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ANTIOXIDANT CAPABILITIES OF *Litsea garciae* BARK EXTRACTS AND THEIR RELATION TO THE PHYTOCHEMICAL COMPOSITIONS

SITI ZALEHA RADUAN[1,2], QAMAR UDDIN AHMED[3*], ABDUL RAZAK KASMURI[1], MUHAMAD RUSDI AHMAD RUSMILI[1], MD ABDUR RASHID MIA[4], WAN MOHD AZIZI WAN SULAIMAN[5], MUHAMMAD HAMDI MAHMOOD[2] and MOHD FAROOQ SHAIKH[6]

[1]*Department of Basic Medical Sciences, Kulliyyah of Pharmacy, International Islamic University of Malaysia*
[2]*Department of Paraclinical Sciences, Universiti Malaysia Sarawak*
[3]*Department of Pharmaceutical Chemistry, Kulliyyah of Pharmacy, International Islamic University of Malaysia*
[4]*Department of Pharmaceutical Technology, Kulliyyah of Pharmacy, International Islamic University of Malaysia*
[5]*Faculty of Health Sciences, PICOMS International University College*
[6]*Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia*
[*]*E-mail: quahmed@iium.edu.my*

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ABSTRACT

The plant species belonging to the *Litsea* genus are widely investigated due to their nutritional and medicinal purposes. In this regard, this study is another similar sincere effort in which the antioxidant property and phytochemical composition of *Litsea garciae* (*L. garciae*) bark's hexane, chloroform, methanol, and aqueous extracts were evaluated to confirm its traditional benefits. The total flavonoid content (TFC) and total phenolic content (TPC) were determined first, followed by an assessment of in vitro antioxidant activity using the DPPH and FRAP assays. The composition of the secondary metabolites was determined using Ultra-High-Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS). As a result, methanol extract was recorded to have the highest TPC value aligned with its positive appearance in phytochemical screening. Its antioxidant capacity indicated the least IC_{50}. The results indicated that the significant free radical scavenging activity was due to the methanolic extract's high phenolic content. The secondary metabolites found in the methanol extract varied significantly according to UHPLC-MS analysis. The major phenolic compounds were found including N-*trans*-feruloyl-4-O-methyldopamine, N-*cis*-feruloyltyramine, epicatechin-(4beta->6)-epicatechin-(2beta->7,4beta->8)-epicatechin, 7-Hydroxy-3-(4-methoxyphenyl)-4-propyl-2H-1-benzopyran-2-one and 9-O-Methylneodunol. In general, the results indicate that *L. garciae* bark may be a promising source of novel natural compounds with antioxidative properties.

Key words: Antioxidant, aqueous, chloroform, hexane, *Litsea garciae*, methanol

INTRODUCTION

Plants are an essential component of traditional medicine, as they contain a diverse array of bioactive compounds that are useful against several ailments. According to Kuruppu *et al*. (2019), around 70,000 plant species ranging from lichens to trees have been shown to have the ability to treat a variety of ailments.

Litsea garciae S. Vidal is a Sarawak native plant that is usually referred to as Engkala (Lim, 2012). It is one of the 50 genera that comprise the Lauraceae family (Yen *et al*., 2008). Additionally, it is endemic in Malaysia's southwest Sabah region, Kalimantan, Indonesia, the Philippines, and Taiwan (Lim, 2012). The plant grows in inland riparian forests, secondary woods, and on rare occasions, mixed dipterocarp forests. It is a medium-sized tree with a loose crown (long brittle spreading branches) and lanceolate or obovate leaves, 25 cm long or more and hairless. Flowers are borne on the branches with pale yellow color (Chai, 2006). The wild edible fruit of pale greenish-white can be found seasonally and will turn pink or red at maturity (Lim, 2012). Historically, several tribes, notably those in Sarawak, have used various components of this plant (i.e. leaves, bark, & wood chips) as traditional herbal medicines to cure various ailments. For instance, the Iban (Lim, 2012) and Bidayuh (Chai, 2006) have been using the lightly burned bark (ash) to ease pain caused by caterpillar stings. The Selako tribe utilized a pounded poultice of the leaves or young shoots combined with fennel seed and shallot to treat skin disorders and infections of the

* To whom correspondence should be addressed.

palms and fingers, (Chai, 2006; Lim, 2012) including skin burns (Lim, 2012). The Kayan tribe warms the leaves and uses them as a treatment against beriberi. The Kelabit people use warm scrapings of the root bark as a treatment for sprains. The Iban tribe uses pounded bark or young leaves as a plaster to extract pus from boils and a bark decoction to treat blood in stools. Equal parts of the bark and durian (*Durio zibethinus*) bark are crushed and applied to snakebite wounds as an antidote (Chai, 2006). The Penan tribe pound, reheat, and poultice the bark to treat muscular problems, sprained ankles, and knees (Chai, 2006; Lim, 2012).

Oxidative stress has been linked to a wide variety of diseases. According to the extent to which oxidative stress contributes to the etiology of these pathologies, they have been classified as follows: first, oxidative stress as the primary cause of pathology (including radiation and paraquat toxicity, as well as atherosclerosis); second, oxidative stress as a secondary contributor to disease progression (as in COPD, hypertension, & Alzheimer disease) (Forman & Zhang, 2021). Antioxidants prevent or eliminate oxidative stress-related disorders by neutralizing the damaging effect of reactive oxygen species (ROS). Antioxidants neutralize free radicals and are critical for maintaining adequate cellular processes. Traditional medicinal plants have been reported to produce various types of potent antioxidants including a well-reputed antioxidant i.e. quercetin. The antioxidant activity, including the radicals scavenging and redox potential of polyphenols, is generally thought to be a reason behind their beneficial effect on human health (Maliński *et al*., 2021). In this regard, *L. garciae* could also prove to be another source of potent antioxidants that may provide a lead for the synthesis of safe and potent antioxidants envisaged to treat various ailments associated with the manifestation of deleterious oxidative reactions in the human body. Hence, keeping view of its traditional claims by tribes mentioned above in the management of several diseases primarily associated with the outcome of oxidative reactions to prove its antioxidative potential, Wulandari *et al*. (2018) evaluated the stem/ branch, bark, and leaf part of *L. garciae* hexane, ethyl acetate, and ethanolic extracts. According to the findings, the ethanol extract of bark exhibited the highest antioxidant activity compared to the branch and leaf parts. Additionally, total phenol content (TPC) and total flavonoid content (TFC) of ethanol-soluble extracts were determined to be relatively high. Hassan *et al*. (2013) found that the stem cap part of *L. garciae* fruit methanolic and aqueous extracts possessed the highest ferric reducing activity and recorded the highest TPC and TFC values than seed and flesh part, further confirming the antioxidative potential of *L. garciae*. A study carried out by Rafidah (2017) also showed a high value of TPC and TFC in various parts of superheated-steam drying (SHSD) and freeze-drying (FD) *L. garciae* fruits. The study found that TPC may contribute to *L. garciae*'s substantial radical scavenging activity, with FD and SHSD seed sections exhibiting significantly higher antioxidant activity than cupule, pulp, and peel. A recent study demonstrated the polyphenol-rich fraction of *Litsea quinqueflora* (Dennst.) Suresh possessed statistically significant antioxidant capabilities as measured by its scavenging free radicals against DPPH and ABTS (Jose & Anilkumar, 2021).

Numerous in vitro antioxidative evaluations of various parts of the *L. garciae* plant have been conducted to confirm its antioxidant potential. However, no study discusses the possible active principles in the *L. garciae* plant identified through the liquid chromatography-mass spectrometry (LC/MS) analytical technique and its relationship to the antioxidative effect. Thus, the purpose of this study was to determine the phytochemical compositions and antioxidant capabilities of hexane, chloroform, methanolic, and aqueous extracts of *L. garciae* bark, which may have potential effects as a natural antioxidant beneficial to human health.

MATERIALS AND METHODS

Plant material and chemicals

The bark of *L. garciae* was gathered from a mature plant at Kampung Sentosa Salim, Sibu Sarawak (coordinates: 2.2735800, 111.8665960) and identified by a botanist from the Sarawak Biodiversity's Centre (SBC) with the research permit and voucher specimen no. SBC-2018-RDP-15-SZR. The plant material was collected in bulk (approximately 2 kg cumulatively), dried under shade, and pulverized in a commercial blender (Waring Commercial, USA). The coarse powder was kept dry in a desiccator for further studies.

All chemicals used were of analytical grade reagents. n-hexane (C_6H_{14}), chloroform ($CHCl_3$) and methanol (CH_3OH), hydrochloric acid 37% (HCl), Dragendorff's reagent spray solution, magnesium turnings (Mg), zinc dust (Zn), ferric (III) chloride ($FeCl_3$), sodium hydroxide (NaOH), acetic anhydride ($C_4H_6O_3$), gallic acid anhydrous ($C_7H_6O_5$) were obtained from Merck (Germany). Mayer's reagent was from LabChem Inc (USA). Ethanol absolute (C_2H_5OH) was from HmbG Chemicals (Germany). Sulfuric acid 96% (H_2SO_4) was from Fischer Scientific (UK). Quercetin ($C_{15}H_{10}O_7$) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were from Sigma Aldrich (USA). Folin & Ciocateu's Phenol Reagent was from R&M Chemicals (UK). Aluminum chloride anhydrous powder ($AlCl_3$) was from Fischer Scientific (UK). L-ascorbic acid ($C_6H_8O_6$) was from BDH (Dublin).

Extraction of the sample

The extraction process was modified slightly using the Opeyemi *et al*. (2019) method. In brief, the coarse dry powder of bark was macerated successively with the solvents in ascending order of polarity (hexane < chloroform < methanol < water). The powder was first left soaked in hexane solvent for 42 h with the ratio of dried powder to solvent 1:10. It was soaked three times (until exhaustion) to obtain the maximum yield of hexane soluble compounds of bark. Following that, the filtrate was dried using a rotary evaporator (Buchi, Switzerland). After allowing the residue to dry in the fume hood, the same maceration method was done successively with chloroform and methanol to get chloroform and methanol extracts, respectively. As to obtain the aqueous extract, the dried residue after having undergone maceration with methanol was soaked in distilled water with the ratio of 1:10 and left on the water bath (Memmert, Shwabach) for 1 h (95 ºC). It was then vacuum filtered and the entire process was repeated three times to obtain the maximum yield. The filtrate was freeze-dried (Martin Christ, Germany) for 72 to 96 h. The residue was discarded.

Before Ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) of *L. garciae* bark analysis, the suspension (1:100 extract to methanol) was sonicated for 180 min in an ultrasonic bath. The supernatants were then passed through PTFE membrane (0.45 µm; 13 mm) sterile syringe filters.

Determination of percentage yield

The dry weight of respective crude over the powder of bark used was recorded to determine the percentage yield (% w/w) as Equation 1:

(weight of dried extract/ weight of dried powder) ×100

Phytochemical composition

Phytochemical Screening

a. Test for Alkaloids

A total of 0.5 g of crude *L. garciae* extract (hexane, chloroform, methanol, or aqueous) was combined with 5 mL of 1% HCl, heated in a water bath, and thoroughly sonicated. Following that, the solution is filtered while it is still hot. Following that, the filtrate was subjected to the following tests;

1. Mayer's reagent test

Mayer's reagent was added to 1 mL of filtrate (Patle *et al*., 2020). Precipitates of white or cream color suggested the presence of alkaloids (Tiwari & Gupta, 2020).

2. Dragendroff's test

0.5 mL of Dragendroff's reagent was added to 1 mL of filtrate. Orange-yellow precipitate development suggested the presence of alkaloids (Tiwari & Gupta, 2020).

b. Test for Flavonoids

1 g of *L. garciae* crude extract (hexane, chloroform, methanol, or aqueous) was mixed with 10 mL ethanol, boiled in a water bath, and sonicated thoroughly. The hot solution was then filtered. The following reagents were used to test the filtrate;

1. Shinoda's Test

A few drops of concentrated HCl and magnesium turnings were added to 3 mL of extract. Colors ranging from orange to red indicated flavones, red to crimson indicated flavonols, and crimson to magenta indicated flavanones (Odebiyi & Sofowora, 1978)

2. Zinc-hydrochloride test

A pinch of zinc dust and a few drops of concentrated HCl were added to 3 mL of extract. The presence of crimson red or magenta colors suggested the presence of flavonoids (Krishnaveni & Dhanalakshmi, 2014; Vimalkumar *et al*., 2014)

c. Test for Saponins (Froth Formation Test)

The crude extract (0.5 g) was combined with 10 mL of distilled water, boiled in a water bath, and thoroughly sonicated (Edeoga *et al*., 2005). After that, the solution is filtered while it is still hot. For at least 15 min, the filtrate was vigorously agitated in a graduated cylinder for 1 to 2 min to obtain a stable, persistent foam, which was interpreted as the presence of saponins (Singh & Kumar, 2017).

d. Test for Tannins and Phenolic compounds

The 0.5 g crude extract was combined with 10 mL distilled water, boiled in a water bath, and sonicated thoroughly. After that, the heated solution is filtered. Preliminary phytochemical analysis of the filtrate was performed as follows;

1. Ferric chloride test 5%

The test was performed with slight modification based on Prashanth and Krishnaiah (2014), Tiwari and Gupta (2020), Uma *et al*. (2017), and Singh and Kumar (2017) . 2 mL of aqueous 5% ferric chloride solution was added to 2 mL of extract. Blue, green, or violet colors were produced to indicate the presence of phenolic compounds.

2. Ferric chloride test 10% (Braymer's test)

The test was adapted with a minor based on Singh and Kumar (2017) and Uma *et al*. (2017). 2 mL of extract was added to 2 mL of a 10% alcoholic ferric chloride solution. The presence of tannins was detected by the production of a blue or greenish color.

3. Hydrolyzable tannin

The assay was adapted and modified based on Singh and Kumar (2017). 4 mL of 10% NaOH solution was added to 2 mL of extract. The assay resulted in the

formation of an emulsion, indicating the existence of hydrolyzable tannin.

e. Test for Terpenoids (Salkowski Test)

The 0.5 g extract was combined with 2 mL chloroform, and 3 mL concentrated H_2SO_4 to generate a layer. A reddish-brown color determined the presence of terpenoids (Ayoola *et al*., 2008).

f. Test for Steroids (Liebermann-Buchard Test)

The 2 mL acetic anhydride and 2 mL H_2SO_4 were added to 0.5 g crude extract. The change in color from violet to blue or green was seen as proof of the presence of steroids. (Edeoga *et al.*, 2005).

g. Secondary metabolite profiling

The RP-UHPLC-MS analysis was used to profile secondary metabolites. Agilent 1290 Infinity LC system was utilized with Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with a dual ESI source. Agilent Zorbax Eclipse XDB-C18, narrow-bore 2.1 × 150 mm, 3.5 m (P/N: 930990-902) were the column specifications. The temperature of the column and auto-sampler were kept at 25 ºC and 4 ºC, respectively. The flow rate was 0.5 mm/min. The 0.1% formic acid in water was utilized, and 0.1% formic acid in acetonitrile was employed as the mobile phase. The injection volume was set at 1.0 µL. The run took 25 min, while the recovery time was 5 min. A complete scan using an electrospray ion source in positive mode, MS analysis was performed over the m/z range of 100-1000. Nitrogen was delivered at flow rates of 25 and 600 mL/h as nebulizing and drying gas, respectively. The temperature of the drying gas was 350 ºC. The voltage for fragmentation was set to 125 V. A 3500 V capillary voltage was used for the analysis. Agilent Mass Hunter Qualitative Analysis B.05.00 was used to process the data. Compounds were identified using Search Databases such as METLIN (Saleem *et al*., 2019), SPECTRA BASE, LIPID MAPS, and EPA DSSTox.

Antioxidant measurements

Total phenolic content (TPC)

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu method with slight modification (Amalia *et al*., 2019; Keypour *et al*., 2019). The standard calibration curve was developed using gallic acid. Gallic acid standard solutions were prepared in serial dilutions with methanol (200, 100, 50, 25, 12.5 & 6.25 µg/mL). Standard gallic acid solutions or extracts (25 µL) and 100 µL (25%) Folin-Ciocalteu were pipetted into a flat bottom 96-well microplate (Corning, USA), homogenized for 60 s with a shaker and then left at room temperature (RT) for 4 min in the dark. Then, 75 µL of sodium carbonate solution (7.5%) was added and mixed for 60 s. After that, the mixture was incubated at RT in the dark for 2 h. The absorbance was determined at a wavelength of 750 nm using a microplate reader (Tecan, Austria).

The calibration plot was used to determine the total phenolic content of the test samples. y=0.0061x + 0.0558, R^2=0.987, where y is the yield of gallic acid equivalents (GAE) (total phenolic content) and x is the absorbance of gallic acid or extract. The results were represented in µg (GAE) per mg of the dry weight of the extracts (µg GAE/mg DW extracts). All measurements were made in triplicate.

Total flavonoid content (TFC)

The aluminum chloride colorimetric method by Chandra *et al*. (2014) was used to determine total flavonoid content (TFC) for all extracts with slight modification. The standard calibration curve was created using standard quercetin. The stock quercetin solution was made by dissolving 5 mg quercetin in 1 mL methanol, and then serial dilutions were used to make the standard quercetin solutions (200, 100, 50, 25, 12.5 µg/mL).

In a flat-bottom 96-well microplate, 25 µL of diluted standard quercetin solutions or extracts (Herald *et al*., 2012) were mixed with 25 µL of 2% aluminum chloride. After that, the mixture was incubated at RT for 60 min. The absorbance at 420 nm was determined using a microplate reader (Tecan, Austria).

The total flavonoid content of the test samples was determined using the calibration plot y=0.0031x + 0.0085, R_2= 0.9932, where y denotes the yield of QE (total flavonoids content) and x denotes the absorbance of quercetin or extract. The results were represented as µg quercetin equivalents (QE) per mg extract dry weight (µg QE/mg DW extracts). All measurements were made in triplicate.

Antioxidant capacity assessment

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric tripyridyltriazine (Fe III-TPTZ) complex was reduced to the ferrous ion (Fe II) at a low pH, resulting in a solid blue color. Compounds with an antioxidant activity work by donating a hydrogen atom to break the free radical chain. The method was executed based on Sarian *et al*. (2017) with slight modification. The standard calibration curve was developed using ascorbic acid. Serial dilutions of ascorbic acid (AA) in methanol were used to establish the standard solutions (100, 50, 25, 12.5, 6.25, & 3.125 µg/mL). The ferric reducing antioxidant power (FRAP) reagent was made (1 mL of a 10 mM TPTZ solution in 40 mM HCl, 1 mL of 20 mM $FeCl_3$, and 10 mL of 0.1 M acetate buffer pH 3.6) and incubated for 10 min at 37 ºC. Then, in a 96-well plate, 20 µL of extract or AA (standard) and 40 µL of FRAP reagent were added to 140 µL of dH_2O, yielding a blue-colored solution. The solutions were maintained at

room temperature for 20 min in the dark before being measured with a microplate reader at 593 nm using a reagent blank made up of 40 µL of FRAP reagents in 140 µL dH2O with 20 µL of 100% methanol.

The calibration plot was used to calculate the total reduction power of the test samples. y=0.8833x + 0.0961, R^2= 0.9587, where y is the yield of AA (reducing power) and x is the absorbance of AA or extract. The results were represented in µg AA equivalents (AAE) per mg of dry weight extracts (µg AAE/mg DW extracts). All measurements were made in triplicate.

2,2-Diphenyl-1-picrylhydrazyl free radical scavenging (DPPH) Assay

The radical scavenging activity was assessed with slight modifications as described by Sarian *et.al* (2017). In 50 mL of methanol, 3.94 mg of DPPH was dissolved. Around 80 ul of DPPH methanolic solution (0.2 mM) was added to 20 µL of 200 µg/mL extract for the sample (serial dilution of 200, 100, 50, 25, 12.5 µg/mL were prepared) in a microplate 96-well (the absorbance reading was deducted later with the absorbance reading of sample blank). As for control, the absorbance value of blank control was subtracted from the absorbance value of blank. AA 25 µg/mL was utilised as a positive control (serial dilution of 100, 50, 25, 12.5, 6.25 µg/mL were prepared). After 10 min, it was placed in the dark at room temperature, and the absorbance at 540 nm was measured. All measurements were made in triplicate. The percentage inhibition (%) of the DPPH radical by the samples was estimated using the following formula from Equation 2:

$$[(OD_{control} - OD_{sample})/OD_{control}] \times 100\%$$

Where $OD_{control}$ is the absorbance control and OD_{sample} is the absorbance of the sample.

(Sample blank referred to a mixture of 80 µL absolute methanol and 20 µL extract; blank control referred to a mixture of 80 µL methanolic solution of DPPH and 20 µL absolute methanol, and blank referred to the 100 µL of absolute methanol).

Statistical Analysis

Triplicates of all experiments were performed. The mean ± standard error of the mean (SEM) was used to describe the data. A one-way analysis of variance (ANOVA) was used to determine the significance at $p<0.05$. Turkey's multiple comparison test was used to examine whether there were statistically significant differences between groups. Additionally, Pearson's coefficient correlation test was used to establish the association between the DPPH and FRAP assays and the TPC.

RESULTS

Extraction yield

The yield of extracts obtained using the maceration process is listed in Table 1. Methanol extract had the highest yield, followed by aqueous, chloroform, and hexane extracts. The yield of methanol extract was 3.71 (as % w/w of *L. garciae* bark on a dry weight basis).

Phytochemical Composition

Phytochemical Screening

The phytochemical screening of the *L. garciae* bark extracts is shown in Table 2 which reveals that methanol extract is abundant in phenolic (flavonoids & tannins) contents.

Secondary metabolites profiling

Liquid chromatography-mass spectrometry (LC/MS) was used to profile the secondary metabolite components of *L. garciae* bark of hexane, chloroform, methanol, and aqueous extracts. The sample was chromatographically separated using standard optimum conditions.

The total ion chromatogram (TIC) of the *L. garciae* bark of hexane extract with positive ion mode mass spectrometric detection revealed intricate patterns of peaks, as illustrated in Figure 1. The extract's LC/MS analysis revealed 48 different compounds listed in Table 3.

The TIC of the *L. garciae* bark of chloroform extract was determined using mass spectrometry in positive ion mode and revealed complex patterns of peaks, as illustrated in Figure 2. The extract's LC/MS analysis revealed 29 different compounds presented in Table 4.

The TIC of the *L. garciae* bark of methanolic extract detected in positive ion mode by mass spectrometry revealed complex patterns of peaks, as illustrated in Figure 3. The extract's LC/MS analysis revealed 35 different compounds presented in Table 5.

The TIC of the *L. garciae* bark of aqueous extract was determined using mass spectrometry in positive ion mode and revealed intricate patterns of peaks, as illustrated in Figure 4. The extract's LC/MS analysis revealed 36 different compounds presented in Table 6.

Antioxidant measurements

The total phenolic content (TPC) and total flavonoid content (TFC) are depicted in Figure 2 and summarised in Table 7.

The TPC was quantified in gallic acid equivalents (mg GAE/mg extract). The TPC was highest in the methanol extract (34.82 ± 1.12 g GAE/mg). The TPC analysis were significantly different ($p<0.05$) between extracts, with methanol > aqueous > chloroform > hexane following the trend order (Figure 5a).

The TFC was quantified as quercetin equivalent (mg of QE/mg of extract). The results indicated that chloroform extract contained the highest concentration of TFC (24.03 ± 4.15 g QE/mg). The analysis, on the other hand, was not significantly different (p>0.05) between extracts, with chloroform > methanol > hexane > aqueous being the trend order (Figure 5b).

Antioxidant capacity assessment

The FRAP assay and DPPH free radical scavenging assay are illustrated in Figure 3 and presented in Table 8.

In FRAP assay, antioxidants exerted their effect by their electron-donating action. The result for the FRAP assay was expressed in ascorbic acid equivalents (mg of AAE/mg of extract). All of the extracts showed no significant (p<0.05) antioxidant capacity compared to the standard AA. The highest antioxidant capacity exerted by chloroform (0.167 ± 0.003 mgAAE/mg) among the extracts following the trend order (chloroform > methanol > hexane > aqueous) (Figure 6a).

The IC_{50} value is defined as the concentration of *L. garciae* bark extract required to scavenge 50% of radicals, as indicated by the regression curve. Since it is a measure of inhibitory concentration, lower IC_{50} reflects higher antioxidant activity and *vice versa*. The methanol extract of *L. garciae* bark had the lowest IC_{50} of 280.010 ± 58.047µg/mL DPPH radical scavenging capacity and was comparable to the standard ascorbic acid (IC_{50}= 44.310 ± 1.952 µg/mL). The trend order of IC_{50} obtained in the extract was hexane> chloroform > aqueous > methanol (Figure 6b).

Table 1. *Litsea garciae* bark extracts yield

Extract	Hexane	Chloroform	Methanol	Aqueous
Yield (% w/w)	0.55	2.06	3.71	2.49

Table 2. Phytochemical constituents of hexane, chloroform, methanol, and aqueous extracts of *Litsea garciae* bark

Extract	Test	Hexane	Chloroform	Methanol	Aqueous
Phytochemical Constituents					
Alkaloids	Mayer's	+	+	+	-
	Dragendroff's	+	+	+	+
Flavonoids	Shinoda's	-	+	+	-
	Zinc-hydrochloride	-	+	+	-
Saponins	Froth formation	-	-	+	+
Phenol	Ferric chloride test 5%	-	-	+	+
Tannins	Ferric chloride test 10%	-	-	+	+
	Hydrolyzable tannin	+	+	+	+
Triterpenes	Salkowski	+	+	+	+
Steroids	Liebermann-Buchard Test	+	+	+	+

Note: (-) negative test (+) positive test

Table 3. UHPLC-MS of *Litsea garciae* bark hexane extract

CPD	RT (min)	Base peak (m/z)	Proposed compounds	Compound class	Mol. formula	Mol. mass
4	7.757	314.1388	N-*cis*-Feruloyltyramine	Phenol derivative	$C_{18}H_{19}NO_4$	313.1316
5	8.763	328.1543	6-Acetylmorphine	Alkaloid	$C_{19}H_{21}NO_4$	327.147
6	8.841	312.1234	9-O-Methylneodunol	Pterocarpan	$C_{18}H_{14}O_4$	294.0896
7	8.885	566.4277	UNKNOWN	UNKNOWN	$C_{29}H_{49}N_{12}$	565.4203
9	9.036	163.1327	(3R,7R)-1,3,7-Octanetriol	Fatty alcohol	$C_8H_{18}O_3$	162.1254
10	9.201	679.5115	UNKNOWN	UNKNOWN	$C_{34}H_{64}N_9O_5$	678.5035
12	9.72	310.1078	Cauliflorin A	Flavonoid	$C_{18}H_{12}O_4$	292.0739
13	10.026	320.0551	Hernandonine	Alkaloid	$C_{18}H_9NO_5$	319.0478
19	11.315	237.1848	Cyperolone	Sesquiterpene	$C_{15}H_{24}O_2$	236.1775
23	12.093	274.2746	C16 Sphinganine	Sphingoid	$C_{16}H_{35}NO_2$	273.2672
25	12.144	230.2475	Xestoaminol C	Sphingoid	$C_{14}H_{31}NO$	229.2402
30	12.25	290.269	16-hydroxy hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_3$	272.235
34	12.792	288.2527	Prosopinine	Sphingoid	$C_{16}H_{33}NO_3$	287.2456
36	13.007	263.1268	Imiquimod	Imidazoquinoline	$C_{14}H_{16}N_4$	240.1378
37	13.129	382.2205	UNKNOWN	UNKNOWN	$C_{18}H_{26}N_3O_5$	364.1868
42	13.778	221.1879	10E-Tridecen-2S-ol	Fatty alcohol	$C_{13}H_{26}O$	198.1984
43	13.873	445.2116	UNKNOWN	UNKNOWN	$C_{25}H_{26}N_5O_3$	444.203
46	14.424	313.2006	(7Z)-14-hydroxy-10,13-dioxoheptadec-7-enoic acid	Fatty acid	$C_{17}H_{28}O_5$	312.1933
47	14.465	227.1993	UNKNOWN	UNKNOWN	$C_{12}H_{24}N_3O$	226.192
51	14.747	366.2263	Chlorovulone III	UNKNOWN	$C_{20}H_{28}O_5$	348.1933
56	15.259	507.2282	UNKNOWN	UNKNOWN	$C_{32}H_{30}N_2O_4$	506.2208
60	15.48	297.2035	4,12-dihydroxy-pentadecanoic acid	Fatty acid	$C_{15}H_{30}O_4$	274.2143
63	15.622	566.4279	UNKNOWN	UNKNOWN	$C_{30}H_{55}N_5O_5$	565.4204
75	16.079	434.3984	6α-hydroxycholestanol(d7)	UNKNOWN	$C_{27}H_{41}D_7O_2$	411.4089
81	16.266	295.2268	α-9(10)-EpODE	Epoxy fatty acid	$C_{18}H_{30}O_3$	294.2195
85	16.432	199.168	UNKNOWN	UNKNOWN	$C_{10}H_{20}N_3O$	198.1607
106	17.107	243.1954	4-keto myristic acid	Fatty acid	$C_{14}H_{26}O_3$	242.1882
107	17.133	434.3996	22α-Hydroxy-5α-campestan-3-one	Glycerolipid	$C_{28}H_{48}O_2$	416.3657
113	17.791	263.1275	Helenalin	Sesquiterpenoid	$C_{15}H_{18}O_4$	262.1202
115	17.797	574.3019	UNKNOWN	UNKNOWN	$C_{31}H_{40}O_9$	556.2671
120	18.148	320.2216	Dihydrotetrabenazine	Tetrahydroisoquinoline	$C_{19}H_{29}NO_3$	319.2149
124	18.281	423.3108	19-(3-methyl-butanoyloxy)-villanovane-13alpha,17-diol	Isoprenoid	$C_{25}H_{42}O_5$	422.3035
127	18.298	416.3891	Dihydrotachysterol	Vitamin D derivative	$C_{28}H_{46}O$	398.3552
130	18.465	522.3791	Tomentosic acid	Triterpenoid	$C_{30}H_{48}O_6$	504.3452
132	18.507	250.2166	2,4-Dodecadienoic acid pyrrolidide	n-acylpyrrolidines	$C_{16}H_{27}NO$	249.2092
133	18.524	309.2426	methyl 15,16-epoxy-9,12-octadecadienoate	Fatty acid	$C_{19}H_{32}O_3$	308.2352
134	18.654	442.4041	Oleic Acid-2,6-diisopropylanilide	Fatty acid	$C_{30}H_{51}NO$	441.3964
136	18.835	432.3835	28:5(10Z,13Z,16Z,19Z,22Z)	Fatty acid	$C_{28}H_{46}O_2$	414.3494
145	19.194	426.3233	UNKNOWN	UNKNOWN	$C_{25}H_{36}N_4O$	408.2894
152	19.315	482.3504	UNKNOWN	UNKNOWN	$C_{30}H_{45}N_2O_3$	481.3435
156	19.56	262.2164	Cryptophorine	Alkaloid	$C_{17}H_{27}NO$	261.209
158	19.671	444.4208	Cycloartenol	Triterpenoid	$C_{30}H_{50}O$	426.3863
159	19.688	275.2482	Dispiro(cyclohexane-1,2'(3'H)-quinazoline-4'(4'aH),1''-cyclohexane), 5',6',7',8'-tetrahydro-	Heterocyclic derivative	$C_{18}H_{30}N_2$	274.2408
160	19.704	616.5308	UNKNOWN	UNKNOWN	$C_{39}H_{69}NO_4$	615.5232
163	19.96	389.3055	1α,25-dihydroxy-26,27-dinorvitamin D3 / 1α,25-dihydroxy-26,27-dinorcholecalciferol	Vitamin D3 derivative	$C_{25}H_{40}O_3$	388.2982
167	20.073	477.3574	11-acetoxy-3beta,6alpha-dihydroxy-9,11-seco-5alpha-cholest-7-en-9-one	Cholesterol derivative	$C_{29}H_{48}O_5$	476.35
170	20.225	540.3804	UNKNOWN	UNKNOWN	$C_{25}H_{49}N_9O_2S$	539.3726
178	24.398	386.3414	Fistulosin	Indole derivative	$C_{26}H_{43}NO$	385.3341

Table 4. UHPLC-MS of *Litsea garciae* bark chloroform extract

CPD	RT (min)	Base peak (m/z)	Proposed compounds	Compound class	Mol. formula	Mol. mass
4	3.429	113.0597	Parasorbic acid	Lactone	$C_6H_8O_2$	112.0526
5	7.197	328.1543	Bracteoline	Alkaloid	$C_{19}H_{21}NO_4$	327.1469
9	7.855	328.1545	Norisocorydine	Alkaloid	$C_{19}H_{21}NO_4$	327.147
20	9.018	322.0715	3-(3-(4-Nitrophenyl) acryloyl)-2H-chromen-2-one	Coumarin	$C_{18}H_{11}NO_5$	321.0637
22	9.103	342.1344	Cassythine	Alkaloid	$C_{19}H_{19}NO_5$	341.1267
23	9.199	679.5136	UNKNOWN	UNKNOWN	$C_{38}H_{68}N_3O_7$	678.5054
25	9.674	338.1027	Atheroline	Alkaloid	$C_{19}H_{15}NO_5$	337.0955
26	9.718	310.1078	Cauliflorin A	Flavonoid	$C_{18}H_{12}O_4$	292.074
29	11.022	222.1852	N-3,7-Dimethyl-2,6-octadienylcyclopropylcarboxamide	Monoterpenoid	$C_{14}H_{23}NO$	221.1777
33	11.624	332.2218	Megastachine	Azaspiro derivative	$C_{20}H_{29}NO_3$	331.2143
35	12.116	226.2151	UNKNOWN	UNKNOWN	$C_{12}H_{25}N_4$	225.2077
37	12.196	301.2848	Prosafrinine	Sphingoid	$C_{17}H_{33}NO_2$	283.2508
39	12.669	366.2269	Chlorovulone III	UNKNOWN	$C_{20}H_{28}O_5$	348.1932
41	13.875	445.2119	Asperglaucide	Phenylalanine derivative	$C_{27}H_{28}N_2O_4$	444.2046
45	14.108	315.2513	UNKNOWN	UNKNOWN	$C_{16}H_{32}N_3O_3$	314.244
46	14.428	313.2003	(7Z)-14-hydroxy-10,13-dioxoheptadec-7-enoic acid	Fatty acid	$C_{17}H_{28}O_5$	312.1932
51	14.879	271.1886	UNKNOWN	UNKNOWN	$C_{13}H_{24}N_3O_3$	270.1814
54	15.082	211.1679	UNKNOWN	UNKNOWN	$C_{11}H_{20}N_3O$	210.1607
56	15.624	566.4266	UNKNOWN	UNKNOWN	$C_{28}H_{53}N_8O_4$	565.4191
57	15.685	279.2295	2-hexyl-decanoic acid	Fatty acid	$C_{16}H_{32}O_2$	256.2398
63	16.269	277.2162	5Z,8Z,11Z,14Z-octadecatetraenoic acid	Fatty acid	$C_{18}H_{28}O_2$	276.2088
64	16.269	295.2274	α-9(10)-EpODE	Epoxy fatty acid	$C_{18}H_{30}O_3$	294.22
67	16.508	299.2214	Plakortic acid	Dioxanes	$C_{17}H_{30}O_4$	298.2141
71	16.677	149.0236	1,3-isobenzofurandione	Phthalic anhydride	$C_8H_4O_3$	148.0163
75	18.287	521.3472	Cucurbitacin P	Triterpenoid	$C_{30}H_{48}O_7$	520.3403
78	19.066	523.3625	UNKNOWN	UNKNOWN	$C_{30}H_{50}O_7$	522.3554
83	19.479	609.2716	Harderoporphyrin	Porphyrin derivative	$C_{35}H_{36}N_4O_6$	608.2642
88	20.07	477.3582	11-acetoxy-3beta,6alpha-dihydroxy-9,11-seco-5alpha-cholest-7-en-9-one	Cholesterol derivative	$C_{29}H_{48}O_5$	476.351
89	20.173	593.2719	Khayanthone	Triterpenoid	$C_{32}H_{42}O_9$	570.2827

Table 5. UHPLC-MS of *Litsea garciae* bark methanolic extract

CPD	RT (min)	Base peak (m/z)	Proposed compounds	Compound class	Mol. Formula	Mol. Mass
10	0.962	190.0708	Glutarylglycine	Amino acid	$C_7H_{11}NO_5$	189.0635
15	1.909	204.0864	N2-Acetyl-L-aminoadipate	Acid	$C_8H_{13}NO_5$	203.0792
16	2.062	389.2029	Ile-Gln-Glu	Peptide	$C_{16}H_{28}N_4O_7$	388.1956
17	2.301	220.1181	Pantothenic acid	Acid derivative	$C_9H_{17}NO_5$	219.1108
18	3.26	371.1928	UNKNOWN	UNKNOWN	$C_{16}H_{26}N_4O_6$	370.1855
19	6.912	476.2121	Eugenol O-[a-L-Arabinofuranosyl-(1->6)-b-D-glucopyranoside]	Phenolic glycoside	$C_{21}H_{30}O_{11}$	458.1782
20	7.193	328.1549	Bracteoline	Alkaloid	$C_{19}H_{21}NO_4$	327.1475
21	7.286	344.1485	N-*trans*-Feruloyl-4-O-methyldopamine	Phenol derivative	$C_{19}H_{21}NO_5$	343.1414
23	7.44	496.2024	Gentiobiosyl 2-methyl-6-oxo-2E,4E-heptadienoate	Glycoside	$C_{20}H_{30}O_{13}$	478.1684
24	7.603	314.1384	N-*cis*-Feruloyltyramine	Phenol derivative	$C_{18}H_{19}NO_4$	313.1315
26	7.726	314.1368	UNKNOWN	UNKNOWN	$C_{16}H_{17}N_4O_3$	313.1298
28	7.854	344.1854	3-Hydroxyestra-1,3,5(10)-trien-17-one O-(carboxymethyl)oxime	Steroid	$C_{20}H_{25}NO_4$	343.1779
29	7.97	346.1629	Alloxydim	Oxime O-ether	$C_{17}H_{25}NO_5$	323.1736
35	8.195	865.1982	Epicatechin-(4beta->6)-epicatechin-(2beta->7,4beta->8)-epicatechin	Flavonoid	$C_{45}H_{36}O_{18}$	864.1904
40	8.548	300.1595	Hydrocodone	Alkaloid	$C_{18}H_{21}NO_3$	299.1521
41	8.575	286.144	Hydromorphone	Alkaloid	$C_{17}H_{19}NO_3$	285.1368
44	8.742	328.1542	7-Hydroxy-3-(4-methoxyphenyl)-4-propyl-2H-1-benzopyran-2-one	Coumarin	$C_{19}H_{18}O_4$	310.1205
45	8.785	342.1708	Cularine	Alkaloid	$C_{20}H_{23}NO_4$	341.1634
46	8.812	312.1223	9-O-Methylneodunol	Flavonoid	$C_{18}H_{14}O_4$	294.0887
50	9.038	679.2283	UNKNOWN	UNKNOWN	$C_{30}H_{28}N_{15}O_3S$	678.2211
51	9.047	358.1647	Desacetylcolchicine	Alkaloid	$C_{20}H_{23}NO_5$	357.1573
53	9.206	679.5109	UNKNOWN	UNKNOWN	$C_{34}H_{64}N_9O_5$	678.5037
54	9.682	338.1018	Atheroline	Alkaloid	$C_{19}H_{15}NO_5$	337.0945
56	9.937	331.1537	Tetrahydrosappanone A Trimethyl Ether	Alcohol	$C_{19}H_{22}O_5$	330.1464
62	11.427	295.2264	α-9(10)-EpODE	Epoxy fatty acid	$C_{18}H_{30}O_3$	294.2191
63	11.429	348.2743	5,8,12-trihydroxy-9-octadecenoic acid	Fatty acid	$C_{18}H_{34}O_5$	330.2403
64	12.133	274.274	C16 Sphinganine	Sphingoid	$C_{16}H_{35}NO_2$	273.2666
71	14.623	239.1629	UNKNOWN	UNKNOWN	$C_{12}H_{20}N_3O_2$	238.1557
72	14.872	277.2165	5Z,8Z,11Z,14Z-octadecatetraenoic acid	Unsaturated fatty acids	$C_{18}H_{28}O_2$	276.2092
74	15.625	566.4265	UNKNOWN	UNKNOWN	$C_{28}H_{53}N_8O_4$	565.4188
75	15.685	279.2318	9Z,12Z,15E-octadecatrienoic acid	Fatty acid	$C_{18}H_{30}O_2$	278.225
84	16.915	310.2337	UNKNOWN	UNKNOWN	$C_{12}H_{25}N_{10}$	309.2266
86	18.287	521.3474	Cucurbitacin P	Triterpenoid	$C_{30}H_{48}O_7$	520.3403
90	18.944	523.3624	UNKNOWN	UNKNOWN	$C_{29}H_{44}N_7O_2$	522.3553
91	19.065	523.363	UNKNOWN	UNKNOWN	$C_{30}H_{50}O_7$	522.3558

Table 6. UHPLC-MS of *Litsea garciae* bark aqueous extract

CPD	RT (min)	Base peak (m/z)	Proposed compounds	Compound class	Mol. formula	Mol. mass
17	0.871	511.0691	UNKNOWN	UNKNOWN	$C_{18}H_{26}N_2O_7S_4$	510.0627
26	0.982	321.1657	Acarbose (component 1)	UNKNOWN	$C_{13}H_{21}NO_7$	303.1317
30	1.176	284.0989	Guanosine	Purine	$C_{10}H_{13}N_5O_5$	283.0919
31	1.176	229.1548	Metaproterenol	Resorcinol	$C_{11}H_{17}NO_3$	211.1208
34	1.797	166.0852	UNKNOWN	UNKNOWN	$C_7H_9N_4O$	165.078
36	2.009	389.2034	Ile-Gln-Glu	Peptide	$C_{16}H_{28}N_4O_7$	388.196
39	3.252	371.1923	Phe-Ser-Thr	Peptide	$C_{16}H_{23}N_3O_6$	353.1585
40	6.333	287.1599	UNKNOWN	UNKNOWN	$C_{13}H_{22}N_2O_5$	286.1526
41	6.745	303.1549	Glycine, N, N'-(1,9-dioxo-1,9-nonanediyl) bis-	Amino acid derivative	$C_{13}H_{22}N_2O_6$	302.1476
42	6.92	261.112	Citrusal	Coumarin	$C_{15}H_{16}O_4$	260.1051
43	6.92	476.2129	Eugenol O-[a-L-Arabinofuranosyl-(1->6)-b-D-glucopyranoside]	Phenolic glycoside	$C_{21}H_{30}O_{11}$	458.1789
45	7.307	344.1496	N-*trans*-Feruloyl-4-O-methyldopamine	Phenol derivative	$C_{19}H_{21}NO_5$	343.1425
46	7.443	496.2028	Gentiobiosyl 2-methyl-6-oxo-2E,4E-heptadienoate	Glycoside	$C_{20}H_{30}O_{13}$	478.1692
47	7.627	314.1385	N-*cis*-Feruloyltyramine	Phenol derivative	$C_{18}H_{19}NO_4$	313.1312
49	7.865	328.155	Norisocorydine	Oligopeptide	$C_{19}H_{21}NO_4$	327.1476
51	7.985	346.1646	Aknadicine	Alkaloid	$C_{19}H_{23}NO_5$	345.1573
53	8.12	330.1697	(R)-Reticuline	Alkaloid	$C_{19}H_{23}NO_4$	329.1626
54	8.158	314.1592	Trimethylolpropane triacrylate	Ester	$C_{15}H_{20}O_6$	296.1259
56	8.161	279.1223	Artecanin	Sesquiterpenoid	$C_{15}H_{18}O_5$	278.115
60	8.449	252.0863	N-Feruloylglycine	Amino acid	$C_{12}H_{13}NO_5$	251.0791
61	8.457	505.2641	(3S,7E,9R)-4,7-Megastigmadiene-3,9-diol 9-[apiosyl-(1->6)-glucoside]	Glycoside	$C_{24}H_{40}O_{11}$	504.2565
62	8.459	453.3427	UNKNOWN	UNKNOWN	$C_{22}H_{42}N_7O_3$	452.3353
65	8.721	584.2692	UNKNOWN	UNKNOWN	$C_{26}H_{36}N_3O_{11}$	566.2353
68	8.856	312.1224	(+)-Norushinsunine N-oxide	Sesquiterpenoid	$C_{18}H_{17}NO_4$	311.1152
69	8.891	566.4292	UNKNOWN	UNKNOWN	$C_{32}H_{57}N_2O_6$	565.4215
73	9.067	358.1649	Desacetylcolchicine	Alkaloid	$C_{20}H_{23}NO_5$	357.1578
74	9.089	535.2761	3-Hydroxy-beta-ionol 3-[glucosyl-(1->6)-glucoside]	Terpene glycoside	$C_{25}H_{42}O_{12}$	534.2683
75	9.207	679.5139	UNKNOWN	UNKNOWN	$C_{37}H_{62}N_{10}O_2$	678.5053
78	9.744	519.2801	Blumenol C O-[rhamnosyl-(1->6)-glucoside]	Glycoside	$C_{25}H_{42}O_{11}$	518.2724
80	10.038	345.1331	Arjunone	Flavonoid	$C_{19}H_{20}O_6$	344.1259
81	11.43	295.2266	α-9(10)-EpODE	Epoxy fatty acid	$C_{18}H_{30}O_3$	294.2193
82	11.431	348.2747	5,8,12-trihydroxy-9-octadecenoic acid	Fatty acid	$C_{18}H_{34}O_5$	330.2408
84	12.194	230.2478	Xestoaminol C	Sphingoid	$C_{14}H_{31}NO$	229.2404
85	12.224	318.3007	Phytosphingosine	Aminoalcohol	$C_{18}H_{39}NO_3$	317.2934
86	12.298	290.269	16-hydroxy hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_3$	272.2351
92	16.728	279.1588	Emmotin A	Sesquiterpene	$C_{16}H_{22}O_4$	278.1516

Table 7. Total phenol and flavonoid contents of *Litsea garciae* bark extracts

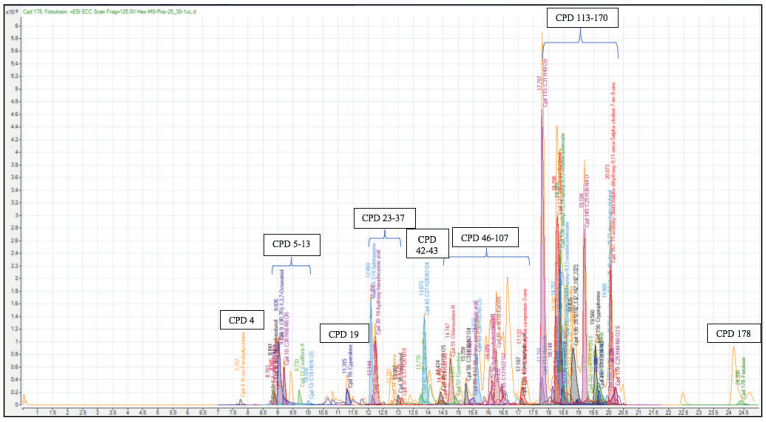
Extract	Total Phenol Content (μg GAE/mg)	Total Flavonoid content (μg QE/mg)
Hexane	7.88 ± 0.17^{a}	14.55 ± 0.24^{abcd}
Chloroform	16.05 ± 0.23^{b}	24.03 ± 4.15^{abc}
Methanol	34.82 ± 1.12^{c}	16.18 ± 2.13^{abc}
Aqueous	29.40 ± 1.76^{d}	3.84 ± 1.59^{ad}

Note: Values are mean ± SEM of triplicate analyses. Values followed by different letters are significantly different (P<0.05)

Table 8. Antioxidant capacity assessment of *Litsea garciae* bark extracts

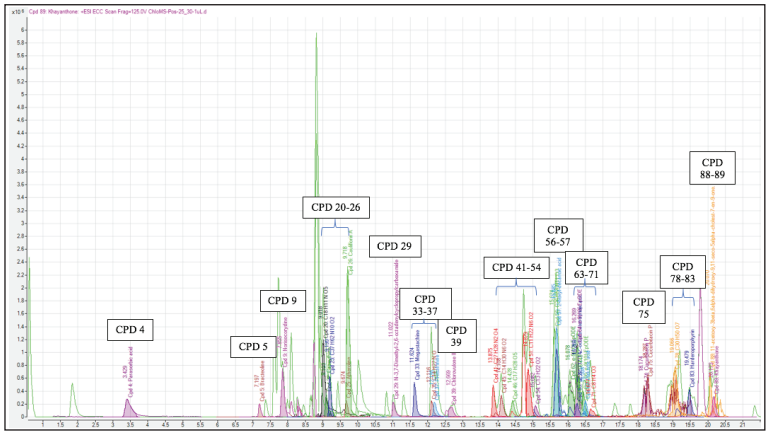
Sample	FRAP Assay (μgAAE/mg)	DPPH Assay IC50 (μg/mL)
Hexane	0.120 ± 0.006^{abcd}	2539.137 ± 143.024^{a}
Chloroform	0.167 ± 0.003^{abcd}	994.533 ± 193.257^{bd}
Methanol	0.130 ± 0.006^{abcd}	280.010 ± 58.047^{cde}
Aqueous	0.073 ± 0.003^{abcd}	740.010 ± 66.597^{bcd}
Ascorbic acid	8.197 ± 0.423^{e}	44.310 ± 1.952^{ce}

Note: Values are mean ± SEM of triplicate analyses. Values followed by different letters are significantly different (P<0.05)



Counts vs Acquisition Time (min)

Fig. 1. Total ion chromatogram (TIC) of *Litsea garciae* bark hexane extract in positive $(M+H)^{+}$ ionization mode.



Counts vs Acquisition Time (min)

Fig. 2. Total ion chromatogram (TIC) of *Litsea garciae* bark chloroform extract in positive $(M+H)^{+}$ ionization mode.

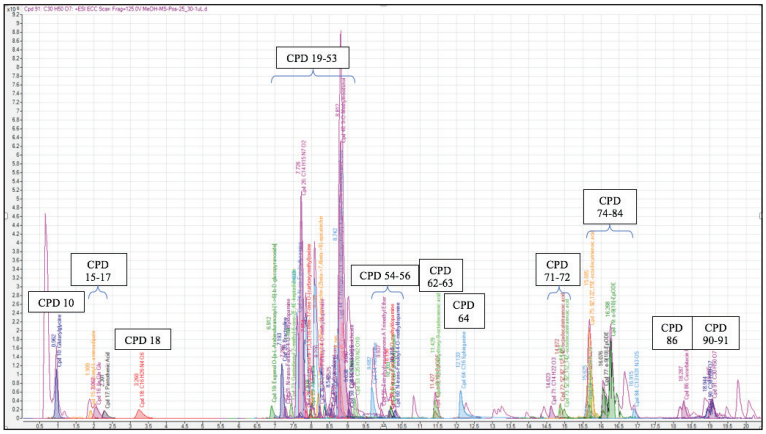


Fig. 3. Total ion chromatogram (TIC) of *Litsea garciae* bark methanolic extract in positive $(M+H)^+$ ionization mode.

Correlation between TPC and antioxidant capacity

To ascertain the significance of various parameters in assessing the antioxidant capabilities of plant extract, it is necessary to examine their relationship to one another. Figure 4 illustrates the association between TPC and the antioxidant capacity of *L. garciae* bark extracts in the DPPH and FRAP assays.

The findings of the DPPH assay revealed a robust negative association (r=-0.8812). Increases in the TPC value were highly linked with decreases in the IC_{50} values of the extracts. Additionally, there was a strong negative association (p<0.05) between the DPPH and TPC assay results.

The findings of the FRAP assay revealed no correlation (r=-0.3518). Thus, no significant correlation (p>0.05) between the FRAP assay result and the TPC was seen.

DISCUSSION

The purpose of this study was to ascertain the ability of *L. garciae* bark extracts as potential antioxidant agents. The antioxidant activity was determined using the FRAP and DPPH assays. The FRAP assay is based on the iron's reductive capacity and analyses the antioxidants' ability to reduce the ferric ion (Fe^{3+})-ligand complex to the highly blue-colored ferrous (Fe^{2+}) complex in an acidic solution (Spiegel *et al*., 2020). Meanwhile; the DPPH assay is used to determine an antioxidant's capacity to scavenge DPPH radicals (Molyneux, 2004). The FRAP test (Halvorsen & Blomhoff, 2011) and the DPPH assay (Ácsová *et al*., 2019) can quantify most hydrophilic and lipophilic substances with antioxidant characteristics satisfactorily. It is feasible to extract both hydrophilic and hydrophobic compounds with the maceration technique using polar and non-polar solvents.

No single method is adequate to determine the total antioxidant capacity of a plant extract because the extract may contain multiple types of antioxidants, thus, to ensure total antioxidant capacity with multiple modes of action of antioxidants present in the extract, various types of antioxidant assays must be considered before evaluation. This is because each assay reflects a distinct component of a plant extract's antioxidant activity. By using two separate assays, such as DPPH and FRAP. It may increase the overall estimation of the antioxidant capacity of the plant extracts. Müller *et al*. (2011) demonstrated that several approaches based on various mechanical principles must be utilized in tandem because the results of other methods are frequently inconsistent. The TPC assay is frequently used in conjunction with DPPH and FRAP assays, presumably to expand the database of information on a specific plant extract.

Antioxidant measurements

The Folin-Ciocalteu reagent was used to quantify TPC, resulting in improved sensitivity and reproducibility. This reagent combines phosphotungstic acid ($H_3PW_{12}O_40$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) that forms chromogens when combined with phenols and non-phenolic reducing chemicals. The latter can be determined photometrically because the oxotungstate and oxomolybdate generated in this redox reaction have a blue coloration proportionate to the concentration of phenolic compounds under alkaline circumstances. (Vasco *et al*., 2008).

Extraction techniques and solvents are responsible for dissolving endogenous compounds in plants. Natural compounds, as is well known, can be polar or nonpolar. Due to the presence of a hydroxyl group, phenolic compounds are more soluble in polar organic solvents (Wang & Weller, 2006) relevant to the methanolic extract that possessed the highest TPC compared with other extracts (Figure 5a & Table 7).

The significantly high TPC (Figure 5a & Table 7) detected in the methanolic extract aligned with the

preliminary screening findings in which both tannins and phenolic compounds were detected positives (Table 2). Besides, the compounds listed by LC/MS displayed varieties of phenolic compounds Figure 3 & Table 5).

Thus, the methanolic extract's highest TPC value contributed to the lowest IC_{50} value exhibited in the DPPH assay (Figure 6b & Table 8). It was demonstrated by the significant (p<0.05) negative association between the TPC value and the IC_{50} value of the crude extracts determined using the DPPH assay (Figure 7b). According to Clarke *et al*. (2013), high antioxidant activity is associated with high phenolic content. Antioxidant properties of phenolic substances are structurally determined. Indeed, phenolic compounds are made of one (or more) aromatic rings with one (or more) hydroxyl groups immediately attached to the benzene ring and can quench free radicals through the formation of resonance-stabilized phenoxyl radicals (Bors & Michel, 2002). According to Irshad *et al*. (2012), phenolic compounds possess redox characteristics that enable them to operate as reductants, hydrogen donors, and singlet oxygen quenchers. The redox potential of phenolic compounds was critical in establishing their antioxidant capacity. Additionally, LC/MS results displayed varieties of phenolic acids, an essential group of phenolic compounds (Figure 3 & Table 5).

The aluminum chloride method was used to determine TFC. In flavonols and flavones, aluminum chloride forms a stable combination with a carbonyl group at C4 and hydroxyls at C3 and C5, respectively. Additionally, it may form labile acid complexes with flavonoids' B ring hydroxyls at the ortho position (Chang *et al*., 2002).

Flavonoids are a plant's most abundant and bioactive phenolic chemicals (de la Rosa *et al*., 2019). Flavonoids are secondary metabolites widely recognized for their antioxidant properties (Matos *et al*., 2020). Flavonoids' efficacy is determined by the amount and position of free OH groups (Panche *et al*., 2016). The high result of TFC (Figure 5b & Table 7) detected in the methanolic extract aligned with the preliminary screening findings in which flavonoids were detected positives (Table 2). The results indicated that the extracts included a variety of commonly occurring flavonoids, as identified by LC/MS (Figure 3 & Table 5). The presence of flavonoids may contribute to the highest antioxidant capacity of methanolic extracts as exhibited in DPPH (Figure 6b & Table 8).

Overall, TPC and TFC levels may indicate the plant's antioxidant capacity by measuring the antioxidants. As a result, it is hypothesized that the phenolic and flavonoid groups play a significant role in the antioxidant activity of *L. garciae* bark extracts.

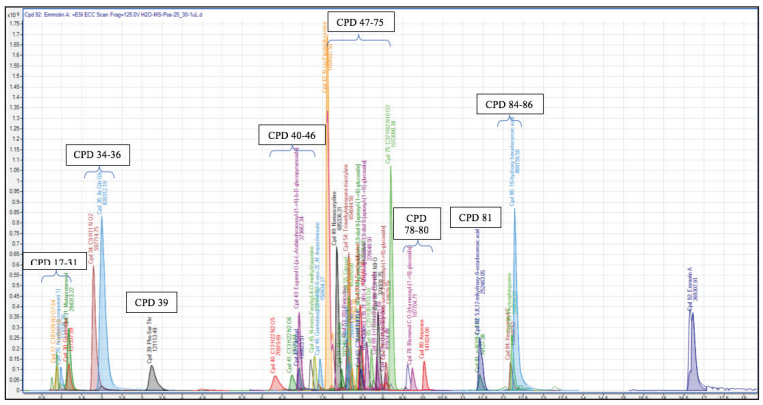


Fig. 4. Total ion chromatogram (TIC) of *Litsea garciae* bark aqueous extract in positive $(M+H)^+$ ionization mode.

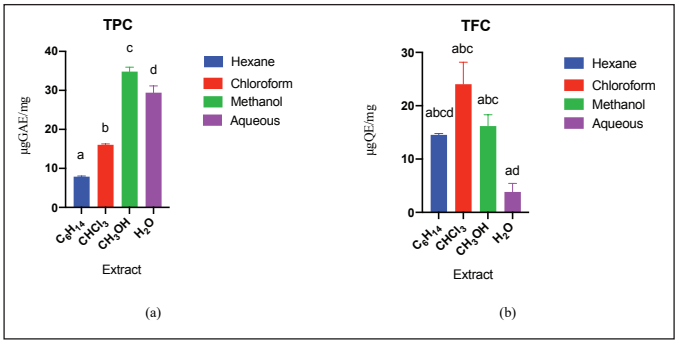


Fig. 5. Total phenol (a) and flavonoid (b) contents of *Litsea garciae* bark extracts.

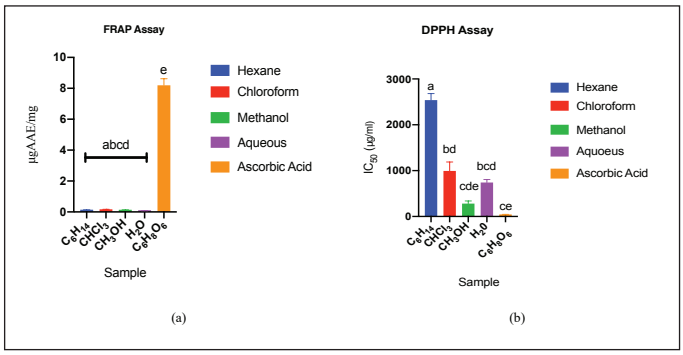


Fig. 6. Antioxidant capacity assessment FRAP (a) and DPPH (b) of *Litsea garciae* bark extracts.

Antioxidant capacity assessment

The chloroform extract demonstrated the most remarkable ability to reduce Fe^{3+} in this study. (Table 8). Based on the LC/MS result (Figure 2 & Table 4), it was found that there were phenolic compounds under the class of coumarins and flavonoids which may contribute to the FRAP result. Katalinic *et al.* (2006) asserted that the ferric reducing potential is proportional to the phenolic concentration (Figure 8).

However, antioxidant capacity values detected from the FRAP assay demonstrated a poor correlation to TPC. The FRAP assay accurately quantifies solely the ferric ion's reducing capacity, which is irrelevant to antioxidant activity mechanistically and physiologically (Prior *et al.*, 2005). Non-significant (p>0.05) association between the TPC value and the antioxidant capacity of the *L. garciae* bark extracts as determined by FRAP (Figure 7a).

This approach's most frequently discussed component is that the results vary depending on the period employed (Prior *et.al*, 2005). Fast-reacting phenols that bind iron or degrade into compounds with lower or different reactivity are best analyzed with short reaction times, i.e., 4 to 6 min, but phenolic compounds containing acids such as caffeic acid, tannic acid, and ferulic acid, or quercetin, can take up to an hr to react (San Miguel-Chávez, 2017). As referred to in the LC/MS analysis (Figure 3 & Table 5), methanolic extract was detected with the presence of N-*trans*-Feruloyl-4-O-methyldopamine, a derivative from ferulic acid. Thus, it may influence the extract's capacity to reduce Fe^{3+} in a shorter time based on the current study's standard methodology.

According to Clarke *et.al* (2013), the FRAP test inferred two mechanisms: interference caused by the color in some extracts and sluggish color development. The acidic pH may have caused this in the FRAP assay, but it was considerably less of an issue with the DPPH assay.

The DPPH radical scavenging analysis is the most extensively used and straightforward approach for determining antioxidant activity capacity. Due to the delocalization of its spare electrons across the molecule, it is a stable purple-colored free radical. The degree of color shift (to the pale yellow of reduced DPPH) is proportional to the concentration and efficacy of the antioxidants. The optical absorbance of the solution lowers as a result of the reaction (Pyrzynska & Pękal, 2013).

In the DPPH scavenging assay, a proportional drop in the reaction mixture's absorbance shows that the chemical has considerable free radical scavenging activity. The test findings demonstrated that all *L. garciae* bark extracts had a DPPH radical scavenging activity. The IC_{50} values in this investigation ranged from 2539 to 280 μg/mL. A low IC_{50} value indicated the extract's strong ability to act as a DPPH scavenger, which was observed in the methanolic extract (Figure 6b & Table 8). It was comparable with standard ascorbic acid. Meanwhile, high IC_{50} values indicated low scavenging activity which was observed in hexane extract (Figure 6b & Table 8).

It is generally predicted that DPPH radical scavenging activity is strongly affected by the number of phenolic compounds (Figure 9). Consistent with Irshad *et al*. (2012) and Jose and Anilkumar (2021), the radical scavenging effect of the extract was found to be directly related to its phenolic content. Based on the LC/MS results (Figure 3 and Table 5), there were various types of phenolic compounds found in the methanolic extract including N-*trans*-feruloyl-4-O-methyldopamine, N-*cis*-feruloyltyramine, epicatechin-(4beta->6)-epicatechin-(2beta->7,4beta->8)-epicatechin, 7-Hydroxy-3-(4-methoxyphenyl)-4-propyl-2H-1-benzopyran-2-one and 9-O-Methylneodunol. These findings demonstrated a connection between the content of phenolic compounds in methanolic *L. garciae* bark extract and their ability to scavenge free radicals. As a result, the presence of phenolic compounds in plant extracts greatly enhances their antioxidant capacity.

Antioxidant potential of alkaloids

The genus *Litsea* contains structurally diverse and biologically active alkaloids (Wang *et al*., 2016). Varieties subclasses of alkaloids were detected in the extracts, particularly in the methanolic extract. Aporphine alkaloids are isoquinoline-type alkaloids that process pharmacological activity, such as antioxidants (Chen *et al*., 2013). The aporphine group was found to be the most abundant in *Litsea* (Wang *et.al*, 2016). In a recent study, aporphine alkaloids of bracteoline and atheroline were found in the methanolic extract (Figure 3 & Table 5), which aligned with the preliminary screening findings, in which alkaloid was detected positives (Table 2). The finding of atheroline was first reported in this genus. Previous studies have reported various isoquinoline alkaloids (Noureddine *et al*., 2013; Salleh & Farediah, 2017), particularly aporphine alkaloids (Liu *et al*., 2014) potentially to exert antioxidant. Isoquinoline alkaloids can be viewed as a therapeutically effective agent in antioxidative treatment from the thermodynamic and kinetic perspectives of the computational density functional theory (DFT) method (Dung *et al*., 2020). According to Chahardoli *et al*. (2018), alkaloids, including bracteoline of *Nigella arvensis,* may reduce Ag^{+} ions. Its biosynthesized/phytogenic of AgNPs exhibited moderate antioxidant activity through the DPPH assay.

Additionally, a recent study discovered the isoquinoline alkaloid cularine. According to Wang *et al*. (2017), cularine exhibited the most potent antioxidant in the multiherbal formula, Xiexin Tang.

Based on the previous report's findings, in addition to the role of phenolic compounds, it may be assumed that alkaloids detected by LC/MS may contribute to the methanolic extract of *L. garciae* bark's potent antioxidant activity.

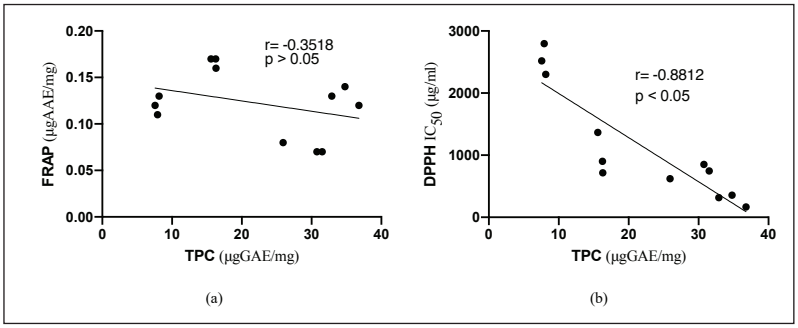


Fig. 7. Correlation between TPC and antioxidant capacity of *Litsea garciae* bark extracts in FRAP (a) and DPPH (b) assay.

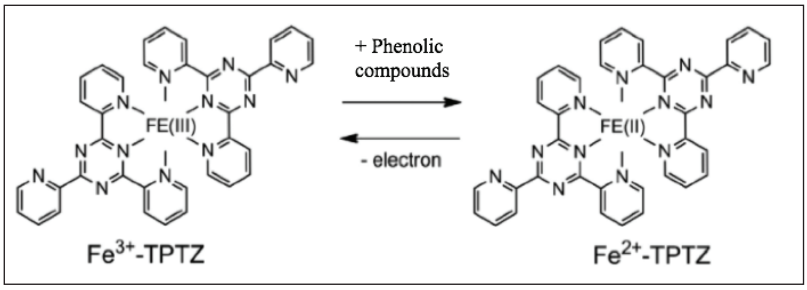


Fig. 8. Antioxidant activity of phenolic compounds in FRAP Assay. Illustration modified from Xiao *et al*. (2020).

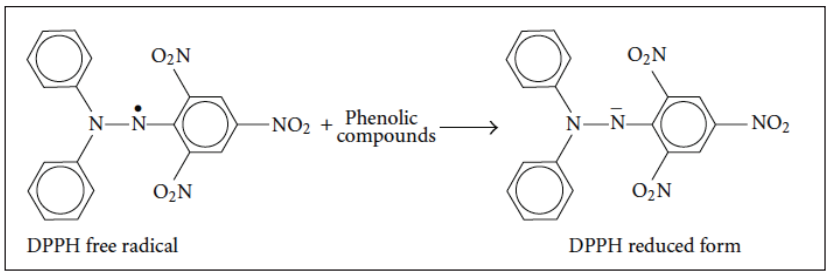


Fig. 9. Antioxidant activity of phenolic compounds in DPPH Assay. Adopted from Irshad *et al*. (2012).

CONCLUSION

According to related compounds discovered using LC/MS data and the results in the phytochemical screening, the antioxidant capacity assessment in this work indicates that *L. garciae* methanolic bark extract with high phenolic and flavonoid content may be a significant natural antioxidant source. In addition, alkaloids discovered via LC/MS and positive detection in the phytochemical screening may also contribute to the antioxidant effects of this compound. While the parameters employed in this study were not disease-specific, quantifying antioxidants can help biologically orient the use of *L. garciae* bark in ROS-related disease research. Perhaps the substantial antioxidant activity of methanolic bark extract could be used to inhibit harmful oxidation in the treatment of neurological illnesses such as Alzheimer's and Parkinson's disease. A recent study provided novelty of proposed compounds data through LC/MS analysis for hexane, chloroform, methanolic, and aqueous *L. garciae* bark extract.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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