (µg/mL)	162.36 ± 6.01	91.58 ± 1.81	IC_{50} (µg/mL)	2.01 ± 0.08

*Values are mean of three replicate determinations (n = 3) ± standard deviation.

To date, both *D. maritima* and *I. philippinensis* have been recognised as sources of bioactive compounds with potential antioxidant properties. Previous literature has reported that *D. maritima* may contain ample potent free radical scavenging and therapeutic compounds (Rauf et al., 2017, 2024). One of these is the plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), a quinoid compound which can be isolated from different parts of this species in previous studies (Gu et al., 2004; Higa et al., 2017; Isnansetyo et al., 2022; Kuo et al., 1997; Matsutake et al., 1998). Plumbagin exhibits broad bioactivities, including anticancer, antimicrobial, antioxidant, and anti-inflammatory effects, but also possesses cytotoxicity, making it an important target for pharmacological and agrochemical research (Gu et al., 2004; Isnansetyo et al., 2022; Tan et al., 2011). However, our results showed that the methanolic crude extract of *D. maritima* fruit only exhibited moderate DPPH radical scavenging activity compared with other species (Abbas et al., 2025; Clearn et al., 2025; Mal et al., 2024). In this context, Kapur et al. (2018) reported that plumbagin may function predominantly as a pro-oxidant by increasing intracellular reactive oxygen species (ROS), rather than as a direct free radical scavenger in chemical antioxidant assays such as the DPPH assay. Furthermore, Gao et al. (2011) and Yuwanda et al. (2019) have demonstrated a strong correlation between total phenolic content (TPC) and antioxidant capacity in *Diospyros* species. These findings suggest that phenolic compounds, rather than plumbagin, are likely the primary contributors to free radical scavenging activity in *Diospyros* fruits. Accordingly, the relatively moderate DPPH radical scavenging activity observed in *D. maritima* fruits may be explained by a lower abundance or absence of phenolic and flavonoid compounds in the fruit tissue. Previous phytochemical surveys have demonstrated considerable interspecific variation in the presence of phenolic and flavonoid compounds among *Diospyros* fruits, with some species exhibiting low levels or absence of these metabolites (Maridass, 2008). Such interspecific variability is likely influenced by differences in phytochemical composition, geographic distribution, microhabitat conditions, and genetic factors. Given the well-established positive correlation between total phenolic content and antioxidant capacity in *Diospyros* species (Gao et al., 2011; Yuwanda et al., 2019), the moderate DPPH radical scavenging activity observed in *D. maritima* fruits may be attributed, at least in part, to a relatively low phenolic content. However, comprehensive quantitative data on TPC in the fruits of *D. maritima* remain lacking. Therefore, further systematic studies are required to clarify the contribution of TPC to the antioxidant activity observed in the present study. For the *I. philippinensis*, a previous study by Ragasa et al. (2015) has reported that the stem and leaves contain various phytochemical including pinoresinol (3,4-divanillyltetrahydrofuran), syringaresinol (3,5-dimethoxy-4-hydroxyphenyl lignan), and isoscopoletin (6-hydroxy-7-methoxy-2H-chromen-2-one), which have been reported to possess antioxidant potential. In the present study, the strong DPPH radical scavenging activity observed suggests a new potential utilisation of the methanolic crude extract of *I. philippinensis* fruit. However, information about the chemical constituents of its fruit is still scarce and remains largely unexplored. Previous studies have suggested that fruits of the *Ixora* genus possess promising potential for food and medicinal applications (Nadeem et al., 2024; Sunitha et al., 2015). For instance, Shreelakshmi et al. (2021) reported that fruits of *Ixora coccinea* L. (*I. coccinea*) are rich in phenolic and flavonoid compounds and exhibit significant in vitro antioxidant and anticancer activities. In addition, Torey et al. (2010) reported that *I. coccinea* exhibits strong antioxidant activity in its leaves, stems, and flowers, indicating the broad utility of the whole plant. Similarly, Kalusalingam and Balakrishnan (2022) reported antioxidant activity in the leaves, stems, and roots of *Ixora notoniana* Wall., supporting its potential medicinal applications. Regarding this, *I. philippinensis* is characterised by diverse phytochemical profiles across plant parts (Ragasa et al., 2015), suggesting that it may possess functional and bioactive properties beyond antioxidant activity (Shreelakshmi et al., 2021; Torey et al., 2010). Our results align with previous studies showing strong DPPH radical scavenging and antioxidant activity within the *Ixora* genus. Nevertheless, comprehensive information on compound isolation, identification, and underlying mechanisms of action within the genus *Ixora* remains limited and warrants further investigation (Nadeem et al., 2024). Therefore, any future study should conduct comparative studies on different plant parts of *I. philippinensis* to properly identify, characterise, and document the distribution of its antioxidant and other bioactive compounds. In Malaysia, several species of the *Diospyros* genus have been reported to be traditionally utilised. In particular, the ripe fruits of *Diospyros discolor* Willd. are known to be used as food in Malaysia (Syazwani, 2024). Similarly, limited consumption of *D. maritima* fruits was recorded on Mantanani Besar Island, suggesting a previously under-recognised utilitarian potential of this species. In addition, ethnobotanical information from the island indicates that *D. maritima* has been used for medicinal skincare. Despite this local knowledge, no published literature has documented the use of Malaysian *Diospyros* species in skincare applications. In this context, Nematollahi et al. (2011) reported that the fruits of *Diospyros wallichii* King & Gamble exhibit antioxidant and anti-inflammatory properties, which are important evaluation indicators for cosmetic and skincare applications. These findings suggest that Malaysian *Diospyros* species may represent promising candidates for future studies focusing on skincare-related bioactivities. On Mantanani Besar Island, the fruits of *I. philippinensis* are widely utilised as a food source by the local community. In contrast, there have been no prior reports documenting the use of *Ixora* species as an edible fruit in Malaysia. Therefore, the present study provides novel evidence that *I. philippinensis* fruit is consumed locally and exhibits antioxidant DPPH radical scavenging activity, indicating a previously unreported potential for its utilisation. Nevertheless, forest degradation associated with development has been reported as a growing concern on Mantanani Besar Island (Yuta et al., 2025). Island ecosystems are widely recognised to harbor distinct ethnic traditions and cultural practices (Grydehøj et al., 2020). Particularly on Malaysian islands, ongoing development may lead to the loss of unique traditional knowledge and cultural practices (Tharmabalan, 2023). Therefore, there is an urgent need to conserve ethnobotanical knowledge while simultaneously enhancing the scientific understanding of plant resources and their functional properties.

This study represents the pioneer evaluation of the antioxidant potential conducted on the methanolic crude extracts of *D. maritima* and *I. philippinensis* fruit. Based on IC_{50} values, the DPPH radical scavenging activity of *D. maritima* was classified as moderate, while *I. philippinensis* showed comparatively higher activity. These findings suggest that both species contain bioactive compounds with antioxidant potential, even though their activity is lower than that of well-established antioxidant-rich species. More importantly, this study provides the first scientific evidence supporting the potential utilisation of these fruits in Malaysia. Scientific data on phytochemical composition and antioxidant property of these fruits remains scarce and largely unexplored. Hence, future research could be focused on various qualitative and quantitative phytochemical determinations and compound isolation to identify the specific bioactive constituents responsible for their antioxidant activity. Other bioactive potential of different plant parts such as antimicrobial or anti-inflammatory assessments could be performed in order to further elucidate their potential in pharmaceutical and nutraceutical applications.

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DECLARATIONS

Research permit(s). This study was conducted with the approval of the Sabah Biodiversity Council [Access License Ref. JKM/MBS.1000-2/2 JLD. 14 (2)].
Ethical approval/statement. Not applicable.
Generative AI use. The author(s) declare that generative AI has been used in compliance with the JTBC policies, and that I/we have reviewed and edited the content after using this tool/service and I/we take(s) full responsibility for the content of the publication.

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