



## RESEARCH ARTICLE

# Phytochemical Contents and Antioxidant Activity of Medicinal Plants from the Rubiaceae Family in Thailand

Rungcharn Suksungworn & Sutsawat Duangsrirai\*

Phyto-Chemodiversity and Ecology Research Unit, Department of Botany, Faculty of Science, Kasetsart University, Bangkean, Bangkok 109 00, Thailand

\*Email: [fscissw@ku.ac.th](mailto:fscissw@ku.ac.th)

## ARTICLE HISTORY

Received: 02 August 2020

Accepted: 19 October 2020

Published: 01 January 2021

## KEYWORDS

Anti-radical  
Traditional medicine  
Herbal medicine  
Nature products

## ABSTRACT

Several plants of the Rubiaceae family possess potential pharmacological properties, such as antioxidant activity, for subsequent drug development. We investigated the methanolic extracts from the bark and wood of five Rubiaceae species for phenolic and flavonoid contents and antioxidant activity. Regarding the phytochemical contents and antioxidant activity, *Mitragyna diversifolia* wood ( $437.57 \pm 9.90$  mg GAE  $g^{-1}$ ) and *Haldina cordifolia* wood ( $30.11 \pm 0.20$  mg QE  $g^{-1}$ ) displayed the highest total phenolic content (TPC) and total flavonoid content (TFC) respectively. *Morinda coreia* bark followed the highest antioxidant activities ( $IC_{50} = 360.58 \pm 19.28$   $\mu g$   $ml^{-1}$ ) in the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH), *Catunaregam tomentosa* bark ( $IC_{50} = 13.96 \pm 5.32$   $\mu g$   $ml^{-1}$ ) in the nitric oxide radical scavenging activity (NO), *M. coreia* wood ( $IC_{50} = 918.27 \pm 0.16$   $\mu g$   $ml^{-1}$ ) in the superoxide radical scavenging activity (SO) and *M. coreia* wood ( $IC_{50} = 236.65 \pm 1.66$   $\mu g$   $ml^{-1}$ ) in ferric reducing antioxidant power activity (FRAP). The TPC and TFC displayed strong correlations with DPPH in *M. diversifolia* wood and with FRAP in *M. diversifolia* bark and wood. We found high correlation between TFC and FRAP in all plant extracts except *C. tomentosa* wood, while no relation was detected between TFC and NO in all plant extracts. Comparing Rubiaceae species, the highest antioxidant potential were showed in *C. tomentosa* bark. Overall, it is worth mentioning that the Rubiaceae species exhibit potential as a promising source of natural antioxidants.

## Introduction

Reactive oxygen species (ROS) are a class of chemical products that are formed from oxygen metabolism and largely contribute to oxidative stress, which damages lipids, proteins and DNA (1, 2). Common ROS include the hydroxyl radical, hydrogen peroxide, superoxide radicals, hydroxyl ion and nitric oxide. Various environmental stresses lead to excessive ROS production, causing progressive oxidative damage, such as high irradiance, pollution, metal toxicity, UV radiation and pathogenic infection (2, 3). Since oxidation and oxidative damage to cellular components and biomolecules have been related to several diseases, many studies have investigated the relationship between oxidative damage and cancer (4), liver disease (5), Alzheimer's disease (6), aging (7), arthritis (8), inflammation (9), diabetes (10), Parkinson's disease (11), atherosclerosis (12) and

AIDS (13). Based on such literature, medicinal plants are commonly used to treat diseases due to their therapeutic properties and powerful antioxidant activity.

Rubiaceae is a one of the largest families of angiosperms, well known for its high diversity of secondary metabolites. Various species from the Rubiaceae family have proven to be a promising source for the development of new potential metabolites and drug prototypes because of their diversity and pharmacology properties (14). Their wide range of secondary metabolites include anthraquinones, alkaloids, coumarins, flavonoids and terpenes, which display pharmacological properties (15). Based on literature, medicinal plants from the Rubiaceae family have been reported to possess rich antioxidant activity (16–20), supporting their use in therapeutics.

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**To cite this article:** Suksungworn R, Duangsrirai S. Phytochemical Contents and Antioxidant Activity of Medicinal Plants from the Rubiaceae Family in Thailand *Plant Science Today*. 2021;8(1):24–31. <https://doi.org/10.14719/pst.2021.8.1.882>

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For this study, the plant samples were obtained from a woody plant to evaluate the phenolic compounds and potential biological properties, especially in the bark (21). Moreover, the woody part contains various secondary metabolites with potential biological activities (22). Hence, this study investigated the antioxidant activity and quantified phenolic and flavonoid content of selected woody medicinal plants from the Rubiaceae family in Thailand.

## Materials and Methods

### Collection of plant material

Five species of the Rubiaceae family, including *Catunaregam tomentosa* (Blume ex DC.) Tirveng., *Haldina cordifolia* (Roxburgh) Ridsdale., *Mitragyna diversifolia* (Wallich ex G. Don) Haviland., *Mitragyna rotundifolia* (Roxburgh) Kuntze. and *Morinda coreia* Buch.-Ham. were collected same location at Chainat province, Thailand in March 2015. We identified the plants at Bangkok Forestry Herbarium (BKF in Bangkok, Thailand). The voucher specimens of the plants (PCERU\_CT1, PCERU\_HC1, PCERU\_MD1, PCERU\_MR1 and PCERU\_MC1) were deposited in the Department of Botany, Kasetsart University, Bangkok, Thailand.

Methanol (analytical grade) was obtained from Merck (Darmstadt, Germany). Other reagents and chemicals included 2,2-diphenyl-1-picrylhydrazyl (DPPH; Merck, USA), tris-HCl buffer (Tris; Amresco, USA), nitroblue tetrazolium (NBT; Sigma-Aldrich, China), *b*-nicotinamide adenine dinucleotide (NADH; Sigma, Germany), phenazine methosulfate (PMS; Sigma, Ukraine), sodium nitroprusside (SNP; Himedia, India), sulfanilamide (Carlo Erba, France), phosphoric acid (Macron Fine Chemicals, China), naphthaylethylenediamine hydrochloride (AppliChem Panreac, Germany), 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ; Fluka, Switzerland), ferric chloride (Chem-supply, Australia), Folin-Ciocalteu (Merck, USA), sodium carbonate (Merck, Germany), gallic acid (Merck, USA), aluminum trichloride (Univar, New Zealand), quercetin acid (Sigma-Aldrich, Germany), Whatman® grade 1 filtration paper (Sigma Aldrich, USA), Büchi Rotavapor® R-210 (Mumbai, India) and T60-Visible spectrophotometer (PG Instruments, United Kingdom).

### Preparation of crude extract

The bark and wood samples were powdered coarsely using a mechanical grinder and macerated in absolute methanol for 7 days in the dark at room temperature (23). The extracts were filtered through filtration paper, evaporated to dryness in vacuum at 40 °C using a rotary evaporator, then stored at -20 °C in the dark.

### Quantitative analysis of phenolic and flavonoid compounds

#### Total phenolic content

The phenolic content of plant extracts was determined according to a previously described procedure (24). The sample (0.5 ml) was mixed with

0.2 N Folin-Ciocalteu reagent (2.5 ml), left for 5 min at room temperature, added solution of Na<sub>2</sub>CO<sub>3</sub> (75 g l<sup>-1</sup> in water, 2 ml). After incubation for 1 hr, the absorbance was measured at 765 nm. A calibration of standard curve was produced using gallic acid (0-300 mg l<sup>-1</sup>). The results are expressed as mg of gallic acid equivalents (mg GAE g<sup>-1</sup>).

#### Total flavonoid content

The total flavonoid was measured based on the method described in a previous work (25). The sample (1.5 ml) was mixed with 2% AlCl<sub>3</sub> in methanol (1.5 ml). The absorbance was read at 415 nm after 15 min against a blank sample. Quercetin (0-50 mg l<sup>-1</sup>) was used as a standard compound to plot the curve. The results are expressed as gm of quercetin equivalents (mg QE g<sup>-1</sup>).

#### DPPH radical scavenging activity

DPPH scavenging activity was demonstrated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (26). The sample (1.5 ml) was mixed with 0.2 mM DPPH solution in methanol (1.5 ml). After incubation for 30 min, the absorbance was read at 520 nm.

#### Nitric oxide radical scavenging activity

Nitric oxide, generated from SNP in solution at physiological pH, interacts with oxygen to produce nitrite ions, which can be measured by the Griess reaction (27). The reaction mixture (3 ml), containing sodium nitroprusside in phosphate PBS buffer and the extracts were incubated. After incubation for 150 minutes, the reaction mixture (0.5 ml) was removed, then Griess reagent (1% sulfanilamide, 2% H<sub>3</sub>PO<sub>4</sub> and 0.1% naphthaylethylene diamine hydrochloride, 0.5 ml) was added. The absorbance was measured at 546 nm.

#### Superoxide radical scavenging activity

The NADH-PMS condition produced superoxide radicals according to a previously described procedure (28). The mixture contained samples (1 ml), 936 µM NADH (1 ml) and 300 µM NBT (1 ml). After 10 minutes, the reaction was initiated by adding 120 µM PMS (1 ml). After incubation for 5 min, the absorbance at 560 nm was compared against blank samples.

#### Ferric reducing antioxidant power (FRAP) activity

The ferric reducing antioxidant power (FRAP) activity was evaluated using the followed method from previous report (29). Fresh FRAP solution, containing 300 mM acetate buffer (100 ml), 10 mM TPTZ solution (10 ml) and 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O (10 ml), was kept warmed at 37 °C until subsequently used. The sample (0.15 ml) was mixed with FRAP solution (2.85 ml) in the dark. After 30 min, the absorbance was read at 593 nm. The FRAP content in the sample was reported as mg trolox equivalent (TE) g extract<sup>-1</sup>.

#### Data analysis

The experimental treatments were performed in three independent replicates. Values are displayed as mean with standard deviation. The results of ANOVA analysis and Dunnett's multiple comparisons tests

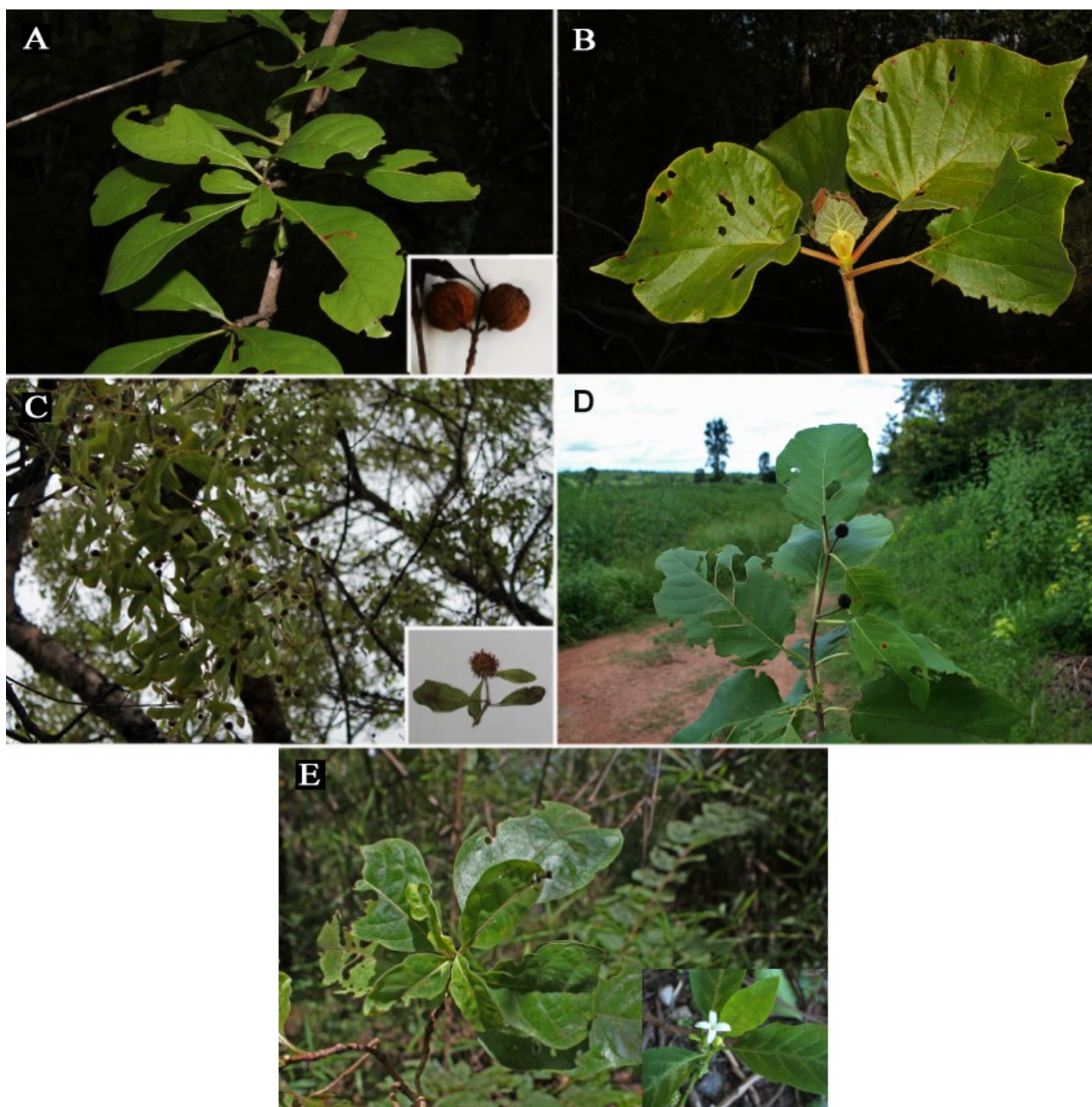


Fig. 1. Description of five Rubiaceae species; (A) *Catunaregam tomentosa*, (B) *Haldina cordifolia*, (C) *Mitragyna diversifolia*, (D) *Mitragyna rotundifolia* and (E) *Morinda coreia*.

were analyzed using GraphPad Prism 6 Software (San Diego, CA, USA) for statistical comparison. The  $p$ -value less than 0.05 were considered statistically significant.

## Results and Discussion

In order to fully reflect the antioxidant activity of the five Rubiaceae species (Fig. 1), four well-known antioxidant methods, including 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO), superoxide (SO) radical scavenging activity, and ferric reducing antioxidant power activity (FRAP), were employed. It is pertinent to measure various types of antioxidant activity because most antioxidants have several functions (30). Despite numerous studies on *C. tomentosa* fruit (31), *H. cordifolia* bark, leaves, stem and root (32-36) and *M. rotundifolia* bark and leaves (37), no existing work considers the chemical

composition and antioxidant activity of *M. diversifolia* and *M. coreia* bark and wood.

### Determination of total phenolic and flavonoid content

Quantification of total phenolic content (TPC) is usually carried out by the Folin-Ciocalteu method, while the  $AlCl_3$  method is used for determination of the total flavonoid content (TFC). The amount of TPC and TFC in the plant extracts are presented in Table 1. The TPC of plant extracts is presented the highest in *M. diversifolia* wood ( $437.57 \pm 9.90$  mg GAE  $g^{-1}$ ) and the lowest in *M. coreia* bark ( $119.48 \pm 0.41$  mg GAE  $g^{-1}$ ). On the other hand, the flavonoid content of plant extracts shown the highest in *H. cordifolia* wood ( $30.11 \pm 0.20$  mg QE  $g^{-1}$ ) and the lowest in *M. coreia* bark ( $0.74 \pm 0.01$  mg QE  $g^{-1}$ ). In particular, phenols are one of the most effective antioxidants (38, 39), which possess strong redox properties that play a role in neutralizing and absorbing free radicals, quenching

**Table 1.** Total phenolic content (TPC) and total flavonoid content (TFC); the results are presented as mean  $\pm$  standard in triplicate ( $n = 3$ ).

Plant extracts	Part	TPC (mg GAE g <sup>-1</sup> )	TPC ranking	TFC (mg QE g <sup>-1</sup> )	TFC ranking
<i>Catunaregam tomentosa</i>	bark	396.38 $\pm$ 0.41****	4	4.32 $\pm$ 0.02****	5
<i>Catunaregam tomentosa</i>	wood	285.67 $\pm$ 7.01****	7	1.49 $\pm$ 0.01****	8
<i>Haldina cordifolia</i>	bark	359.24 $\pm$ 3.67****	6	5.05 $\pm$ 0.13****	4
<i>Haldina cordifolia</i>	wood	258.29 $\pm$ 3.27****	9	30.11 $\pm$ 0.20	1
<i>Mitragyna diversifolia</i>	bark	382.33 $\pm$ 3.30****	5	3.15 $\pm$ 0.10****	7
<i>Mitragyna diversifolia</i>	wood	437.57 $\pm$ 9.90	1	1.48 $\pm$ 0.01****	9
<i>Mitragyna rotundifolia</i>	bark	411.62 $\pm$ 10.72****	3	3.68 $\pm$ 0.01****	6
<i>Mitragyna rotundifolia</i>	wood	412.81 $\pm$ 0.41****	2	5.65 $\pm$ 0.02****	3
<i>Morinda coreia</i>	bark	119.48 $\pm$ 0.41****	10	0.74 $\pm$ 0.01****	10
<i>Morinda coreia</i>	wood	261.14 $\pm$ 0.71****	8	7.40 $\pm$ 0.01****	2

Values followed by the asterisk symbol (\*) are statistically significant ( $p < 0.05$ ) compared to the highest value of each activity based on Dunnett's multiple comparisons test.

of singlet and triplet oxygen, and the ability to chelate metals (40, 41). In addition, flavonoids are a group of naturally occurring polyphenolic compounds that serve as excellent hydrogen-/electron-donors. The resulting flavonoids radical is relatively stable due to electron delocalization and intramolecular hydrogen bonding (42). Compared to bark, some reports suggest that wood contains higher phenolic levels (43, 44) and is richer in polyphenols and resin acids (diterpenes). In the heartwood part, most soluble sugars such as xylose, mannose and arabinose can be derived from hydrolyses (45). Previous reports have demonstrated that the wood part of plants have various secondary metabolites with potential biological activities (43, 46, 47). In this study, the highest TPC and TFC were found in *M. diversifolia* wood and *H. cordifolia* wood.

#### DPPH radical scavenging activity

DPPH radical scavenging activity has been widely used to evaluate the free radical scavenging activity of antioxidants or hydrogen donors. As shown in Table 2, the scavenging effects of plant extracts on DPPH radical were found the highest in *M. coreia* bark ( $IC_{50} = 360.58 \pm 19.28 \mu\text{g ml}^{-1}$ ) and the lowest in *M. coreia* wood ( $IC_{50} = 2408.18 \pm 73.82 \mu\text{g ml}^{-1}$ ). The DPPH method is based on scavenging through the addition of a radical species or antioxidant with visually noticeable discoloration. The DPPH method is based on scavenging through the addition of a radical species or antioxidant with visually noticeable discoloration. The level of color change is proportional to the concentration and potency of the antioxidants (48). Concerning the  $IC_{50}$  value of *H. cordifolia*, the bark methanol extract from a previous study ( $56.1 \mu\text{g ml}^{-1}$ ) was found to be 7.3-fold higher than

our DPPH test ( $411.80 \mu\text{g ml}^{-1}$ ) (35). This result suggests that Rubiaceae plants are capable of donating hydrogen to a free radical to scavenge potential damage.

#### Nitric oxide radical scavenging activity

Nitrite ( $\text{NO}^{2-}$ ) can be metabolized into nitric oxide (NO), which is an essential bio-regulatory molecule with physiological processes and functions. However, excessive NO can interact with the superoxide anion to form peroxynitrite ion ( $\text{ONOO}^-$ ). Nitrite is detected and analysed by the formation of  $\text{NO}^{2-}$  containing the Griess reagent, while nitric oxide scavengers compete with oxygen, leading to reduced production of nitrite ions. According to the  $IC_{50}$  values in Table 2, the inhibition of nitric oxide radical scavenging ability were presented the highest  $IC_{50}$  value ( $13.96 \pm 5.32 \mu\text{g ml}^{-1}$ ) in *C. tomentosa* bark and the lowest  $IC_{50}$  value ( $651.74 \pm 7.68 \mu\text{g ml}^{-1}$ ) in *M. coreia* bark. Nitric oxide plays an important role in various inflammatory processes (27). The nitric oxide radical generated from sodium nitroprusside interacted with oxygen to form nitrite (49). Comparing  $IC_{50}$  values of *H. cordifolia*, the bark methanol extract from a previous study ( $125.7 \mu\text{g ml}^{-1}$ ) was found to be 2.7-fold higher than our result ( $342.57 \mu\text{g ml}^{-1}$ ) (35). The Rubiaceae extracts from our study inhibit nitrite formation by competing with oxygen to react with nitric oxide.

#### Superoxide radical scavenging activity

Superoxide radical scavenging activity (SO) is an antioxidant enzyme related to ROS scavengers and mediators in oxidative chain reactions. To evaluate the  $\text{O}_2^-$  scavenging activity of antioxidants, the PMS-NADH-NBT system was employed. As shown in Table 2, the scavenging activity of superoxide in plant

**Table 2.** Anti-oxidant activity of plant extracts; the results are presented in mean  $\pm$  standard in triplicate ( $n = 3$ ).

Plant extracts	Part	$IC_{50}$ of DPPH ( $\mu\text{g ml}^{-1}$ )	$IC_{50}$ of NO ( $\mu\text{g ml}^{-1}$ )	$IC_{50}$ of SO ( $\mu\text{g ml}^{-1}$ )	FRAP (mg TE g <sup>-1</sup> )
<i>Catunaregam tomentosa</i>	bark	540.32 $\pm$ 27.37 <sup>ns</sup>	13.96 $\pm$ 5.32	4150.13 $\pm$ 45.35****	206.86 $\pm$ 1.33****
<i>Catunaregam tomentosa</i>	wood	380.60 $\pm$ 99.65 <sup>ns</sup>	81.33 $\pm$ 9.83**	8295.09 $\pm$ 641.25****	96.10 $\pm$ 6.29****
<i>Haldina cordifolia</i>	bark	411.80 $\pm$ 10.69 <sup>ns</sup>	342.57 $\pm$ 8.56****	5236.43 $\pm$ 41.50****	199.98 $\pm$ 0.44****
<i>Haldina cordifolia</i>	wood	532.73 $\pm$ 29.23 <sup>ns</sup>	434.56 $\pm$ 14.37****	1204.94 $\pm$ 19.25 <sup>ns</sup>	61.54 $\pm$ 0.07****
<i>Mitragyna diversifolia</i>	bark	1769.41 $\pm$ 382.95****	73.31 $\pm$ 9.27****	3615.47 $\pm$ 7.69****	153.31 $\pm$ 0.39****
<i>Mitragyna diversifolia</i>	wood	795.34 $\pm$ 8.95**	259.69 $\pm$ 14.98****	4516.30 $\pm$ 16.07****	62.30 $\pm$ 0.32****
<i>Mitragyna rotundifolia</i>	bark	2225.30 $\pm$ 64.51****	52.65 $\pm$ 6.66*	3179.04 $\pm$ 22.44****	95.97 $\pm$ 0.39****
<i>Mitragyna rotundifolia</i>	wood	716.53 $\pm$ 60.43*	183.94 $\pm$ 15.14****	7036.50 $\pm$ 92.69****	222.76 $\pm$ 0.26****
<i>Morinda coreia</i>	bark	360.58 $\pm$ 19.28	651.74 $\pm$ 7.68****	6622.71 $\pm$ 25.92****	18.75 $\pm$ 0.52****
<i>Morinda coreia</i>	wood	2408.18 $\pm$ 73.82****	183.52 $\pm$ 31.27****	918.27 $\pm$ 0.16	236.65 $\pm$ 1.66

$IC_{50}$  = the half maximal inhibitory concentration

Values followed by the asterisk symbol (\*) are statistically significant ( $p < 0.05$ ) compared to the highest value of each activity based on Dunnett's multiple comparisons test.

extracts decreased the highest value in *M. coreia* wood ( $IC_{50} = 918.27 \pm 0.16 \mu\text{g ml}^{-1}$ ) and the lowest in *C. tomentosa* wood ( $IC_{50} = 8295.09 \pm 641.25 \mu\text{g ml}^{-1}$ ). Reactive oxygen species, such as superoxides, give rise to the generation of dangerous hydroxyl radicals, which contribute to oxidative stress and damage lipids, proteins and DNA (50). The results of our study reveal that Rubiaceae plants have an effective capacity in scavenging for superoxide radical.

### Ferric reducing antioxidant power activity

The ferric reducing capacity of plant extracts may serve as an indicator of its potential antioxidant activity, where the presence of an antioxidant reduces  $Fe^{3+}$ -TPTZ to  $Fe^{2+}$  under acidic conditions. Thus, ferric reducing ability can be evaluated by the formation of a  $Fe^{2+}$ -TPTZ complex. According to the FRAP activities in Table 2, the ferric reducing effects of plant extracts have the highest value in *M. coreia* wood ( $236.65 \pm 1.66 \text{ mg TE g}^{-1}$ ) and lowest value in *M. coreia* bark ( $18.75 \pm 0.52 \text{ mg TE g}^{-1}$ ). The reducing power of the test, the presence of reductants (antioxidants) in the solution causes the reduction of the  $Fe^{3+}$ -TPTZ complex to ferrous form (51). Our results show that the extracts act as an electron donor and thus, influence the reduction of the  $Fe^{3+}$ -TPTZ complex to ferrous form.

### Ranking of antioxidant activity

As shown in Table 3, the antioxidant activity of the extracts in our study are ranked as follows: *C. tomentosa* bark > *M. coreia* wood > *M. diversifolia* bark > *M. rotundifolia* bark > *C. tomentosa* wood and *H. cordifolia* bark > *M. rotundifolia* wood > *H. cordifolia* wood > *M. diversifolia* wood > *M. coreia* bark. The comparison of these results suggests that the level of anti-oxidant of species of Rubiaceae in different bark and wood part are depend on the part and the species. Regarding the highest value of TPC, TFC and each antioxidant activity (Table 1 and 3), our results show that antioxidants in wood extracts are more effective than those in the bark extracts in TPC, TFC, NO radical scavenging, and FRAP activities. Other studies also suggest that wood extract has higher antioxidant activity than bark that is directly

activity to polyphenols, which may be contributable to proteins, that could be oxidized using the Folin reagent and aluminum chloride (53–55). This may be further explained by the interference of other chemical components in the extract (56).

### Correlation with phytochemical constituents and $IC_{50}$ values of antioxidant

The correlation between the TPC and TFC and antioxidant activity, including DPPH, NO and SO radical scavenging activity and FRAP activity of bark and wood were analysed (Tables 4 and 5). Specifically, a correlation coefficient of  $(r) > 0.6$  shows extracts with strong antioxidant activity (57). Analysis of the strong correlation between the phenolic content and antioxidant activities showed that the TPC exhibits radical scavenging ability as follow; DPPH scavenging activity of *H. cordifolia* bark ( $r = 0.9667$ ) and wood ( $r = 0.8048$ ), *M. diversifolia* wood ( $r = 0.8922$ ), *M. rotundifolia* wood ( $r = 0.9977$ ) and *M. coreia* bark ( $r = 0.6386$ ); NO scavenging activity of *C. tomentosa* bark ( $r = 0.8879$ ) and wood ( $r = 0.7538$ ), *H. cordifolia* wood ( $r = 0.6858$ ), *M. rotundifolia* wood ( $r = 0.9479$ ) and *M. coreia* wood ( $r = 0.6869$ ); SO scavenging activity of *M. diversifolia* bark ( $r = 0.8102$ ) and *M. coreia* bark ( $r = 0.8061$ ); and FRAP of *H. cordifolia* wood ( $r = 0.7559$ ), *M. diversifolia* bark ( $r = 0.9449$ ) and wood ( $r = 0.8030$ ) and *M. rotundifolia* bark ( $r = 0.9449$ ). The correlation analysis of flavonoid content and antioxidant activities show as follow; DPPH scavenging activity of *C. tomentosa* wood ( $r = 0.6171$ ), *H. cordifolia* bark ( $r = 0.7763$ ), *M. diversifolia* wood ( $r = 0.8372$ ), *M. rotundifolia* bark ( $r = 0.9983$ ), and *M. coreia* wood ( $r = 0.8445$ ); and SO scavenging activity of *C. tomentosa* bark ( $r = 0.9895$ ), *M. rotundifolia* wood ( $r = 0.9342$ ), and *M. coreia* wood ( $r = 0.9100$ ) respectively. Interestingly, the correlation of flavonoid content and FRAP showed that all plant extracts exhibit the high correlation coefficient except *C. tomentosa* wood ( $r = -0.4825$ ), while no correlation was observed between flavonoids and nitric oxide radicals or between phenolic and flavonoid contents and antioxidant activities. Antioxidant activity can be determined by ranking TPC and TFC from greatest to least, which

**Table 3.** Ranking of anti-oxidant activity, total phenolic and total flavonoid content.

Plant extracts	Part	DPPH ranking	NO ranking	SO ranking	FRAP ranking	Antioxidant rank
<i>Catunaregam tomentosa</i>	bark	5 <sup>ns</sup>	1	5 <sup>****</sup>	3 <sup>****</sup>	1
<i>Catunaregam tomentosa</i>	wood	2 <sup>ns</sup>	4 <sup>**</sup>	10 <sup>****</sup>	6 <sup>****</sup>	5
<i>Haldina cordifolia</i>	bark	3 <sup>ns</sup>	8 <sup>****</sup>	7 <sup>****</sup>	4 <sup>****</sup>	5
<i>Haldina cordifolia</i>	wood	4 <sup>ns</sup>	9 <sup>****</sup>	2 <sup>ns</sup>	9 <sup>****</sup>	8
<i>Mitragyna diversifolia</i>	bark	8 <sup>****</sup>	3 <sup>**</sup>	4 <sup>****</sup>	5 <sup>****</sup>	3
<i>Mitragyna diversifolia</i>	wood	7 <sup>**</sup>	7 <sup>****</sup>	6 <sup>****</sup>	8 <sup>****</sup>	9
<i>Mitragyna rotundifolia</i>	bark	9 <sup>****</sup>	2 <sup>*</sup>	3 <sup>****</sup>	7 <sup>****</sup>	4
<i>Mitragyna rotundifolia</i>	wood	6 <sup>*</sup>	6 <sup>****</sup>	9 <sup>****</sup>	2 <sup>****</sup>	7
<i>Morinda coreia</i>	bark	1	10 <sup>****</sup>	8 <sup>****</sup>	10 <sup>****</sup>	10
<i>Morinda coreia</i>	wood	10 <sup>****</sup>	5 <sup>****</sup>	1	1	2

Values followed by the asterisk symbol (\*) from  $IC_{50}$  are statistically significant ( $p < 0.05$ ) compared to the highest value of each activity based on Dunnett's multiple comparisons test.

related to the phenolic content, which agrees with the highest value of phenolic content in wood extracts (44, 52). However, a non-significant correlation was observed between the TPC and TFC and antioxidant

varies depending on plant species, growth and developmental stages, stress conditions and other factors (58, 59). Similar to previous reports, our results reveal a positive relationship between TPC

**Table 4.** Correlation between total phenolic content (TPC) and anti-oxidant activities of each plant extract; correlation coefficients were analysed at the 95% confidence interval.

Plant extracts	Part	correlation coefficient (r)			
		DPPH	NO	SO	FRAP
<i>Catunaregam tomentosa</i>	bark	-0.2603	0.8879	-0.1446	0.0275
<i>Catunaregam tomentosa</i>	wood	-0.6171	0.7538	0.2438	0.4825
<i>Haldina cordifolia</i>	bark	0.9667	-0.5081	-0.9541	0.2127
<i>Haldina cordifolia</i>	wood	0.8048	0.6858	-0.9958	0.7559
<i>Mitragyna diversifolia</i>	bark	-0.5000	-0.8874	0.8102	0.9449
<i>Mitragyna diversifolia</i>	wood	0.8922	-0.7995	-0.0262	0.8030
<i>Mitragyna rotundifolia</i>	bark	0.4490	-0.8470	0.1742	0.9449
<i>Mitragyna rotundifolia</i>	wood	0.9977	0.9479	-0.6307	-0.9707
<i>Morinda coreia</i>	bark	0.6386	-0.9953	0.8061	-0.8660
<i>Morinda coreia</i>	wood	-0.8198	0.6869	0.0316	-0.6099

**Table 5.** Correlation between total flavonoid content (TFC) and anti-oxidant activities of each plant extract. Correlation coefficient was analysed at 95% confidence interval.

Plant extracts	Part	correlation coefficient (r)			
		DPPH	NO	SO	FRAP
<i>Catunaregam tomentosa</i>	bark	-0.9655	-0.4600	0.9895	0.9996
<i>Catunaregam tomentosa</i>	wood	0.6171	-0.7538	-0.2438	-0.4825
<i>Haldina cordifolia</i>	bark	0.7763	-0.9953	-0.8042	0.9148
<i>Haldina cordifolia</i>	wood	0.1612	-0.0176	-0.7763	0.9982
<i>Mitragyna diversifolia</i>	bark	-0.9286	-0.9824	0.2740	0.9177
<i>Mitragyna diversifolia</i>	wood	0.8372	-0.9199	-0.8788	0.9177
<i>Mitragyna rotundifolia</i>	bark	0.9983	-0.8839	-0.7657	0.7559
<i>Mitragyna rotundifolia</i>	wood	-0.8302	-0.9802	0.9342	0.9608
<i>Morinda coreia</i>	bark	-0.9857	0.5818	-0.9155	0.8660
<i>Morinda coreia</i>	wood	0.8445	-0.9354	0.9100	0.9664

and TFC and antioxidant ability from *Trifolium pratense* (60). However, no significant correlation was observed between highest TPC and TFC and antioxidant activity in samples, which may be because of interference from other antioxidant chemical components (49). Moreover, phenolic content was correlated with DPPH radical scavenging and ferric reducing antioxidant potential activities, suggesting a relation between TPC and TFC and antioxidant activity in *Garcinia lasoar* bark, as similarly quantified by researchers (61). Our results revealed that *M. rotundifolia* revealed that the correlation between the TFC and DPPH radical scavenging and FRAP activities were relatively higher (DPPH;  $R^2 = 0.7612$ , FRAP;  $R^2 = 0.751$ ). The data of antioxidant activity corresponding to DPPH and TPC had a very low correlation coefficient ( $R^2 = 0.00008$ ), which has also been reported (37). Further, our analysis shows no significant correlation between TFC and NO radical scavenging activity, agreeing with a published report that TPC and TFC of *Launaea procumbens* extract have no correlation with NO radical scavenging activity (62). While several flavonoids may scavenge NO pro-oxidant properties by increasing superoxide, flavanones and flavonoid glycosides did not show significant inhibition of NO production up to 100  $\mu$ M (63). Besides NO, under oxidative stress conditions, flavonoids may also protect NO from  $O_2$ -driven inactivation and inhibit NO-scavenging effects. Therefore, the effect of flavonoids on NO levels depends on the structure and the concentrations (64). Our results further suggest that the plant extracts contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage.

## Conclusion

This work reveals the antioxidant ability of Rubiaceae species due to the methanolic extracts from TPC in *M. diversifolia* wood and TFC in *H. cordifolia* wood. Further, *C. tomentosa* bark presented the highest antioxidant activity. However, it is pertinent to note that the categorization established among the species depends on the method used. To the best of our knowledge, this is the first report on the phytochemical contents and antioxidant activity of *M. diversifolia* and *M. coreia*. The results of our study reveal that medicinal plants of the Rubiaceae family offer a potential source of natural antioxidants.

## Acknowledgements

This research was supported by “the Strengthening and Developing New Researcher Plan, in conformance with the Research and Innovation of Graduate Study Strategy of the National Research Council of Thailand (NRCT) as of the fiscal year 2019” and “The Graduate Program Scholarship from the Graduate School of Kasetsart University.”

## Authors' contributions

SD provided conceptualisation and resources, review and edited the manuscript. RS collect plant samples, investigated experiment, analysed data, and wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

## Conflict of interests

The authors declare no conflict of interest.

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