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COMPARISON OF EXTRACTION TECHNIQUES FOR THREE *Calophyllum* SPECIES AND THEIR ANTIOXIDANT ACTIVITY

(Perbandingan Teknik Pengekstrakan untuk Tiga Spesies *Calophyllum* dan Aktiviti Antioksidan)

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Abstract

Calophyllum is a pan-tropical genus belonging to the Guttiferae family and is known in Malaysia as '*bintangor*'. There has been continual interest to further investigate the phytochemistry of *Calophyllum* species since this genus is a rich source of active secondary metabolites, which show anti-HIV, cytotoxicity, and antimicrobial properties. This study was conducted to investigate the effect of extraction techniques on the phytochemicals content and antioxidant activity of the barks, leaves, and heartwood extracts of three *Calophyllum* species, namely *C. incrassatum*, *C. rubiginosum*, and *C. canum*. Soxhlet and maceration extraction techniques by using methanol as solvent were chosen in this study. The maceration extraction technique produced higher percentage yield as compared to Soxhlet extraction for leaves and barks of the three *Calophyllum* species. The highest percentage yield was obtained from the bark extract of *C. canum* (21.76%), followed by the bark extract of *C. rubiginosum* (20.24%) and leaves extract of *C. rubiginosum* (19.34%). Meanwhile, the Soxhlet extraction technique gave higher percentage yield as compared to the maceration technique for heartwood extracts of all samples. The phytochemical screening test revealed that all extracts contained tannin, phenol, flavonoid, terpenes, cardiac glycoside, coumarin, and phytosterol. The highest total phenolic content was obtained from Soxhlet extraction technique. The bark extract of *C. canum* displayed the highest phenolic content (461.90 mg GAE/g), followed by the bark extract of *C. incrassatum* (394.52 mg GAE/g) and leaves extract of *C. incrassatum* (227.89 mg GAE/g). The bark extract of *C. canum* showed the lowest IC_{50} value (3.07 µg/mL), followed by the bark extract of *C. incrassatum* (5.12 µg/mL) and leaves extract of *C. incrassatum* (5.93 µg/mL). Pearson's correlation test showed a positive correlation between total phenolic content and DPPH radical scavenging activity.

Keywords: *Calophyllum*, *C. incrassatum*, *C. rubiginosum*, *C. canum*, antioxidant

Abstrak

Calophyllum ialah sejenis genus pan-tropika milik keluarga Guttiferae dan dikenali di Malaysia sebagai *'bintangor'*. Terdapat minat yang berterusan untuk mengkaji fitokimia *Calophyllum* spesies memandangkan genus ini merupakan sumber yang kaya dengan metabolit sekunder aktif yang menunjukkan ciri-ciri anti-HIV, sitotoksisiti dan antimikrob. Kajian ini dijalankan untuk mengkaji kesan teknik pengekstrakan terhadap kandungan fitokimia dan aktiviti antioksidan daripada ekstrak kulit, daun dan batang daripada tiga spesies *Calophyllum*, *C. incrassatum*, *C. rubiginosum* dan *C. canum*. Teknik pengekstrakan Soxhlet dan rendaman dengan menggunakan metanol sebagai pelarut telah dipilih dalam kajian ini. Teknik pengekstrakan rendaman menghasilkan hasil peratusan yang lebih tinggi berbanding dengan pengekstrakan Soxhlet untuk daun dan kulit ketiga-tiga spesies *Calophyllum*. Hasil peratusan paling tinggi diperoleh daripada ekstrak kulit *C. canum* (21.76%) diikuti oleh ekstrak kulit *C. rubiginosum* (20.24%) dan ekstrak daun *C. rubiginosum* (19.34%). Sementara itu, teknik pengekstrakan Soxhlet memberikan hasil peratusan yang lebih tinggi berbanding dengan teknik rendaman untuk ekstrak batang dari semua sampel. Ujian saringan fitokimia menunjukkan semua ekstrak mengandungi tanin, fenol, flavonoid, terpena, kardiak glikosida, komarin dan fitosterol.

Jumlah kandungan fenolik tertinggi diperolehi daripada teknik pengekstrakan Soxhlet. Ekstrak kulit *C. canum* menunjukkan kandungan fenolik tertinggi (461.90 mg GAE/g) diikuti oleh ekstrak kulit *C. incrassatum* (394.52 mg GAE/g) dan ekstrak daun *C. incrassatum* (227.89 mg GAE/g). Ekstrak kulit *C. canum* menunjukkan nilai IC_{50} terendah (3.07 µg/mL) diikuti oleh ekstrak kulit *C. incrassatum* (5.12 µg/mL) dan ekstrak daun *C. incrassatum* (5.93 µg/mL). Ujian korelasi Pearson menunjukkan korelasi positif antara jumlah kandungan fenolik dan aktiviti pemerangkapan radikal DPPH.

Kata kunci: *Calophyllum, C. incrassatum, C. rubiginosum, C. canum,* antioksidan

Introduction

Calophyllum, or locally known as '*bintangor*' is one of the genera in the Guttiferae family. It had been used traditionally to treat a variety of health conditions, such as malaria, bronchitis, inflammation, diabetes, and other health problems [1]. Researchers have developed interest to investigate this species due to the isolation of coumarins known as '*the calanolides*' from *C. lanigerum* against HIV-1 replication [2]. In addition, phytochemical studies reporting on other *Calophyllum* species revealed that they are rich with bioactive compounds such as xanthones, flavonoids, and triterpenoids [1]. These compounds display a broad spectrum of bioactivity, e.g. antioxidant, anti-HIV, anti-fungal, anti-malarial, and cytotoxicity [3].

Plant extraction is the most important step in a phytochemical study since the quality and quantity of the extracts are the key factors that influence the type of isolated compounds and bioactivity results. There are limited numbers of phytochemical studies reported on these three *Calophyllum* species, i.e. *C. incrassatum, C. rubiginosum, and C. canum*. Hence, the best extraction technique suitable for these three species is unknown. The commonly used extraction technique reported for other *Calophyllum* species is maceration [4-5]. However, there are also several reports on the *Calophyllum* species that employed Soxhlet extraction [6-7]. Hence, this study was designed to compare the best extraction technique between maceration and Soxhlet techniques for *C. incrassatum, C. rubiginosum,* and *C. canum* since the best techniques for these species are yet to be determined. In this study, the extracts from both extraction techniques were evaluated qualitatively through phytochemical screening, and quantitatively through antioxidant assay. The reliability and correlation of the data obtained through antioxidant assay was determined by performing statistical analyses, namely *t*-test and Pearson's correlation.

Materials and Methods

Chemicals and reagents

Ferric (III) chloride, sodium hydroxide, chloroform, sulphuric acid, glacial acetic acid, acetic anhydride and sodium carbonate were obtained from R&M Chemicals. Meanwhile, gallic acid, 2,2 diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, butylated hydroxyl toluene (BHT) and Folin-Ciocalteu's reagent were obtained from Sigma Aldrich.

Extraction of *Calophyllum* species

The samples of leaves, barks and heartwoods of all *Calophyllum* species were dried and ground before subjected into extraction process. Soxhlet and maceration extractions were performed employing methanol as the solvent. For Soxhlet, dried samples (100 g) were extracted with methanol (900 mL) for 16 hours and 30 minutes. For maceration, dried samples (50 g) were soaked with methanol (450 mL) in closed beaker for 72 hours. The maceration process was repeated for three times. The extracts were filtered by using filter paper and concentrated under reduced pressure by using rotary evaporator to yield the crude extracts. The crude extracts were kept in vials at 4 ^{o}C before subjected to phytochemical screening and antioxidant assay.

Phytochemical screening of *Calophyllum* species

Test for tannin

A small quantity of extracts was dissolved in a test tube containing methanol (1 mL) followed by the addition of distilled water (2 mL) and ferric (III) chloride solution (10% w/v, 4 drops). The presence of tannin was indicated by formation of greenish brown precipitate [8].

Test for phenol
A small quantity of extract was dissolved in a test tube containing methanol (2 mL) followed by the addition of distilled water (2 mL) and ferric (III) chloride solution (10% w/v, 10 drops). The presence of phenol was indicated by change in colour of extract solution to yellowish green [9].

Test for flavonoid
A small quantity of extract was dissolved in a test tube containing methanol (3 mL) followed by the addition of distilled water (10 mL). The test tube was gently shaken before the addition of sodium hydroxide solution (10% w/v, 1 mL). The presence of flavonoid was indicated by change in colour of extract solution to yellow [10].

Test for terpene
A small quantity of extract was dissolved in a test tube containing methanol (1 mL) followed by the addition of chloroform (2 mL). Then, concentrated sulphuric acid (1 mL) was added carefully along the side of test tube. The presence of terpene was indicated by formation of reddish brown ring [11].

Test for cardiac glycoside
Phytochemical screening for cardiac glycoside was performed according to Killer-Killiani's test. A small quantity of extract was dissolved in a test tube containing methanol (1 mL). Glacial acetic acid containing ferric (III) chloride solution (10% w/v, 1 mL) was added into the test tube followed by addition of concentrated sulphuric acid (1 mL). The presence of cardiac glycoside was indicated by formation of brown ring at the interface [12].

Test for coumarin
A small quantity of extracts was dissolved in a test tube containing methanol (2 mL) followed by the addition of sodium hydroxide solution (10% w/v, 3 mL). The presence of coumarin was indicated by change in colour of extract solution to yellow [13].

Test for phytosterol
Phytochemical screening for phytosterol was performed according to Libermann-Burchard's test. The extract (2 mg) was dissolved in acetic anhydride (1 mL) and heated to boiling. The hot extract solution was immediately cooled in ice bath. Concentrated sulphuric acid (1 mL) was carefully added along the side of test tube. The presence of phytosterol was indicated by formation of brown coloured ring at the interface of two layers [14].

Test for saponin
A small quantity of extracts was dissolved in a test tube containing methanol (3 mL) followed by addition of distilled water (2 mL). The tube was vigorously shaken. The presence of saponin was indicated by formation of frothing layer [10].

Antioxidant assay
Total phenolic content
The total phenolic content of all extracts was assessed by using Folin-Ciocalteau's assay [15]. Samples (40 µL) with concentrations ranging from 1000 until 7.81 µg/mL were mixed with Folin-Ciocalteau's reagent (20 µL) in 96-well microplate. The mixture was allowed to incubate for 5 minutes at room temperature. Upon incubation, sodium carbonate solution (6% w/v, 80 µL) followed by distilled water (60 µL) was added to the reaction mixture and kept in the dark for 90 minutes. The absorbance was recorded at 760 nm. A calibration graph of standard gallic acid was constructed and the total phenolic content of the extracts was expressed as mg Gallic acid equivalent (GAE) per gram of extract.

DPPH radical scavenging activity

DPPH radical scavenging activity of all extracts were evaluated following the method described by Kassim et al. [15] with minor modifications. DPPH reagent (2000 µM, 15 µL) was added to the samples (85 µL) with concentration ranging from 1000 to 7.81 µg/mL in methanol obtained from two-fold serial dilution in 96-well microplate. The reaction mixture was allowed to incubate in dark conditions at room temperature for 30 minutes.

The colour conversion from purple to yellow illustrates the samples are active as antioxidant agents. The absorbance of DPPH radical scavenging activity was measured at 517 nm. The radical scavenging activity were analyzed and compared with ascorbic acid and butylated hydroxytoluene (BHT) as the positive controls. The percentage of DPPH scavenging inhibition was calculated by using the following formula [15]:

$$\text{Scavenging concentration (\%)} = \frac{A_{\text{DPPH blank}} - (A_{\text{sample}} - A_{\text{blank sample}})}{A_{\text{DPPH blank}}} \times 100 \quad (1)$$

where, $A_{\text{DPPH blank}}$ is the absorbance of DPPH solution, A_{sample} is the absorbance of tested sample and DPPH solution and $A_{\text{blank sample}}$ is the absorbance of tested sample without DPPH solution.

Statistical analysis

Triplicates data of each sample were used for statistical analysis with values reported as mean ± standard deviation. Standard curves were generated and calculation of the 50% inhibitory concentration values was performed using GraphPad Prism software. The statistical analysis was performed by using SPSS for Windows (version 22). A *t*-test was performed to determine the significant difference between treatment of samples and control. Pearson's correlation coefficient test was used to determine the correlation between the two antioxidant assays.

Results and Discussion

Extraction yield of *Calophyllum* species

This study proposed two types of extraction method, namely Soxhlet extraction and maceration extraction techniques, to obtain the crude extracts from different parts of *C. incrassatum*, *C. rubiginosum*, and *C. canum*. The percentage yield of extract is shown in Table 1. The maceration technique was identified to provide better extraction yield for the leaves and barks of the three *Calophyllu*m species. The highest yield was obtained from the bark extract of *C. canum* (21.76%), followed by the bark extract of *C. rubiginosum* (20.24%) and leaves extract of *C. rubiginosum* (19.34%). In contrast, the heartwood of the three species gave the highest percentage yield of extract by the Soxhlet extraction technique. The heartwood extract of *C. canum* (9.62%) gave the highest yield, followed by *C. rubiginosum* (6.40%) and *C. incrassatum* (4.39%).

Table 1. Percentage yield of extracts from *Calophyllum* species

Sample	Crude Extract	Percentage Yield (%)
C. incrassatum	CILS	14.38
	CILM	19.34
	CIBS	12.30
	CIBM	18.00
	CIHS	4.39
	CIHM	3.66
C. rubiginosum	CRLS	9.18
	CRLM	14.28
	CRBS	14.67
	CRBM	20.24
	CRHS	6.40
	CRHM	3.84

Table 1(cont'd). Percentage yield of extracts from *Calophyllum* species

Sample	Crude Extract	Percentage Yield (%)
C. canum	CCLS	13.46
	CCLM	13.82
	CCBS	20.90
	CCBM	21.76
	CCHS	9.62
	CCHM	3.46

CILS: *C. incrassatum* leaves Soxhlet; CILM; *C. incrassatum* leaves maceration; CIBS: *C. incrassatum* barks Soxhlet; *C. incrassatum* barks maceration; CIHS; *C. incrassatum* heartwoods Soxhlet; CIHM: *C. incrassatum* heartwoods maceration; CRLS; *C. rubiginosum* leaves Soxhlet; CRLM; *C. rubiginosum* leaves maceration; CRBS: *C. rubiginosum* barks Soxhlet; CRBM: *C. rubiginosum* barks maceration; CRHS; *C. rubiginosum* heartwoods Soxhlet; CRHM: *C. rubiginosum* heartwoods maceration; CCLS; *C. canum* leaves Soxhlet; CCLM; *C. canum* leaves maceration; CCBS: *C. canum* barks Soxhlet; CCBM: *C. canum* barks maceration; CCHS; *C. canum* heartwoods Soxhlet; CCHM: *C. canum* heartwoods maceration.

Herein, different extraction techniques had different efficiency and percentage yields due to the degree of availability of extractable compounds. The result indicated that the maceration extraction technique was more effective to produce high percentage yield for the leaves and barks of all *Calophyllum* species. This occurrence was mainly due to the introduction of heat from the Soxhlet extraction technique. Prolonged heating may cause heat sensitive compounds to degrade [16]. Unlike Soxhlet, the maceration technique was able to preserve the heat sensitive compounds. Furthermore, the crudes were extracted thrice in the maceration technique instead of once in Soxhlet. These factors may affect the results and give higher percentage yield from the sample.

Phytochemical screening

Phytochemical analysis of all extracts revealed the presence of tannin, phenol, flavonoid, terpene, cardiac glycoside, coumarin and phytosterol. Meanwhile, saponin was only detected in the bark extract of *C. canum* yielded from Soxhlet extraction technique. The results of the screening were summarized in Table 2.

Table 2. Phytochemical screening of extracts from *calophyllum* species

Sample	Crude	Tannin	Phenol	Flavonoid	Terpene	Cardiac Glycoside	Coumarin	Phytosterol	Saponin
C. incrassatum	CILS	+++	+++	+++	+++	+++	+	+++	-
	CILM	++	+++	++	++	++	+++	+	-
	CIBS	+++	+++	+++	++	++	++	+++	-
	CIBM	+++	++	++	+++	+++	+	+++	-
	CIHS	+	+++	++	++	++	++	++	-
	CIHM	++	++	+	++	++	+	+++	-
C. rubiginosum	CRLS	++	++	+++	+	+	++	++	-
	CRLM	+	++	+++	+	+	++	+	-
	CRBS	+++	+++	++	++	+++	+	++	-
	CRBM	+++	++	+++	+++	+++	++	+++	-
	CRHS	++	++	++	+++	+++	++	++	-
	CRHM	++	+	+	+++	++	+	+	-

Table 2 (cont'd). Phytochemical screening of extracts from *calophyllum* species

Sample	Crude	Tannin	Phenol	Flavonoid	Terpene	Cardiac Glycoside	Coumarin	Phytosterol	Saponin
C. canum	CCLS	+++	+++	++	++	++	+++	+++	+
	CCLM	++	++	++	++	++	++	+	-
	CCBS	+++	+++	++	+++	+++	+++	+++	-
	CCBM	+++	++	+	+++	++	++	++	-
	CCHS	+	++	++	++	++	+	+++	-
	CCHM	+	+	+	+++	++	+	++	-

CILS: *C. incrassatum* leaves Soxhlet; CILM; *C. incrassatum* leaves maceration; CIBS: *C. incrassatum* barks Soxhlet; *C. incrassatum* barks maceration; CIHS; *C. incrassatum* heartwoods Soxhlet; CIHM: *C. incrassatum* heartwoods maceration; CRLS; *C. rubiginosum* leaves Soxhlet; CRLM; *C. rubiginosum* leaves maceration; CRBS: *C. rubiginosum* barks Soxhlet; CRBM: *C. rubiginosum* barks maceration; CRHS; *C. rubiginosum* heartwoods Soxhlet; CRHM: *C. rubiginosum* heartwoods maceration; CCLS; *C. canum* leaves Soxhlet; CCLM; *C. canum* leaves maceration; CCBS: *C. canum* barks Soxhlet; CCBM: *C. canum* barks maceration; CCHS; *C. canum* heartwoods Soxhlet; CCHM: *C. canum* heartwoods maceration; +++ = Highly presence; ++ =Moderately presence; + = Slightly presence; - = Absence

The phytochemical screening test showed the presence of tannin in all extracts from both extraction techniques. From the observation, tannin was observed to be present in a significant amount in all leaves and barks extracts of the three *Calophyllum* species, except the heartwood extracts. The analysis suggested tannin was highly present in the leaves extracts of all *Calophyllum* species obtained through the Soxhlet extraction technique. In contrast, the extraction techniques did not express differences in terms of tannin content for all barks and some heartwood extracts of the *Calophyllum* species. The extracts from leaves, barks, and heartwood of *C. incrassatum*, barks of *C. rubiginosum*, and leaves and barks of *C. canum* obtained from the Soxhlet extraction technique demonstrated the highest phenol content. Meanwhile, for the maceration technique, only the leaves of *C. incrassatum* showed the highest phenol content. Hence, the Soxhlet extraction technique affected the phenol content where most of the extracts displayed higher phenol content as compared to the maceration technique.

The presence of flavonoid was observed in all extracts from both extraction techniques. The extracts from leaves and barks of *C. incrassatum* and leaves of *C. rubiginosum* obtained by the Soxhlet extraction technique expressed higher flavonoid content, which signified that these parts contained the highest amount of flavonoid. Meanwhile for the maceration technique, high content of flavonoid could only be observed in the leaves and barks of *C. rubiginosum*. As a conclusion, the Soxhlet extraction technique demonstrated higher availability of flavonoid content as compared to the maceration technique in most parts of the *Calophyllum* species. In contradiction, the maceration technique yielded better terpene content in most parts of extracts, especially for *C. rubiginosum* and *C. canum*. Terpenes were distributed largely in the extracts from the bark and heartwood of *C. rubiginosum* and bark and heartwood of *C. canum*, respectively.

In general, the qualitative analysis of cardiac glycoside, coumarin, and phytosterol revealed positive presence in all extracts. High content of cardiac glycoside was found in the leaves extract of *C. incrassatum*, bark and heartwood extracts of *C. rubiginosum*, and bark extract of *C. canum* obtained from Soxhlet extraction. Meanwhile, only the leaves and bark extracts of *C. canum* obtained by Soxhlet extraction displayed the highest intensity of coumarin as compared to the other species. Negative presence of saponin was observed in majority parts of extracts from the three *Calophyllum* species. However, saponin was detected from the leaves extract of *C. canum* yielded from the Soxhlet extraction technique in minimum amount.

Antioxidant activity of *Calophyllum* species

The total phenolic content (TPC) of the samples was expressed as mg GAE per gram of extract. The evaluation of TPC was performed by using the calibration curve of standard gallic acid ($y = 0.0021x + 1.7678$, $R^2 = 0.9926$). The TPC of each *Calophyllum* species at the concentration of 1000 µg/mL was calculated and tabulated as displayed in Table 3. The leaves and bark extract of all species from the Soxhlet extraction technique showed higher TPC value as compared to maceration. The highest TPC value was depicted from the bark extract of *C. canum* (461.90 mg

GAE/g), followed by bark extract of *C. incrassatum* (394.52 mg GAE/g), and leaves extract of *C. incrassatum* (227.89 mg GAE/g). Nevertheless, the heartwood extract of *C. incrassatum* displayed the lowest TPC value (2.70 mg GAE/g) as compared to the heartwood of other *Calophyllum* species obtained from the Soxhlet extraction technique.

Table 3. TPC, DPPH SC_{50} and Pearson correlation coefficient values

Sample	**Crude**	**Total Phenolic Content[a] (mg GAE/g of Extract at 1000 µg/mL)**	**DPPH IC_{50} (µg/mL)[a]**	***r* coefficient**
C. incrassatum	CILS	227.89 ± 2.14***	5.93 ± 0.22 $^{*b, ***c}$	0.816*
	CILM	67.10 ± 0.18***	26.57 ± 0.60 $^{***b, ***c}$	0.678
	CIBS	394.52 ± 37.86*	5.12 ± 0.10$^{***b, ***c}$	0.572
	CIBM	84.70 ± 15.60***	11.54 ± 1.30$^{**b, c}$	0.924**
	CIHS	2.70 ± 2.55***	39.39 ± 0.58$^{***b, ***c}$	0.905**
	CIHM	65.19 ± 0.74***	27.45 ± 0.35$^{***b, ***c}$	0.813*
C. rubiginosum	CRLS	126.19 ± 4.51**	11.1 ± 0.17$^{***b, ***c}$	0.665
	CRLM	59.71 ± 1.86***	28.43 ± 1.18$^{***b, ***c}$	0.747*
	CRBS	198.00 ± 35.74***	8.56 ± 0.61$^{*b, ***c}$	0.873**
	CRBM	104.14 ± 2.59***	9.22 ± 0.71$^{*b, **c}$	0.918**
	CRHS	64.33 ± 0.76***	27.68 ± 0.20$^{***b, ***c}$	0.892**
	CRHM	42.35 ± 11.80*	31.02± 0.76$^{***b,*** c}$	0.892**
C. canum	CCLS	181.44 ± 3.82*	17.76 ± 0.40$^{***b, ***c}$	0.684
	CCLM	83.62 ± 4.18***	20.31 ± 0.55$^{**b, **c}$	0.746*
	CCBS	461.90 ± 10.97*	3.07 ± 0.43$^{***b, ***c}$	0.713*
	CCBM	77.11 ± 2.78***	11.89 ± 0.74$^{***b, *c}$	0.858**
	CCHS	69.10 ± 3.98***	24.71 ± 0.75$^{**b, ***c}$	0.809*
	CCHM	21.84 ± 0.93	35.50 ± 0.90$^{***b, ***c}$	0.960**
AA[d]			6.92 ± 0.039	
BHT[d]			13.40 ± 0.12	

[a]: Data represents mean ± standard deviation of three replicate experiments; [b]: Significant different value *P* compared to ascorbic acid; [c]: Significant different value *P* compared to BHT; [d]: Positive control; CILS: *C. incrassatum* leaves Soxhlet; CILS: *C. incrassatum* leaves Soxhlet; CILM; *C. incrassatum* leaves maceration; CIBS: *C. incrassatum* barks Soxhlet; *C. incrassatum* barks maceration; CIHS; *C. incrassatum* heartwoods Soxhlet; CIHM: *C. incrassatum* heartwoods maceration; CRLS; *C. rubiginosum* leaves Soxhlet; CRLM; *C. rubiginosum* leaves maceration; CRBS: *C. rubiginosum* barks Soxhlet; CRBM: *C. rubiginosum* barks maceration; CRHS; *C. rubiginosum* heartwoods Soxhlet; CRHM: *C. rubiginosum* heartwoods maceration; CCLS; *C. canum* leaves Soxhlet; CCLM; *C. canum* leaves maceration; CCBS: *C. canum* barks Soxhlet; CCBM: *C. canum* barks maceration; CCHS; *C. canum* heartwoods Soxhlet; CCHM: *C. canum* heartwoods maceration; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

All extracts were subjected to TPC determination by using Folin-Ciocalteu's reagent. Generally, this spectrophotometric method allows possible evaluation of all phenolic compounds, such as flavonoids, polyphenols and others [17]. Folin-Ciocalteu's reagent consists of the mixture of phosphomolybdic ($H_3PMo_{12}O_{40}$) and phosphotungstic ($H_3PW_{12}O_{40}$) acid complexes [18]. The detection of phenolic compounds in the extract by using Folin-Ciocalteu assay is dependent on the reaction of phenolic compounds in alkaline medium provided by sodium carbonate solution to the metal acid mixtures. This reaction results in the formation of blue complexes of molybdene (Mo_8O_{23}) and tungsten (W_8O_{23}). The degree of the blue complexes represents the amount of phenolic compounds, which can be measured spectrophotometrically at the wavelength of 760 nm [19].

Conventional solvent extraction methods such as Soxhlet and maceration extraction techniques are frequently used in extracting polyphenolic compounds from plants. The chosen extraction technique plays a significant role in determining the quantity of phenolic constituents in extracts. This study suggested the Soxhlet extraction technique was better to extract phenolic constituents from *C. incrassatum*, *C. rubiginosum*, and *C. canum* as compared to maceration based on their higher TPC values. High content of phenolic compounds might be influenced by the introduction of heat from the Soxhlet technique. An increase in temperature would allow cell structures, such as cell wall, membrane layer and organelles, to disintegrate. Thus, the mass transfer between the soluble phenolic constituents into the solvents was improved [20]. As a result, more phenolic constituents will be available in the extract.

The antioxidant activities of all extracts were also evaluated based on the IC_{50} values in comparison to ascorbic acid (IC_{50} 6.92 µg/mL) and butylated hydroxytoluene (IC_{50} 13.40 µg/mL) as the positive controls. The calculated IC_{50} for all extracts is presented in Table 3. All tested samples that showed ($p < 0.05$) were considered as statistically and significantly different as compared to ascorbic acid and BHT as positive controls. The leaves and barks extracts of all species from the Soxhlet extraction technique gave lower IC_{50} values as compared to maceration. The bark extract of *C. canum* (IC_{50} 3.07 µg/mL), followed by the bark extract of *C. incrassatum* (IC_{50} 5.12 µg/mL) and leaves extract of *C. incrassatum* (IC_{50} 5.93 µg/mL) displayed low IC_{50} values comparable to both positive controls. This result indicated that the three extracts were superior in terms of antioxidant activity as compared to the positive controls. The heartwood extracts of *C. incrassatum* displayed the highest IC_{50} values among all heartwood extracts at IC_{50} 39.39 µg/mL.

The Pearson's correlation analysis was performed to establish a relationship between TPC and DPPH radical scavenging activity using different concentrations ranging from 1000 – 7.81 µg/mL. The correlation coefficient, *r*, was evaluated as depicted in Table 3. Most of the extracts demonstrated positive correlation with *r* coefficient value in the range of 0.573 to 0.960 between the two parameters. This statistical data suggested that high TPC would contribute to high antioxidant activity of the extracts. It could be deduced that the phenolic compounds are the major contributor to the antioxidant activity by the DPPH method.

Conclusion

The maceration technique gave better yield of extracts from the leaves and barks of all *Calophyllum* species. On the other hand, the Soxhlet extraction technique produced higher percentage yield of heartwood extracts. Phytochemical screening of all extracts depicted the presence of tannin, phenol, flavonoid, terpene, cardiac glycoside, coumarin, and phytosterol. However, Soxhlet extraction provided the best phytochemicals content as compared to maceration extraction. This preliminary screening will provide beneficial reference for future research in determining the suitable extraction technique to isolate certain desired classes of compounds, since each method has its own advantages. Most of the extracts also showed potent antioxidant activity, suggesting that natural antioxidant compound can be isolated in the future.

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