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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF EXTRACTS FROM *Calophyllum ferrugineum* AND *Calophyllum incrassatum*

(Aktiviti Antibakteria dan Antioksidan Terhadap Ekstrak daripada *Calophyllum ferrugineum* dan *Calophyllum incrassatum*)

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Abstract

Calophyllum is a pan-tropical genus belongs to the Guttiferae family and locally known in Malaysia as '*bintangor*'. There has been a continual interest to further investigate the phytochemistry of *Calophyllum sp* because this genus is a rich source of active secondary metabolites which shows anti-HIV, cytotoxicity and antimicrobial properties. In this study, antibacterial and antioxidant activities of barks and leaves of *C. ferrugineum* and *C. incrassatum* were investigated. Cold extraction method employing dichloromethane, ethyl acetate and methanol as solvent was performed. All extracts were tested for their total phenolic content and antioxidant activities by DPPH radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. The methanol extract from the leaves of *C. ferrugineum* showed the highest TPC value at 122.08 mg GAE/g and the lowest DPPH SC_{50} value at 11.80 µg/mL. The methanol extract from the barks of *C. ferrugineum* was found to have the highest FRAP value among all extracts. The antibacterial activity of all extracts was tested using minimum inhibition concentration (MIC) test against *Bacillus subtilis, Staphylococcus aureus, Escheria coli* and *Pseudomonas aeruginosa*. Only the dichloromethane extract from bark of *C. ferrugineum* showed moderate MIC value against Gram positive bacteria, *B. subtilis* and *S. aureus* at 125 µg/mL.

Keywords: *Calophyllum, C. ferrugineum, C. incrassatum*, antioxidant, antibacterial

Abstrak

Calophyllum adalah genus pan-tropika di dalam keluarga Guttiferae dan dikenali secara tempatan di Malaysia sebagai *'bintangor'*. Terdapat minat berterusan untuk mengkaji lebih mendalam tentang fitokimia *Calophyllum sp* kerana genus ini adalah sumber yang kaya dengan metabolit sekunder aktif yang menunjukkan ciri-ciri anti-HIV, sitotoksiti dan antimikrob. Di dalam kajian ini, aktiviti antibakteria dan antioksidan kulit batang dan daun *C. ferrugineum* dan *C. incrassatum* telah dikaji. Kaedah pengekstrakan sejuk menggunakan diklorometana, etil asetat dan metanol sebagai pelarut telah dilakukan. Semua ekstrak telah diuji untuk kandungan total fenolik dan aktiviti antioksidan melalui cerakin pemerangkapan radikal DPPH dan kuasa antioksidan penurunan Ferik (FRAP). Ekstrak metanol daripada daun *C. ferrugineum* telah menunjukkan nilai TPC tertinggi pada 122.08 mg GAE/g dan nilai SC_{50} DPPH terendah pada 11.80 µg/mL. Ekstrak metanol daripada kulit batang *C. ferrugineum* telah menunjukkan nilai FRAP tertinggi antara semua ekstrak. Aktiviti antibakteria semua ekstrak telah diuji untuk kepekatan rencatan minimum (MIC) terhadap *Bacillus subtilis, Staphylococcus aureus, Escheria coli* dan *Pseudomonas aeruginosa*. Hanya ekstrak diklorometana kulit batang *C. ferrugineum* menunjukkan nilai MIC yang sederhana terhadap bakteria Gram positif, *B. subtilis* dan *S. aureus* pada 125 µg/mL.

Keywords: *Calophyllum, C. ferrugineum, C. incrassatum*, antioksidan, antibakteria

Introduction

Calophyllum is a pan-tropical genus that comprises approximately 180 – 200 species. It is the largest genus in the sub-family Calophylloideae and categorised under the Guttiferae family [1]. In Malaysia, *Calophyllum* is locally known as *'bintangor'*. This plant is used traditionally to treat malaria, bronchitis, gastric and hepatic disturbances pain, wound infections, inflammation, rheumatism, varicose, haemorrhoids, chronic ulcer and acts as diuretic [2]. Apart from medicinal values, *Calophyllum* is often used for decorative purposes such as furniture, parquet flooring, solid door and plywood due to their distinctive colours. The poisonous latex from the bark can also be used to numb fish and kill rats [3]. *Calophyllum* genus has been reported as a plant-rich source of phenolic compounds including xanthones, flavonoids and coumarins. Numerous researches had been conducted on common *Calophyllum* species such as *C. inophyllum* and *C. lanigerum* [4]. The discovery of a series of pyranocoumarins known as 'the calanolides' with strong anti-HIV properties from *C. lanigerum var. austrocoriaceum* [5] has elevated the phytochemical research of this species. This genus also produces chromanone carboxylic acids, terpenoids and phloroglucinol derivatives.

In continuation of phytochemicals and bioactivities studies on several *Calophyllum* species from Malaysia [6–9], the antioxidant and antibacterial activities of *C. ferrugineum* Ridley and *C. incrassatum* M. R. Henderson & Wyatt-Smith were investigated. *C. ferrugineum* is an evergreen tree distributed around Southeast Asia especially in Malaysia. It grows from 5 – 30 m and can be found in lowland or colline mixed dipterocarp forest. The bark is used to make walls and masts for house-building. It is also used as decoction by mothers three days after the childbirth. *C. incrassatum* is distributed in East Malaysia, Borneo and Sulawesi. This tree can grow up from 15 – 36 metres and is usually found in well-drained, mixed dipterocarp lowland forest or sometimes in swamp forest [1]. To date, there is no scientific report on the antioxidant and antibacterial activities for both species. Therefore, herein the authors report the evaluation of antioxidant and antibacterial activities of crude extracts from the barks and leaves of both species.

Materials and Methods

Chemicals and reagents

Folin-Ciocalteu's phenol, gallic acid, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were purchased from Sigma Aldrich. Ascorbic acid (AA) was purchased from Goodrich Chemical Enterprice (GCE) while 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Fluka. 2,4,6-Tri(2-pyridyl)-S-triazine (TPTZ) was purchased from Merck while anhydrous sodium carbonate (Na_2CO_3), sodium acetate trihydrate ($CH_3COONa.3H_2O$), iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) and hydrochloric acid (HCl) were obtained from Qrec. Nutrient agar (NA) and nutrient broth (NB) were purchased from Merck while streptomycin sulphate (SS) and 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium (INT) were obtained from Sigma Aldrich. Sodium chloride (NaCl), tryptone, glycerol and H_2SO_4 (98%) were supplied by Qrec, yeast extract was obtained from Scharlau and Tween 80 was purchased from Fischerbrand.

Microorganism

The bacterial strains acquired were from the American Type Culture Collection (ATCC). The activities of the samples were screened against four strains, two Gram-positive bacteria, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737) and two Gram-negative bacteria, *Escherichia coli* (ATCC 10536) and *Pseudomonas aeruginosa* (ATCC 9027).

Plant material

Sample of *C. ferrugineum* Ridley (SK2587/14) was collected at Hutan Lenggong, Perak on 28 August 2014 while *C. incrassatum* M. R. Henderson & Wyatt-Smith (SK2612/14) was collected from Hutan Gunung Belumut, Kluang, Johor on 1 October 2014. Plant specimens of *C. ferrugineum* and *C. incrassatum* were deposited at Herbarium Universiti Putra Malaysia. All plant samples were identified by Dr Shamsul Khamis, a botanist from Universiti Kebangsaan Malaysia.

Extraction method

The dried and ground barks and leaves of *C. incrassatum* and *C. ferrugineum* were macerated sequentially with dichloromethane, ethyl acetate and methanol for 3 days at room temperature. The extracts were then filtered and similar extraction process was repeated twice. The extracts were then concentrated under reduced pressure by using rotary evaporator. All extracts were stored at 4°C for further use.

Antibacterial activity

The antibacterial activity of all extracts was tested quantitatively by evaluating their minimum inhibition concentration (MIC). Two *Gram* positive bacterial strains of *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737) and two *Gram* negative bacterial strains of *Escheria coli* (ATCC 10536) and *Pseudomonas aeruginosa* (ATCC 9027) were chosen. The MIC was carried out by serial broth microdilution according to our previous reported method [7]. The sample stock solution (1000 µg/mL) was prepared in 5% DMSO in nutrient broth (NB) supplemented with 0.02% (v/v) Tween 80. Further twofold dilution with NB was performed to afford concentration of samples from 1000-7.81 µg/mL. 50 µL of bacteria inocula was dispensed in the 96-well microplate followed by 50 µL of sample solution. Streptomycin sulphate was employed as positive control in this assay. The microplates were pre-incubated for 24 hours at 37 ºC for *S. aureus*, *E. coli* and *P. aeruginosa* and 30 ºC *for B. subtilis*. 25 µL of *p*-iodonitrotetrazolium (INT) (0.2 mg/mL in sterile distilled water) solution was added to all wells and were pre-incubated for at least 30 minutes. Bacterial growth in the wells was indicated by the formation of reddish-pink color while clear well indicates inhibition of bacterial growth by the sample.

Antioxidant activity: Total phenolic content

The total phenolic content (TPC) of the extracts were determined by Folin-Ciocalteau's assay [10]. In brief, sample solution in MeOH (40 µL) with concentrations from 1000-7.81 µg/mL obtained from twofold dilution were mixed with Folin-Ciocalteau's reagent (20 µL) in the 96-well microplate and were incubated for 5 minutes at room temperature in a dark condition. Sodium carbonate (80 µL) was added to all wells followed by distilled water (60 µL). The mixture was kept in the dark for 90 minutes and the absorbances were recorded at 760 nm. A calibration graph of standard gallic acid at concentration 125-7.81 µg/mL versus absorbance was constructed. The total phenolic contents were expressed as mg gallic acid equivalent (GAE)/g of extract.

DPPH radical scavenging assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay of the extracts was carried out following the method by Kassim et al. [10]. In brief, DPPH reagent (30 µL) with final concentration of 300 µM was added to the samples (170 µL) with concentration ranging from 1000-7.81 µg/mL in MeOH obtained from twofold dilution. The reaction mixture was allowed to incubate in dark condition at room temperature for 30 minutes. The $DPPH_{blank}$ wells consisting of DPPH reagent and MeOH were reserved for each microplate. The absorbance for DPPH radical inhibition was measured at 517 nm. The radical scavenging effect was examined and compared with ascorbic acid (AA) and butylated hydroxytoluene (BHT) as the references. The percentage of DPPH radical scavenging inhibition was calculated by using the following formula;

$$\text{Scavenging Concentration (\%SC)} = [(A_{\text{DPPH blank}} - (A_{\text{Sample}} - A_{\text{blank sample}})] / A_{\text{DPPH blank}} \times 100\% \quad (1)$$

where $A_{\text{DPPH blank}}$ is the absorbance of DPPH reagent with MeOH, A_{sample} is the absorbance of sample solution with DPPH reagent and $A_{\text{blank sample}}$ is the absorbance of sample solution.

Ferric reducing antioxidant potential (FRAP) assay

Experiment was carried out following the method by Arriffin et al. [11]. FRAP reagent was freshly prepared, consist of stock solution with ratio 10:1:1 of acetate buffer (300 mM), TPTZ (10 mM) in HCl (40 mM) and $FeCl_3.6H_2O$ (20 mM) solutions. Sample (5 µL) with concentration of 1000-100 µg/mL, methanol (15 µL) and FRAP reagent (150 µL) were added to the 96-well microtiter plate. The absorbance was recorded after 10 minutes of incubation at 37 °C at 573 nm. A calibration graph of standard $FeSO_4.7H_2O$ solution with concentration of 1.0-0.1 mM vs absorbance was constructed. The FRAP activity of extracts was expressed as mM FRAP equivalent to $FeSO_4.7H_2O$

Statistical analysis

Three replicates of each sample were used for statistical analysis with values reported as mean ± SD. Standard curves were generated and calculation of the 50% inhibitory concentration (IC_{50}) values was done using GraphPad Prism for Windows (version 5.02) software. The Student's *t*-test was carried out using SPSS (version 22) software for comparison between treatment of samples and positive controls. Pearson's correlation coefficient was used to determine the correlation between two independent variables. A value of $p < 0.05$ was considered significantly different.

Results and Discussion

Antibacterial activity

The antibacterial activity of crude extracts from two *Calophyllum* species was tested against two Gram-positive bacteria, *B. subtilis* and *S. aureus* and two Gram-negative bacteria, *P. aeruginosa* and *E. coli* through MIC determination (Table 1). Streptomycin sulphate was employed as a positive control. According to Sousa et al., the antibacterial activity of MIC values of crude extracts over 1000 µg/mL is inactive, from 500 to 1000 µg/mL is weak, from 100 to 500 µg/mL is moderate and less than 100 µg/mL is considered good [12]. Rios and Recio also proposed that MIC values below 100 µg/mL for crude extracts are promising [13].

Table 1. Minimum Inhibition Concentration (MIC) of Extracts of *C. ferrugineum* and *C. İncrassatum*

***Calophyllum* Species**	**Part**	**Crude (Abbreviation)**	**Minimum Inhibition Concentration (MIC) (µg/mL)[a]**			
			Gram-positive		**Gram-negative**	
			B. s	***S. a***	***P. a***	***E. c***
Crude Extracts						
C. ferrugineum	B	CH_2Cl_2 (CFBD)	125	125	>1000	>1000
		EtOAc (CFBE)	500	1000	>1000	>1000
		MeOH (CFBM)	>1000	>1000	>1000	>1000
	L	CH_2Cl_2 (CFLD)	1000	>1000	1000	1000
		EtOAc (CFLE)	>1000	1000	500	>1000
		MeOH (CFLM)	>1000	>1000	>1000	>1000
C. incrassatum	B	CH_2Cl_2 (CIBD)	>1000	>1000	>1000	>1000
		EtOAc (CIBE)	>1000	>1000	>1000	>1000
		MeOH (CIBM)	>1000	>1000	>1000	1000
	L	CH_2Cl_2 (CILD)	1000	1000	>1000	>1000
		EtOAc (CILE)	1000	>1000	>1000	>1000
		MeOH (CILM)	>1000	>1000	>1000	>1000
SS[b]			1.56	50	6.25	0.78

[a] Data represent mean ± standard deviation of three replicate experiments; [b] Positive control; B: Barks; L: Leaves; *B. s*: *Bacillus subtilis*; *S. a*: *Staphylococcus aureus*; *P. a*: *Pseudomonas aeruginosa*; *E. c*: *Escherichia coli*; SS: Streptomycin Sulphate.

The dichloromethane extract from bark of *C. ferrugineum* showed moderate MIC value against Gram positive bacteria, *B. subtilis* and *S. aureus* at 125 µg/mL. It was suggested that high content of bark resins in the dichloromethane extracts of *C. ferrugineum* is responsible to the antibacterial activity. This justification is supported by the previous finding on the strong antimicrobial properties of the bark resins from *C. inophyllum* and *C. antillanum* [14]. Chromanone carboxylic acids compounds are presented as major compounds in the bark resin of *Calophyllum* species and these compounds are reported to have antibacterial activities. Previous report on the isolation of six chromanone acids from *C. brasiliense* also demonstrated a moderate-to-strong antibacterial activity especially to Gram-positive bacteria [15], thus supporting these findings. In contrast, the dichloromethane extract from bark of *C. incrassatum* showed inactive antibacterial activity against all strains. This result suggested that there

were no chromanone carboxylic acids types of compound present in the bark extract. The bark of *C. incrassatum* also did not contain resin, thus suggesting that no chromanone carboxylic acid can be isolated from this species. Meanwhile, the other crude extracts showed weak or inactive inhibition towards all bacterial strains tested.

Antioxidant activity

Spectrophotometric assay that involves the use of specific chromophore such as Folin-Ciocalteu (FC) reagent is commonly chosen, since it is simple and less time consuming as well as suitable for screening purposes. In general, yellow coloured FC reagent containing molybdenum, Mo^{6+} will reduce to Mo^{5+} in the presence of reducing agent in basic medium to form dark blue complex [16]. The standard calibration curve of gallic acid (Figure 1) was constructed to calculate the total phenolic content expressed as mg gallic acid equivalent (GAE) per gram of extract ($y = 0.0107x + 2.6402$, $R^2 = 0.9978$). In general, the MeOH extracts showed the highest TPC value followed by EtOAc extracts. It can be deduced from the trend that the TPC increased as the solvent polarity increased since the phenolic compounds normally constitutes in more polar extract. Meanwhile, all dichloromethane extracts tested were devoid to this assay and no phenolic content was detected.

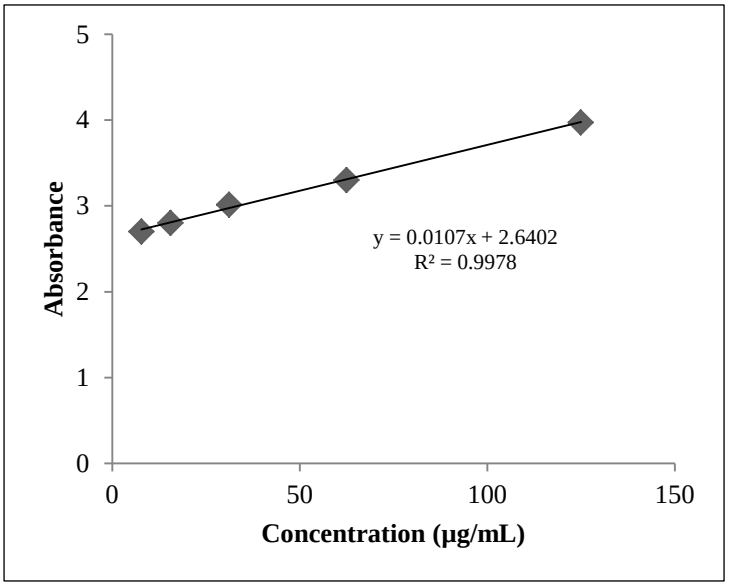

Figure 1. Gallic acid calibration curve

The methanol extract of leaves and barks of *C. ferrugineum* showed the highest TPC value at 122.08 mg GAE/g and 118.48 mg GAE/g, respectively. These TPC values were higher compared to the barks and leaves of *C. incrassatum* at 91.61 mg GAE/g and 45.68 mg GAE/g, respectively. A similar trend was also observed where ethyl acetate extracts from *C. ferrugineum* showed higher TPC values compared to *C. incrassatum*. These results suggested that *C. ferrugineum* is richer with phenolic constituents compared to *C. incrassatum*.

The concentration of sample needed to scavenge 50% of DPPH radical activity (SC_{50}) of the extracts is summarised in Table 2. All tested samples that showed ($P < 0.05$) were considered as statistically significant and different compared to ascorbic acid as the positive control. Blois had classified the antioxidant activity of the tested samples with the SC_{50} value as very strong ($SC_{50} < 50$ µg/mL), strong (50 µg/mL $< SC_{50} <$ 100 µg/mL), moderate (100 µg/mL $< SC_{50} <$ 150 µg/mL), weak (150 µg/mL $< SC_{50} <$ 200 µg/mL), very weak (200 µg/mL $< SC_{50} <$ 250 µg/mL) and inactive ($SC_{50} >$ 250 µg/mL) [17].

Table 2. TPC, DPPH SC_{50} and Pearson correlation coefficient values

***Calophyllum* Species**	**Part**	**Crude (Abbreviation)**	**Antioxidant Activity**		***r* coefficient**
			TPC (mg GAE/g)[a]	**DPPH SC_{50} (µg/mL)[a]**	
C. ferrugineum	B	CH_2Cl_2 (CFBD)	ND	> 1000	0.607
		EtOAc (CFBE)	84.09 ± 0.30*	26.72 ± 0.15***	0.810*
		MeOH (CFBM)	118.48 ± 0.01*	18.82 ± 0.38***	0.473
	L	CH_2Cl_2 (CFLD)	ND	> 1000	0.623
		EtOAc (CFLE)	75.68 ± 0.16*	18.70 ± 1.50**	0.893**
		MeOH (CFLM)	122.08 ± 0.04*	11.80 ± 1.03*	0.867**
C. incrassatum	B	CH_2Cl_2 (CIBD)	ND	> 1000	0.550
		EtOAc (CIBE)	18.29 ± 0.20*	49.90 ± 1.97***	0.800*
		MeOH (CIBM)	91.61 ± 0.01*	17.23 ± 1.21**	0.709*
	L	CH_2Cl_2 (CILD)	ND	> 1000	0.509
		EtOAc (CILE)	35.86 ± 0.01*	52.62 ± 1.38***	0.652
		MeOH (CILM)	45.68 ± 0.10*	53.33 ± 1.98**	0.612
AA[b]			NA	8.90 ± 0.27	NA
BHT[b]			NA	15.30 ± 0.63	NA

[a] Data represent mean ± standard deviation of three replicate experiments; [b] Positive control; B: Barks; L: Leaves; * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$; AA = Ascorbic acid; BHT: Butylated Hydroxytoluene; NA = Not Available.

All dichloromethane crude extracts displayed inactive DPPH radical scavenging activity with SC_{50} values of more than 1000 µg/mL. In contrast, all methanol and ethyl acetate extracts possessed significant DPPH radical scavenging activity in dose-dependent manner as depicted in Figure 2 and Figure 3, respectively. The methanol extract from the leaves of *C. ferrugineum* displayed the lowest SC_{50} value (11.80 µg/mL) compared to the positive control, butylated hydroxytoluene (BHT) (SC_{50} 15.30 µg/mL). In addition, the DPPH radical scavenging activities of methanol extracts from barks of *C. incrassatum* and barks of *C. ferrugineum*, as well as the ethyl acetate extract from the leaves of *C. ferrugineum* were comparable to BHT since their SC_{50} values lies in the range of 17.23 – 20.51 µg/mL. This shows that high TPC values of the methanol extracts are strongly associated with the present findings. Phenolic compounds that are rich in hydroxyl groups serve as a hydrogen donor to the radical thus act as good antioxidants.

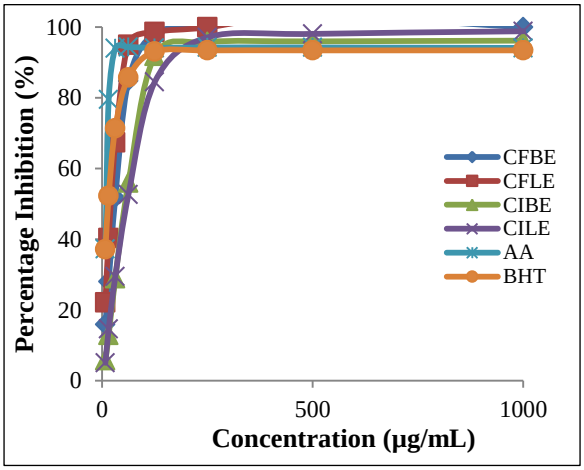

Figure 2. Percentage ınhibition of DPPH radical scavenging activity of EtOAc extracts

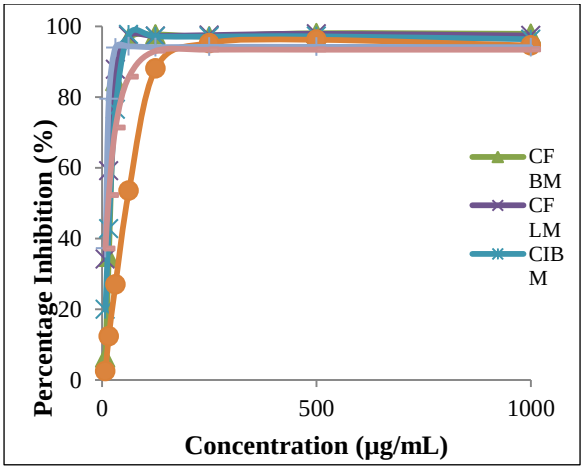

Figure 3. Percentage ınhibition of DPPH radical scavenging activity of MeOH extracts

The correlations between TPC and DPPH radical scavenging activity for each crude extract were evaluated using Pearson's correlation coefficient as tabulated in Table 1. In this study, the two variables were positively correlated with the *r* coefficient value in the range of 0.473 – 0.893. This indicates a positive correlation whereby high total phenolic content is proportional to strong DPPH radical scavenging activity. Previous work on the bioassay-guided fractionation of the methanol and ethanol extracts from several *Calophyllum* species that gave high DPPH radical scavenging activity led to the isolation phenolic compounds; flavonoids and xanthones [18,19].

Ferric Reducing Antioxidant Power (FRAP) involves the redox reaction between ferric (III) tripyridyltriazine complex and a reducing agent to form ferrous (II) tripyridyltriazine complex. The reduction process increases the absorbance value measured at 593 nm due to the colour changes from pale brown to intense blue [20]. The FRAP values were determined using standard calibration curve of standard $FeSO_4.7H_2O$ (y = 0.0005x + 0.0708, R^2 = 0.9992) as shown in Figure 4 and expressed as mM FRAP equivalent to $FeSO_4.7H_2O$.

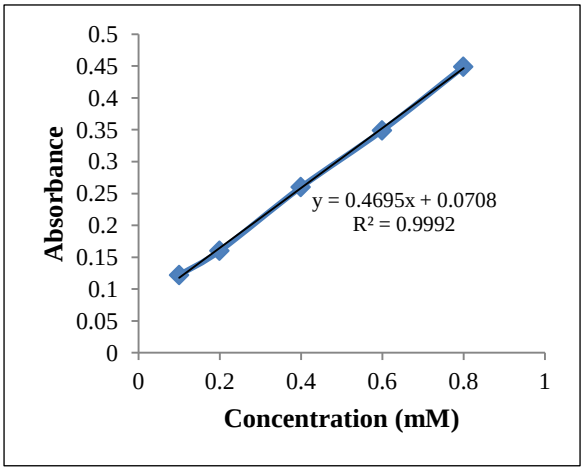

Figure 4. $FeSO_4.7H_2O$ standard calibration curve

The FRAP values of the crude extracts for the concentrations of 1.0, 0.8, 0.6, 0.4, 0.2 and 0.1 mM were summarised in Table 3. The trend for ferric ion reducing activities of the dichloromethane, ethyl acetate and methanol extracts from the barks and leaves of *C. incrassatum* and *C. ferrugineum* are illustrated in Figures 5-7, respectively. The

methanol extracts showed the highest reducing activities followed by ethyl acetate and dichloromethane extracts for all *Calophyllum* species. Similar trend was observed for the total phenolic content determination as discussed earlier. Thus, it can be deduced that high concentration of phenolic compounds in more polar extracts acts as reducing agent in this FRAP assay.

Table 3. FRAP equivalent of the extracts from *Calophyllum* species

***Calophyllum* Species**	**FRAP Equivalent to $FeSO_4.7H_2O$ (mM)[a]**					
	1.0	**0.8**	**0.6**	**0.4**	**0.2**	**0.1**
C. ferrugineum						
CFBD	0.24 ± 0.01 *	0.18 ± 0.02 *	0.16 ± 0.01 *	0.16 ± 0.02 *	0.12 ± 0.01 **	0.11 ± 0.01 **
CFBE	2.98 ± 0.65 **	1.98 ± 0.17 **	1.88 ± 0.79	1.13 ± 0.06 **	0.87 ± 0.21	0.37 ± 0.02 *
CFBM	3.72 ± 0.46 ***	2.74 ± 1.11	2.03± 0.63	1.41 ± 0.25 *	0.74 ± 0.19	0.40 ± 0.08
CFLD	0.37 ± 0.10	0.26 ± 0.02 *	0.20 ± 0.01 *	0.15 ± 0.01 *	0.13 ± 0.01 **	0.10 ± 0.01 **
CFLE	3.24 ± 1.04	1.24 ± 0.14 *	1.17 ± 0.42	0.68 ± 0.10	0.55 ± 0.05	0.22 ± 0.6 *
CFLM	2.57 ± 0.44 **	1.94 ± 0.72	1.37 ± 0.31	0.91 ± 0.12	0.72 ± 0.07	0.32 ± 0.03 *
C. incrassatum						
CIBD	0.23 ± 0.07 *	0.23 ± 0.02 *	0.16 ± 0.02 *	0.12 ± 0.01 *	0.07 ± 0.01 *	0.05 ± 0.01 **
CIBE	1.35 ± 0.04 **	1.15 ± 0.25	0.65 ± 0.09	0.40 ± 0.02	0.18 ± 0.02 *	0.09 ± 0.01 **
CIBM	3.23 ± 0.22 ***	2.44 ± 0.18 ***	2.00 ± 0.22 **	1.40 ± 0.03 ***	0.54 ± 0.04	0.20 ± 0.01 *
CILD	0.17 ± 0.05 *	0.11 ± 0.02 *	0.10 ± 0.02 *	0.10 ± 0.01 *	0.06 ± 0.01 **	0.05 ± 0.01 **
CILE	1.10 ± 0.27	0.62 ± 0.09	0.57 ± 0.11	0.39 ± 0.03	0.22 ± 0.02 *	0.14 ± 0.01 **
CILM	1.41 ± 0.29 *	0.98 ± 0.01	0.84 ± 0.09	0.57 ± 0.06	0.34 ± 0.04	0.22 ± 0.11 *
AA[b]	0.94 ± 0.24	0.29 ± 0.07	0.37 ± 0.09	0.17 ± 0.05	0.08 ± 0.01	0.07 ± 0.01
BHA[b]	1.30 ± 0.33	1.07 ± 0.14	0.83 ± 0.13	0.62 ± 0.11	0.33 ± 0.09	0.22 ± 0.06
BHT[b]	0.59 ± 0.21	0.36 ± 0.06	0.28 ± 0.05	0.21 ± 0.02	0.19 ± 0.04	0.09 ± 0.04

[a] Data represent mean ± standard deviation of three replicate experiments; [b] Positive control; AA = Ascorbic acid; BHT: Butylated Hydroxytoluene; BHA: Butylated Hydroxyanisole; * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$

The dichloromethane extract from the leaves of *C. ferrugineum* (CFLD) displayed the highest FRAP equivalent to $FeSO_4.7H_2O$ among all dichloromethane extracts followed by the bark extract of *C. ferrugineum* and *C. incrassatum* as shown in Figure 5. Meanwhile, the ethyl acetate extract from the bark of *C. ferrugineum* followed by ethyl acetate extract from the leaves of *C. ferrugineum*, ethyl acetate extract from the bark of *C. incrassatum* and ethyl acetate extract from leaves of *C. incrassatum* showed the FRAP equivalent to $FeSO_4.7H_2O$ in decreasing order (Figure 6). Moreover, the methanol extracts from bark of *C. ferrugineum*, bark of *C. incrassatum* and leaves of *C. ferrugineum* also depicted high FRAP equivalent to $FeSO_4.7H_2O$ among all methanol extracts displayed in Figure 7.

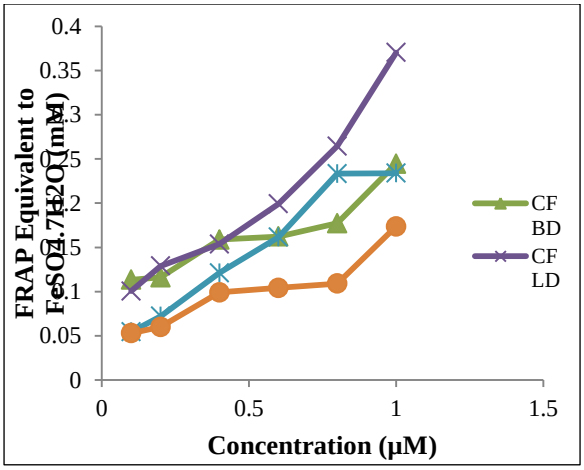

Figure 5. FRAP equivalent to $FeSO_4.7H_2O$ of dichloromethane extracts

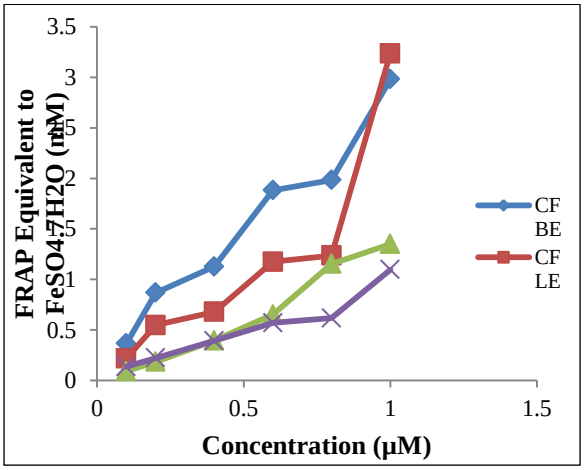

Figure 6. FRAP equivalent to $FeSO_4.7H_2O$ of ethyl acetate extracts

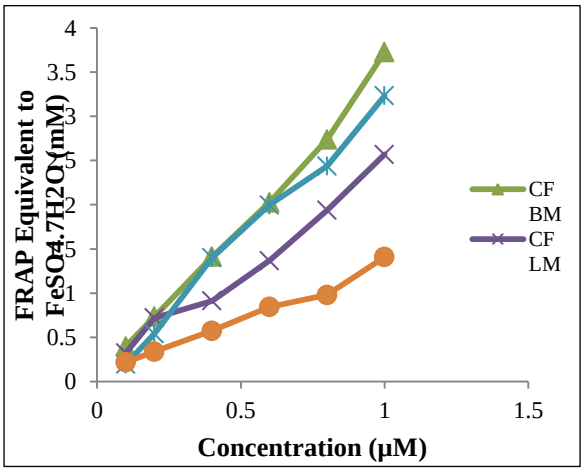

Figure 7. FRAP equivalent to $FeSO_4.7H_2O$ of methanol extracts

The antioxidant powers of CFBM, CFLM, CIBM, CFBE and CFLE extracts that showed highest FRAP equivalent to $FeSO_4.7H_2O$ with respect to their respective type of extracts were compared with antioxidant power of ascorbic acid (AA), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as the positive controls. From the graph (Figure 8), all extracts display higher FRAP equivalent to $FeSO_4.7H_2O$ compared to all positive controls. It justified their stronger antioxidant power compared to the positive controls. The CFBM extract was found to have the highest antioxidant power among all extracts followed by CIBM and CFBE.

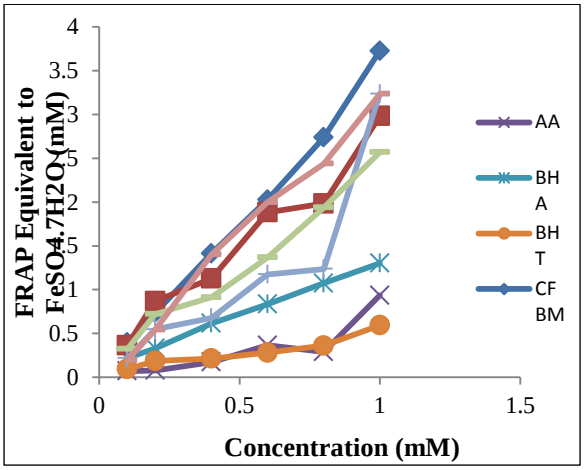

Figure 8. FRAP equivalent to $FeSO_4.7H_2O$ of selected extracts and positive controls

Conclusion

Based on the findings, both *Calophyllum* species showed a strong antioxidant activity especially from the methanol extracts, suggesting potent antioxidant agents may be isolated. Meanwhile, the dichloromethane extract from the bark of *C. ferrugineum* showed potent antibacterial activities selectively against the Gram-positive bacteria, suggesting it may serve as possible source for new antibacterial agents. Mode of antibacterial action of the extracts and isolated phytochemicals specifically against Gram-positive bacteria can be further studied.

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