

# Qualitative Phytochemical Contents and Antioxidant Activity of Dammar and Stembark Extracts of *Rubroshorea parvifolia* (Dyer) P.S.Ashton & J.Heck.

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## Abstract

**Introduction:** *Rubroshorea* species are known for their rich phytochemicals such as phenolics, flavonoids, and terpenoids in the dammar and stembark that possess antioxidant properties. The study aimed to screen the phytochemicals and evaluate the antioxidant activity of methanolic extracts from dammar and stembark of *Rubroshorea parvifolia* by means of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, total phenolic content (TPC) and total flavonoid content (TFC). **Methods:** Methanolic extracts of *R. parvifolia* dammar and stembark were prepared through maceration. Phytochemical screening was conducted to detect different classes of compounds. Antioxidant activity was initially screened by using Dot Blot assay and then quantitatively determined using DPPH radical scavenging assay. TPC and TFC were measured by using Folin-Ciocalteu method and aluminum chloride assay, respectively. **Results:** Phytochemical screening revealed the presence of phenolics, tannins, flavonoids, terpenoids, and triterpenoids in both extracts. Nevertheless, alkaloids and saponins were specifically detected in the dammar and stembark, respectively. The methanolic stembark extract demonstrated superior antioxidant activity with a lower half maximal inhibition concentration (IC<sub>50</sub>) value (16.52 ± 1.57 µg/mL) compared to that of dammar extract (575.68 ± 33.84 µg/mL). The activity of stembark extract is associated with its higher TPC (937.55 ± 2.40 mg GAE/g) and TFC (1016.82 ± 9.64 mg CE/g) than that of dammar extract. **Conclusion:** The strong antioxidant activity of methanolic stembark extract of *R. parvifolia* suggests its potential as natural antioxidant for pharmaceutical and nutraceutical applications. However, dammar extract, with its compelling phytochemical composition, warrants further investigation for other medicinal properties.

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## Introduction

*Rubroshorea* species, are renowned for their dammar and stembark, that have displayed uses as traditional medicine for treating a range of ailments, including gastrointestinal issues, skin diseases, and pain (Kamarozaman et al., 2022). The species are also well-recognised for their ability to synthesis a diverse range of secondary metabolites, such as stilbenes and their resveratrol oligomers, coumarins, and terpenes (Musa et al., 2024). These compounds possess various bioactivities, including antioxidant, anti-inflammatory, cytotoxicity and antimicrobial, which render them promising candidates for medicinal applications (Zain et al., 2010).

According to a recent review by Musa et al. (2024), a total of 113 distinct compounds have been identified across various *Rubroshorea* species. These compounds are particularly present in the stembark, 73% of which are resveratrol oligomers, 16% terpenes and a small percentage of coumarins, flavonoids, and steroids. Dammar is the tree exudate of the genus *Rubroshorea*, *Hopea*, *Balanocarpus* and *Vateria* of the Dipterocarpaceae. It constitutes a complex composition of phytochemicals, largely of triterpenoids particularly the dammarane type and certain percentage of sesquiterpenes and alcohol insoluble polymeric polycadinene. Among the triterpenoids of dammar of *Rubroshorea* species include dammarenediol, ursonic aldehyde, and oleanolic acid (Bonaduce et al., 2016).

*Rubroshorea parvifolia* (Synonym: *Shorea parvifolia*), commonly known as Meranti Sarang Punai or Light Red Meranti, is a large emergent tree native to Southeast Asia, particularly Malaysia. It is recognised for its significant ecological and economic values ("NParks | *R. parvifolia*," 2022). *R. parvifolia* is the main source of light red meranti timber of Malaysia and one of the main dammar producers of the Dipterocarpaceae. The timber primarily serves as building materials for houses, furniture and boats whereas the dammar is used for varnishes (Zain et al., 2010). Despite the traditional use of the stembark in treating ulcers (Syfriana et al., 2020), the medicinal potential of *R. parvifolia* remains

underexplored, and further research into its phytochemistry and biological activities is crucial.

Previously, crude methanolic extract of the stembark of *R. parvifolia* has been screened to contain relatively significant amounts of terpenoids and saponins. It also contains moderate to low amount of tannins, flavonoids and reducing sugars (Kamarozaman et al., 2022). Additionally, the bark contains two resveratrol dimers, namely (-)-ampelopsin F and (-)-laevifonol (Musa et al., 2024) as well as the trimers, identified as (+)- $\alpha$ -viniferin, davidiol and *trans*-miyabenol C (Rosyidah et al., 2010). The presence of different classes of compounds suggests that *R. parvifolia*, like other members of the *Rubroshorea* genus, may offer a wealth of therapeutic potential, particularly as natural sources of antioxidant.

Hence, this study focuses on *R. parvifolia*, aiming to screen the phytochemical composition and antioxidant properties of its dammar and stembark. By measuring the total phenolic and flavonoid contents, the research seeks to highlight the potential of dammar and stembark of *R. parvifolia* as natural sources of antioxidant. As the demand for safer, natural alternatives to synthetic antioxidants grows, understanding the antioxidant activity of the dammar and stembark's extracts of *R. parvifolia* could pave the way for their application in pharmaceutical and nutraceutical products.

## Materials and methods

### Materials

Aged dammar and outer stembark of *R. parvifolia*, sodium nitrite, mercury (III) chloride, Folin-Ciocalteu reagent (EMSURE, Germany), aluminium chloride, ascorbic acid (Alfa Aesar, China), potassium iodide, gallic acid (MERCK, Germany), lead (III) acetate trihydrate (ALDRICH, USA), ferric (III) chloride (Hamburg Chemical GMBH, Germany), dichloromethane (ChemAR, Malaysia), acetic anhydride, ethyl acetate, methanol (R&M Chemical, Malaysia), dimethyl sulfoxide (DMSO) (Thermo Fisher Scientific, United States), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, America), catechin (ChemFaces, Finland).

## Methods

### *Plant Collection and Preparation*

Aged dammar and outer stembark of *R. parvifolia* were collected from the infected and healthy tree, respectively in the IIUM Forest of 'Ilm, International Islamic University Malaysia, Kuantan Campus. The species was identified by a taxonomist, Dr. Shamsul Khamis from UKMB Herbarium, National University of Malaysia. The voucher specimens of dammar (PIIUM 0371) and stembark (PIIUM 0371-1) are deposited at the Herbarium Kulliyyah of Pharmacy, IIUM Kuantan. Both samples were grinded into powder form for experimental use.

### *Extraction of Plant Materials*

100 g of dammar and stembark powder were weighed and macerated in 500 mL of methanol at room temperature for 3 days. The extract was filtered, and the solvent was evaporated using rotary evaporator until the extract was completely dried. The crude methanolic extracts were transferred into a 1.5 mL glass vial and stored in a freezer at 4 °C. The percentage yield (% w/w) of extract from each sample was calculated as below:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried powder}} \times 100\%$$

### *Phytochemical Screening*

Phytochemical screening was performed following the procedures by Iqbal et al. (2015) and Vimalkumar et al. (2014) with slight modifications. For the tests in which water was used as the diluent, dammar extract was dissolved in methanol as it was water insoluble. In contrast, stembark extract was dissolved in ethyl acetate in the test which dichloromethane is used.

### *Frothing test for saponins*

0.5 g of the crude extract was dissolved in 10 mL of distilled water in a test tube. The test tube was shaken vigorously, and the presence of honeycomb froth was recorded.

### *Mayer's test for alkaloids*

10 mg of extract was stirred with 3 mL of aqueous 1% HCl in a water bath for 5 minutes and then filtered. A few drops of Mayer's reagent were added

to the filtrate. Creamish precipitate indicated the presence of alkaloids.

### *Lead acetate test for flavonoids*

0.2 g of extract was dissolved in 2 mL of distilled water in a test tube. The extract was then treated with a few drops of lead acetate solution. The presence of a yellow precipitate indicated flavonoids, while an orange to crimson colour indicated flavanones.

### *Borntrager's test for anthraquinone derivatives*

0.5 g of extract was boiled with 5 mL of dichloromethane in a water bath. The extract was then filtered, and 1 mL of dilute (10%) ammonia was added. The mixture was shaken, and the appearance of a pink to red colour in the ammoniacal layer indicated the presence of anthraquinone derivatives.

### *Borntrager's test for anthraquinone glycosides*

0.5 g of extract was dissolved in 5 mL of water, and 1 mL of 5% H<sub>2</sub>SO<sub>4</sub> was added. The mixture was boiled using a water bath and then filtered. The filtrate was shaken with an equal volume of dichloromethane and left to stand for 5 minutes. The lower dichloromethane layer was then separated, and half of its volume was diluted with 10% ammonia. The appearance of a rose pink to red colour in the ammoniacal layer indicated the presence of anthraquinone glycosides.

### *Ferric chloride test for phenolics*

0.2 g of extract was dissolved with 2 mL distilled water. Next, 1 mL of 5% ferric chloride solution (FeCl<sub>3</sub>) was added to the solution. Blue, green, or violet colour indicated the presence of phenolic compounds.

### *Ferric chloride test for tannins*

0.2 g of extract was dissolved in 4 mL of distilled water and filtered. A few drops of 5% ferric chloride solution were then added to the filtrate. The presence of a blue-black precipitate or colouration indicated condensed tannins, while green precipitation or colouration indicated hydrolysable tannins.

*Salkowski test for terpenoids*

100 mg of extract was dissolved in 2 mL of dichloromethane and filtered. Then, 2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was slowly added along the side of the test tube. The appearance of a reddish-brown colouration at the interface indicated the presence of terpenoids.

*Liebermann-Burchard test for sterol and/ or triterpenoids*

100 mg of extract was dissolved in 2 mL of dichloromethane and filtered. A few drops of acetic anhydride were added to the solution, which was then boiled in a water bath. After cooling the solution rapidly in iced water, 2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> was slowly added along the side of the test tube. The formation of deep red rings at the junction of two layers indicated the presence of triterpenoids. The formation of a brown ring at the junction of two layers, along with the upper layer turning green, indicated the presence of steroids.

*Organoleptic test for aromatic compounds*

The dammar and stembark of *R. parvifolia* samples were crushed using hands. The aromatic smell which indicated the presence of aromatic compounds was observed.

*Dot Blot Assay*

Antioxidant activity screening was performed according to the dot blot assay procedures by Gupta et al. (2016) with slight modifications. Sixteen concentrations were prepared from 10 mg/ml stock solution of extract following a two-fold dilution method in methanol. Then, 5 µL of each dilution of the extract was loaded onto a 2.5 x 2.5 cm grid prepared on 10 x 10 cm thin layer chromatography (TLC) plate in order of decreasing concentration. Then, the drops were allowed to dry. The same procedure was repeated for ascorbic acid (10 mg/mL), which was used as the standard. After the extract completely dried, the silica plates were sprayed with 0.1% DPPH solution in methanol. The radical scavenging activity was observed by the formation of colourless spots at the locations of the drops against a purple background. The minimum concentration required to reduce the colour of DPPH in both extracts and ascorbic acid was

recorded.

*2,2-Diphenyl-1-picrylhydrazyl Assay (DPPH Assay)*

Antioxidant activity was quantitatively determined following a slightly modified DPPH assay procedure by Bobo-García et al. (2015). Samples were assayed at eight concentrations following 2-fold serial dilution of the stock solution. Dammar extract was diluted in methanol as it is incompatible with water and resulted in precipitation whereas stembark extract was prepared in DMSO. The initial assay concentration for dammar extract was 1000 µg/mL whereas 100 µg/mL for stembark extract. 20 µL of each concentration of samples were added in 96 well plate in triplicate. Next, 180 µL of DPPH solution 150 µmol/L in methanol:water (80:20, v/v) was added into each well.

The plate was shaken for 60 seconds and incubated for 40 minutes at room temperature, in the dark room. Next, the absorbance was measured at 515 nm in the microplate reader (TECAN, Switzerland). Ascorbic acid was used as a standard at eight 2-fold dilution of 100 µmol/L in DMSO. 20 µL of diluted extract/sample with 180 µL methanol:water (80:20, v/v) after 40 min was used as blank while 20 µL DMSO with 180 µL DPPH solution after 40 min was used as control. Each sample was assayed in triplicate experiments.

*Total Phenolic Content (TPC)*

TPC was assessed according to the method by Bobo-García et al. (2015) with slight modifications. Dammar extract was prepared at 500 µg/mL and 1000 µg/mL whereas stembark extract at 1000 µg/mL and 10000 µg/mL. Dammar extract is tested at lower concentration than stembark due to its incompatibility with water. A total of 20 µL of a two-fold diluted extract was mixed with 100 µL of 1:4 diluted Folin-Ciocalteu reagent in a flat-bottom 96-well microplate. The mixture was shaken for 60 seconds and then left for 240 seconds. Next, 80 µL of sodium carbonate solution was added and the mixture was shaken at medium-continuous speed for 1 min.

The plate was incubated for 2 hours at room temperature. Then, the absorbance was measured at 750 nm using the microplate reader (TECAN,

Switzerland). The blank that was used for this assay was blank extract which contained 20  $\mu\text{L}$  of extract (at each two-fold diluted concentration tested) and 180  $\mu\text{L}$  water. The second blank was the same reaction mixture for extract, but DMSO was used instead of the extract. Lastly, gallic acid dilutions in DMSO (at final concentration range of 3.13 – 100  $\mu\text{g}/\text{mL}$ ) were used as standards for calibration curve. Each sample was assessed in triplicate experiments.

#### *Total Flavonoid Content (TFC)*

TFC was measured following the procedures by Herald et al. (2012) with minor modifications. Dammar extract was tested at 500  $\mu\text{g}/\text{mL}$  and 1000  $\mu\text{g}/\text{mL}$  whereas stembark extract at 1000  $\mu\text{g}/\text{mL}$  and 10000  $\mu\text{g}/\text{mL}$ . Firstly, 100  $\mu\text{L}$  distilled water was added to each of the 96 wells, then followed by 10  $\mu\text{L}$  of 50 g/L  $\text{NaNO}_2$  and 25  $\mu\text{L}$  of sample solution. Next, the solution was incubated for 5 minutes then followed by adding 15  $\mu\text{L}$  of 100 g/L  $\text{AlCl}_3$  to the mixture.

After that, the mixture was incubated again for 6 minutes, and 50  $\mu\text{L}$  of 1 mol/L NaOH and 50  $\mu\text{L}$  of distilled water was added. Next, the plate was shaken for 30 seconds in the microplate reader (TECAN, Switzerland) prior to absorbance measurement at 510 nm. The blanks that were used for this assay were blank extract containing 25  $\mu\text{L}$  of extract and 225  $\mu\text{L}$  water and the same reaction mixture for extract but DMSO was used instead of the extract. Catechin was assayed at a final concentration range of 9.38  $\mu\text{g}/\text{mL}$  – 300  $\mu\text{g}/\text{mL}$  to generate a calibration curve. Each sample was measured in triplicate experiments.

#### *Statistical Analysis*

Results of the triplicate analysis of each sample were reported as mean  $\pm$  standard deviation (SD). The graph,  $\text{IC}_{50}$ , TPC and TFC values were obtained using Microsoft Excel. The  $\text{IC}_{50}$  for DPPH radical scavenging activity were statistically analysed using one-way analysis of variance followed by Tukey's multiple comparisons test. TPC and TFC results were analysed for statistical comparison using an unpaired *t*-test. The value of  $p < 0.05$  was considered as statistically significant. All statistical analyses were carried out using the GraphPad Prism 10

software.

## **Results and discussion**

### *Extraction of Plant Materials*

Extraction by macerating aged dammar and stembark powder of *R. parvifolia* in methanol at room temperature has furnished 31.43% and 13.08% of their crude methanolic extracts, respectively. The dammar extract was characterised as a shiny brown gummy solid while the stembark extract appeared as dark brown solid. Methanol was chosen as the extraction solvent due to its superior solvent strength and wider solubility profile that is able to extensively extract a broad spectrum of compounds, including flavonoids, terpenoids, phenolics and vitamins (Lee et al., 2024).

In comparison with the previous studies, extraction of *R. parvifolia*'s stembark under reflux produced lower yield (12.97%) possibly due to loss of thermolabile compounds (Kawamura et al., 2011) while repeated maceration in a longer duration improves extraction yield (14.85%) (Syafriana et al., 2020). Meanwhile, dammar is an exudate of the tree that is readily soluble in organic solvents. Thus, higher yield than stembark is produced when it was macerated in methanol. Occasional stirring at longer maceration time might be performed to increase the extract yield (Golam, 2018).

### *Qualitative Phytochemical Content*

Qualitative phytochemical screening of the methanolic extracts of dammar and stembark of *R. parvifolia* has revealed the presence of phenolics, tannins, flavonoids, terpenoids and triterpenoids in both extracts. Phenolics including flavonoids were detected at relatively high amount in the stembark while terpenoids particularly triterpenoids were relatively abundant in dammar extract. Dammar and stembark specifically contained condensed tannins and hydrolysable tannins, respectively. Additionally, alkaloids, anthraquinone derivatives, and aromatic compounds were present in dammar at low concentration while a moderate amount of saponins were detected in stembark extract. A

summary of these findings is provided in Table 1.

In this study, significant presence of flavonoids was detected by using gelatine test in contrary to the low amount resulted from Shinoda test by Kamarozaman et al., (2022). The irreproducible intensity of the positive observations is suggested due to the inconsistency of the phytochemical concentration in the different extracts that are not exactly quantified in the qualitative phytochemical screening (Maheshwaran et al., 2024). Despite

limited studies on the dammar of *R. parvifolia*, the findings, however, align with Yusuf & Srinivasan (2015), who reported the presence of alkaloids, tannins, flavonoids, triterpenoids, and saponins, in the ethanolic extract of oleoresin of *R. robusta*. These phytochemical screening results relates *R. parvifolia* with other *Rubroshorea* species that are excellent sources of bioactive triterpenoids and polyphenolic compounds, particularly resveratrol oligomers in the dammar and stembark, respectively (Musa et al., 2024)

**Table 1:** Qualitative phytochemical contents of methanolic extracts of *R. parvifolia* dammar and stembark

Test	Class of phytochemicals	Dammar		Stembark	
		Observation	Relative intensity	Observation	Relative intensity
Ferric chloride	Phenolics	Blue-black colouration	++	Blue-black colouration	+++
	Tannins	Blue-black precipitate	+	Greenish precipitate	++
Lead acetate	Flavonoids	Yellow precipitate	++	Yellow precipitate	+++
Frothing	Saponins	-	-	Honeycomb froth	++
Salkowski	Terpenoids	Reddish-brown interface	+++	Reddish-brown interface	++
Liebermann-Burchard	Triterpenoids	A deep red ring interface	+++	A deep red ring interface	++
Meyer's	Alkaloids	Creamish precipitate	+	-	-
Bontrager's	Anthraquinone derivatives	Reddish colouration of the ammoniacal layer	+	-	-
	Anthraquinones glycosides	-	-	-	-
Organoleptic	Aromatic compounds	Aromatic smell	++	-	-

\*Note: (+/+/++) positive result with increasing intensity of colour or amount of precipitate; (-) negative result

### ***DPPH Radical Scavenging Antioxidant Activity***

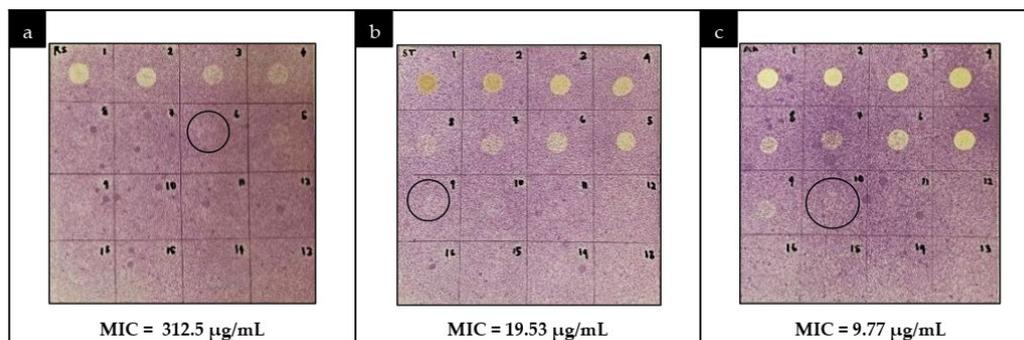
DPPH or 2,2-diphenyl-1-picrylhydrazyl is a highly stable free radical that is frequently used in different qualitative and quantitative assays for assessing antioxidant activity of plant extracts and compounds (Gulcin & Alwasel, 2020; Sherma, 2018). In DPPH antioxidant assay, the radical (DPPH\*) is neutralised by either a hydrogen atom or an electron from a reducing agent, converting it into a reduced

form (DPPH-H). The unpaired electron of DPPH\* absorbs strongly at 515-528 nm, resulting in its intense purple colour. However, when the odd electron pairs with another, the colour fades, eventually turning pale yellow (Sadeer et al., 2020; Xiao et al., 2020).

Initially, the dammar and stembark extracts of *R. parvifolia* were semi-quantitatively analysed by using dot blot and DPPH staining assay that preliminary appraised their antioxidant activity as

shown in Fig. 1. The reduction of purple to yellow or white spots on the TLC plate was reflected from the antioxidant potential of the extracts. Based on the minimum inhibitory concentration (MIC) values, stembark extract displayed a relatively

strong antioxidant activity compared with that of dammar extract. The activity was almost comparable with that of ascorbic acid, a standard known for its potent antioxidant properties.



**Fig. 1:** Dot blot antioxidant activity screening of *R. parvifolia*'s methanolic dammar (a) and stembark (b) extracts, and ascorbic acid standard (c) tested in concentration range from dot 1 (10000 µg/mL) to dot 16 (0.31 µg/mL). The yellow dot for the MIC of each sample is indicated by a black circle.

**Table 2:** DPPH radical scavenging activity, total phenolic content and total flavonoid content of *R. parvifolia*'s dammar and stembark extracts

Sample	IC <sub>50</sub> (µg/mL)	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg CE/g)
Methanolic dammar extract	575.68 ± 33.84 <sup>a</sup>	74.80 ± 16.71 <sup>a</sup>	234.49 ± 9.14 <sup>a</sup>
Methanolic stembark extract	16.52 ± 1.57 <sup>b</sup>	937.55 ± 2.40 <sup>b</sup>	1016.82 ± 9.64 <sup>b</sup>
Ascorbic acid	6.64 ± 0.08 <sup>b</sup>	NA	NA

Values are mean ± SD of three replicate experiments. Different superscript letters within the same column indicate significant difference at  $p < 0.05$ . NA, not applicable.

The ability of *R. parvifolia*'s dammar and stembark extracts in scavenging DPPH free radicals was further quantitatively measured to determine their antioxidant activity. The results and observations

were shown in Table 2 and Fig. 2. A different strength of antioxidant activity was observed, as indicated by their IC<sub>50</sub> values (Table 2) which are aligned with the results of the dot blot assay.

The dammar extract demonstrated a statistically different IC<sub>50</sub> value compared with stembark extract and ascorbic acid ( $p < 0.05$ ), implying its lower antioxidant activity than both samples. Stembark extract requires a smaller concentration to achieve 50% inhibition of the DPPH radical, making it a more effective antioxidant than the resin extract. The extract had no significant difference with the positive control, ascorbic acid ( $p > 0.05$ ), indicating both samples have almost similar antioxidant activity.

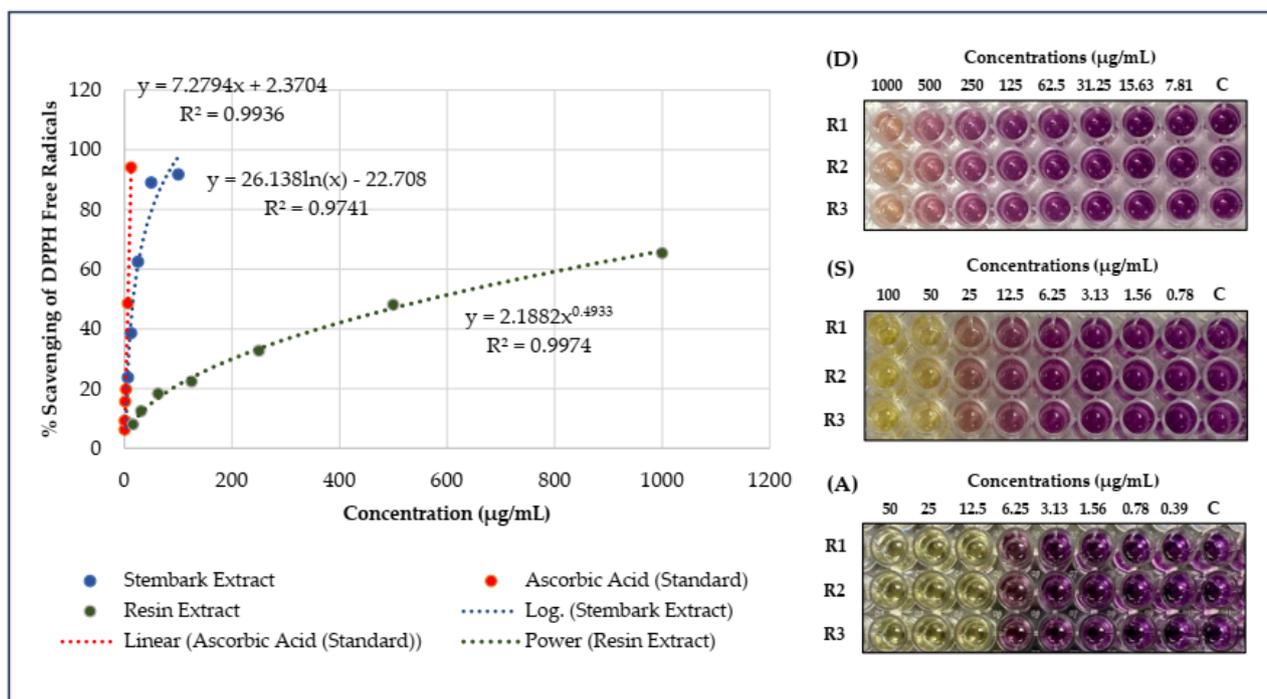
However, ascorbic acid exhibited a slightly lower IC<sub>50</sub> value than that of stembark extract. Ascorbic acid is a well-known antioxidant, and its low IC<sub>50</sub> value supports its strong antioxidant properties, highlighting its applicability as a reliable reference in antioxidant studies (Gegotek & Skrzydlewska, 2022). In comparison with the IC<sub>50</sub> values of extracts from other *Rubroshorea* species from previous studies, the crude methanolic stembark extract of *R. parvifolia* appears to exhibit slightly higher

antioxidant activity than the methanolic fraction of *R. kunsleri* (IC<sub>50</sub>, 18.60 µg/mL) (Daud et al., 2014). The dammar extract, however, demonstrated weaker antioxidant activity than that of the oleoresin of *R. robusta* (IC<sub>50</sub>, 357.44 µg/mL) (Yusuf & Srinivasan, 2015). According to Bornaduce et al., (2016), inconsistency, and high complexity in phytochemical constituents might present in dammar due to different degree of oxidation and degradation that occur during its ageing process. Thus, different strengths of antioxidant activity

might be observed in samples of dissimilar age.

#### Total Phenolic Contents (TPC) and Total Flavonoid Contents (TFC)

The antioxidants in plants are often found in the form of phenolics and flavonoids, and they can be found in different parts of the plant, such as the leaves, flowers, resins, stems, and roots (Jafri et al., 2023).



**Fig. 2:** DPPH radical scavenging activity of *R. parvifolia*'s dammar (D) and stembark (S) extracts, and ascorbic acid (A) represented by the graph (left) and the microplate observation (right), tested in triplicate (R1, R2, R3), and the control DPPH\* (C).

The TPC present in the dammar and stembark of *R. parvifolia* was determined through the Folin-Ciocalteu (F-C) method. According to Pérez et al. (2023), the F-C method operates on the principle of an electron transfer reaction, wherein antioxidant compounds serve as electron donors while the F-C reagent functions as the oxidising agent. This reaction leads to the reduction of anionic derivatives of phosphotungstic and phosphomolybdic acids by antioxidants, resulting in a colour change from yellow to blue. The intensity of this colour shift is

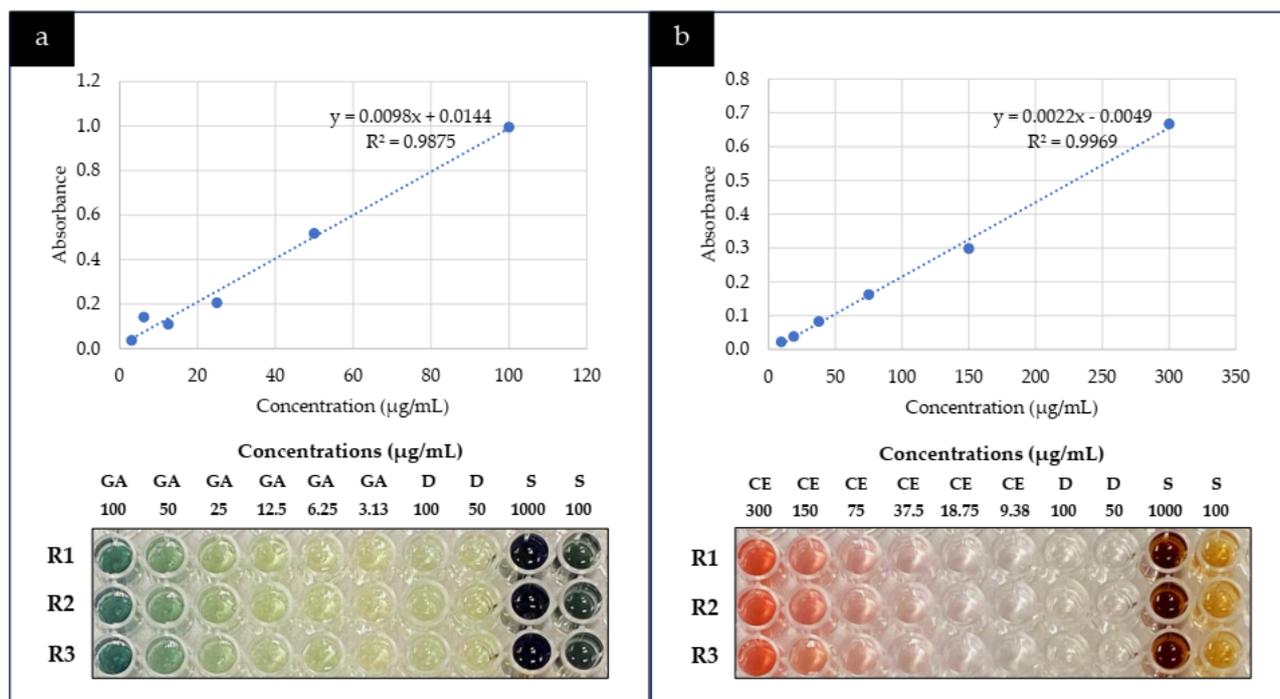
proportional to the phenolic compounds reducing capacity, expressed as gallic acid equivalents (GAE) as depicted by gallic acid standard curve and the assay plate of all samples in Figure 3(a). The results presented in Table 2 indicate that the stembark extract of *R. parvifolia* exhibited a comparatively higher TPC than that of the dammar extract which was statistically significant at  $p < 0.05$ . A prior investigation by Ahmat et al. (2012) on the TPC of stembark extracts from five *Rubroshorea* species, reported the phenolic content, ranging from 24.61 to

27.31 mg GAE/g. Among these, *R. acuminata* exhibited the highest TPC, followed by *R. leprosula*, *R. resinosa*, *R. macroptera*, and *R. bracteolata*. However, due to the scarcity of studies on dammar extracts, the TPC result from the current study cannot be directly compared to findings from previous research. Therefore, the significantly higher TPC observed in the stembark extract of *R. parvifolia* suggests its contribution towards greater antioxidant capacity than the dammar extract, further emphasizing the value of phenolic compounds in mitigating the DPPH radicals.

Plant phenolics often demonstrate strong free radical and reactive oxygen species scavenging abilities, primarily due to their hydroxyl groups on aromatic rings which facilitate reduction by donating a single electron or hydrogen atom (Gulcin & Alwasel, 2023). The strong antioxidant activity of the methanolic stembark extract of *R. parvifolia* could be suggested partly due to the

present of a few powerful antioxidant oligomeric resveratrols including those that had previously been reported, such as (+)- $\alpha$ -viniferin (Musa et al., 2024; Rosyidah et al., 2010). More antioxidant oligomeric resveratrols could be explored in the moderate and polar extracts of *R. parvifolia*'s stembark as *Rubroshorea* species are among their excellent sources (Zain et al., 2010).

Meanwhile, flavonoid is a significant group of plant secondary metabolites, characterised by their polyphenolic structure, that exhibit antioxidant properties (Panche et al., 2016). In this study, the TFC of *R. parvifolia* extracts were measured using the aluminium chloride colorimetric assay which involves a reaction with  $\text{NaNO}_2/\text{AlCl}_3/\text{NaOH}$  system (Herald et al., 2012). Initially, the flavonoid molecule undergoes oxidation by  $\text{NaNO}_2$ , followed by nitrosylation and formation of yellow complex with the aluminium ion. The ion forms a



**Fig. 3:** Standard calibration curves following Folin-Ciocalteu assay of gallic acid (GA) (a) and aluminium chloride assay of catechin (CE) (b) constructed from the mean values of 3 replicates (R1, R2, R3) for the determination of TPC and TFC in *R. parvifolia*'s dammar (D) and stembark (S) extracts.

stable complex with flavonoids especially flavones and flavonols at C4 keto group and the C3 or C5 hydroxyl groups (Guntarti et al., 2017). Addition of NaOH, converts this complex into a red-coloured chelate at a proportional colour intensity to the flavonoid content in the sample (Herald et al., 2012). Figure 3(b) shows the catechin calibration curve and the assay plate of all samples. The stembark extract of *R. parvifolia* exhibited a notably higher TFC than that of the dammar extract. The result was statistically validated as significant and different at  $p < 0.05$  (Table 2).

A previous study by Ahmat et al. (2012) on stembark extracts of five *Rubroshorea* species reported flavonoid contents ranging from 535.93 to 956.73 mg QE/g. Among these species, *R. resinosa* showed the highest TFC, followed by *R. leprosula*, *R. acuminata*, *R. bracteolata*, and *R. macroptera*. However, due to the limited research on dammar extracts, a direct comparison of TFC results between this study and previous studies is not feasible. Referring to Hassanpour & Doroudi (2023), flavonoids exhibit antioxidant activity by donating hydrogen atoms to free radicals. Therefore, the more efficiently the flavonoid structure facilitates hydrogen transfer, the stronger its antioxidant capacity will be. Relating this to the findings of the current study, it is suggested that the stronger antioxidant activity of stembark extract compared with that of dammar extract is primarily accountable to the greater capacity of hydrogen transfer of its rich flavonoids content. This implies that the stembark is potentially offering greater protection against oxidative stress and free radical damage than the dammar extract.

## Conclusion

In conclusion, the findings of this study demonstrate that both dammar and stembark extracts of *R. parvifolia* possessed notable phytochemicals and antioxidant activity, though vary considerably. The phytochemical screening revealed the presence of different types of compounds, particularly phenolics and flavonoids, which are known for their antioxidant effects. The stembark extract exhibited superior antioxidant activity qualitatively and

quantitatively compared with that of dammar extract. Despite exhibiting weaker antioxidant activity, dammar extract still contains valuable compounds, particularly triterpenoids, that could potentially have other pharmacological effects. The stembark extract possessed a great ability to scavenge DPPH free radicals, that is further corroborated with its high total phenolic and flavonoid contents. Hence, the stembark of *R. parvifolia* could serve as a promising source of natural antioxidants. Further research on its antioxidant profiles using advanced phytochemical methods, *in vivo* antioxidant activity, toxicity, and their natural-based formulations is necessary to uncover its potential nutraceutical and pharmaceutical applications.

## Authors contributions

Study design, M.I.A.R. and N.M.H.; Direction and coordination, N.M.H.; Investigation, M.I.A.R.; Resources, N.M.H.; Writing-Original Draft, M.I.A.R.; Writing-Review and Editing, N.M.H. and M.I.A.R.; Supervision, N.M.H.; Project Administration, N.M.H. and M.I.A.R.

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## Conflict of interest

The authors claim that there is no conflict of interest associated with this work.

## Declaration of generative AI and AI-assisted technologies in the writing process

The authors declared that no generative AI and AI-assisted technologies have been utilised during

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