Antioxidant Potential of *Calophyllum gracilentum*: A Study on Total Phenolic Content, Total Flavonoid Content, and Free Radical Scavenging Activities

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Abstract

The most prevalent phytoconstituents of medicinal and aromatic plants are phenols and flavonoids, which are responsible for antioxidant activity. This study aims to determine the total phenolic content (TPC), total flavonoid content (TFC), and free radical scavenging activity of *Calophyllum gracilentum,* an understudied *Calophyllum* species with very limited information. The stem bark extracts were prepared and examined for TPC assay by Folin–Ciocalteu method, while the TFC of the extracts was determined using aluminium chloride colorimetric method and the antioxidant activity was evaluated by free radical scavenging activity using 1,2-diphenyl-2-pricrylhydrazyl (DPPH). The extracts showed methanolic extracts exhibited the highest TPC and TFC with the value of 1542.40 ± 0.0246 mg GAE/g extract and 451.70 ± 0.0003 mg quercetin equivalents (QE)/g extract, respectively. Meanwhile, DPPH scavenging activity data revealed that all extracts had IC50 values ranging from 44.17 1.26 g/mL to 162.61 2.24 g/mL, with the methanolic extract for *C. gracilentum* having the highest antioxidant activity with IC50 values of 44.17 1.26 g/mL. The TPC and TFC assays showed that *C. gracilentum* extracts possess a substantial to moderate phenolic and flavonoid content, while the DPPH free radical scavenging assay revealed that the extracts have strong antioxidant activity. The results demonstrate that *C. gracilentum* contains a high concentration of phenolic and flavonoid chemicals. These discoveries could have significant implications for the prospective use of these plants in traditional medicine and as natural antioxidant sources.

Keywords: *Calophyllum*; DPPH; extracts; total flavonoid content; total phenolic content.

Significance | Study of potential Therapeutic activity of *Calophyllum* herb.

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Introduction

Phytochemicals such as phenols, flavonoids, alkaloids, glycosides, lignins, and tannins are naturally found in plants and plant-based products. The most prevalent phytoconstituents of many fruits, vegetables, medicinal and aromatic plants that are responsible for antioxidant activity are phenols and flavonoids. Natural antioxidants, such as phenols and flavonoid chemicals derived from plants, are gaining appeal due to the potential toxicological consequences of synthetic antioxidants (Dragusha et al., 2023). Phenolic chemicals derived from medicinal plants have a wide range of biological effects and can aid in the prevention of a variety of ailments. They are also well-known in phytotherapy and ethno-veterinary practices for their importance in healthcare.

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

Crude extract	Percentage Yield
n-Hexane extract (CGH)	6.7 %
Chloroform extract (CGC)	10.0 %
Ethyl acetate extract (CGEA)	5.3 %
Methanol extract (CGM)	13.3 %

Table 2. LOD and LOQ of the method validation for TPC and TFC Assay of *C. gracilentum*

Assay	LOD	LOQ
Total Phenolic Content (TPC)	3.3 x (Standard Error) / slope = 3.3 x (0.0302) / 0.4924 = 0.2024 mg/mL	10.0 x (Standard error) / slope = 10 x (0.0302) / 0.4924 = 0.6134 mg/mL
Total Flavonoid Content (TFC)	3.3 x (Standard error) / slope = 3.3 x (0.0196 / 0.3591) = 0.1801 mg/mL	10.0 x (Standard error) / slope = 10 x (0.0196) / 0.3591 = 0.5458 mg/mL

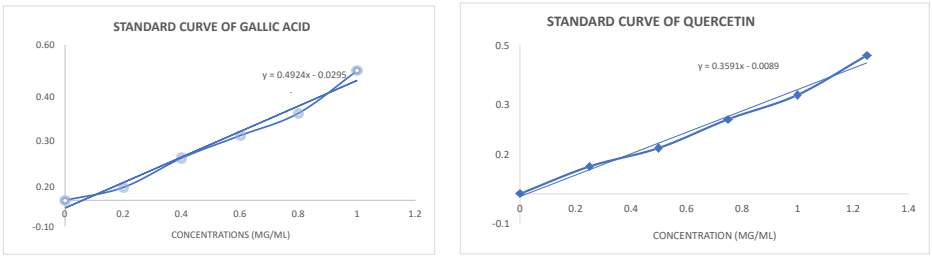
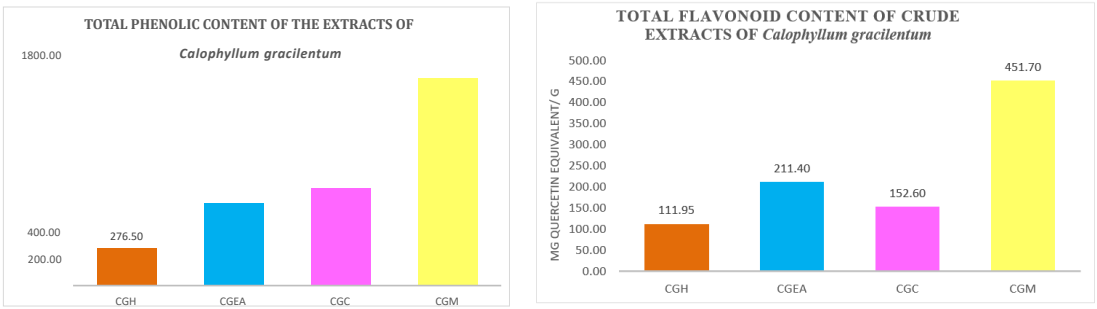


Figure 1. Standard curve of Gallic Acid and Quercetin.



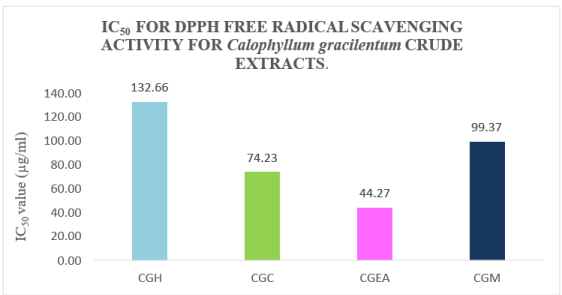


Figure 2. Total Phenolic Conten, total Flavonoid Content and DPPH Free Radical Scavenging Activity for Calophyllum gracilentum Crude extracts

This present study aims to investigate the phenolic and flavonoid content as well as the free radical scavenging activity in the *Calophyllum* species of Sarawak, namely *Calophyllum gracilentum*. The genus *Calophyllum* has about 180–200 species with some indigenous species confined to the Sarawak tropical rainforest in Malaysia. *Calophyllum* is well known for its medicinal uses and has been traditionally used for the treatment of potentially chronic diseases such as peptic ulcers, haemorrhoids, malaria, tumours, infections, high blood pressure, rheumatic disorders, pain, inflammation, and certain venereal diseases (Gupta & Gupta, 2020; Karunakaran et al., 2022; Zamakshshari et al., 2019). In Southeast Asia, especially in Malaysia, local communities have been using various parts of *C. inophyllum* and *C. soulattri* as a remedy for skin diseases, ulcers, rheumatism ailments, psoriasis, and eye infections (Dweck & Meadows, 2002; Shanmugapriya et al., 2017)

According to (Amarowicz & Pegg, 2019), the number of phenolic compounds in plants or extracts impacts their potential antioxidant capacity or the antioxidant activity of their derived products. *Calophyllum* species have been discovered to be a broad collection of plants with a vast variety of chemical profiles, including xanthones, coumarins, flavonoids, triterpenoids, and others. *Calophyllum*'s bioactive chemicals, in particular, are unique to the genus, and they have been utilised as chemical markers to distinguish various species within the genus (Aminudin et al., 2021; Hien et al., 2011; Jong, 2007; Lizazman et al., 2023; Mokhtar et al.). Numerous phytochemical and pharmacological research on *Calophyllum* species have identified interesting responsible bioactive phenolic compounds and their pharmacological properties with considerable biological activities (Gupta & Gupta, 2020; Zailan et al., 2022).

Thus, this study only focusses on the determination of the total phenolic content, total flavonoid content, and the free radical scavenging activity of the various extracts of the *Calophyllum gracilentum*, stem bark part using respective modified methods. *C. gracilentum* from the Sarawak Forest, is largely unexplored due to the limited information available compared to other *Calophyllum* species. While the plant has been identified and described, little research has been conducted, specifically on the possible bioactive chemicals found in this species. Therefore, *C. gracilentum* could have similar medicinal characteristics, but further research is needed to properly comprehend its potential uses and positive benefits.

Materials and Methods

Plant Materials

The stem barks of *C. gracilentum* were gathered from the Bau area, Sarawak under the management of Sarawak Forestry Corporation Sdn. Bhd. A voucher specimen (UITM3042) has been deposited at Universiti Teknologi MARA, Samarahan Campus, Sarawak. The stem barks of *C. gracilentum* were air-dried at room temperature which then chopped and ground to powder for extraction purpose.

Extraction

Powdered air-dried stem barks of *Calophyllum gracilentum* with a weight of 1.5 kg were extracted with solvents (2 L) (n-hexane, chloroform, ethyl acetate and methanol) by cold maceration for 72 hours at room temperature (twice) in a closed vessel. 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 dry vessels.

Total Phenolic Content (TPC)

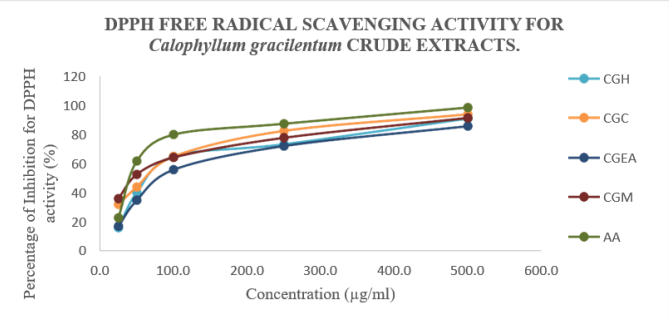
Folin–Ciocalteu method described by (Wani et al., 2019) was employed for the determination of total phenolics content with slight modifications. Three hundred microliters of the diluted extract were mixed with 2.25 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min. Then, 2.25 mL of 6% sodium carbonate (60 g/L) solution was added to the mixture and let set for 90 min at room temperature. The absorbance values were measured at 765 nm detection using a UV- Vis spectrophotometer (Lambda 25, Perkin Elmer, USA) spectrophotometer. Results were expressed as mg gallic acid equivalents in 1g of dried sample (mg GAE/g).

Total Flavonoid Content (TFC)

The TFC of the extracts was determined by using aluminium chloride colorimetric method as described by (Chukwumah et al., 2009) with a slight modification. Briefly, 5 mL of the sample was mixed with 5 mL of 2% aluminium chloride in a vial. The vial was shaken and left for 10 minutes. The analysis was carried out using UV-Vis spectrophotometer with detection of 415 nm by using quercetin (25, 50, 75, 100 and 125 µg/mL) as the reference standard. The results were expressed as mg QE/g extract.

Antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity Assay

The antioxidant activity was evaluated by free radical scavenging activity which was measured by 1,2-diphenyl-2-pricrylhydrazyl (DPPH). The DPPH is a stable free radical, thus the free radical-reducing activity of antioxidants based on the one-electron reduction evaluated by the scavenging of DPPH. In addition, the antioxidant potential of the test sample, which shows its effectiveness, prevention, interception, and repair mechanism against injury in a biological system, can also be determined by the scavenging of DPPH. This DPPH method was carried out according to (Blois, 1958) method as described by (Salleh et al., 2011) with slight modification. A weight of 6 mg of the sample was dissolved in ethanol as a stock solution. It was diluted to the concentrations of 50, 125, 250, 500 and 1000 µL/mL. 1 mL of 2,2-



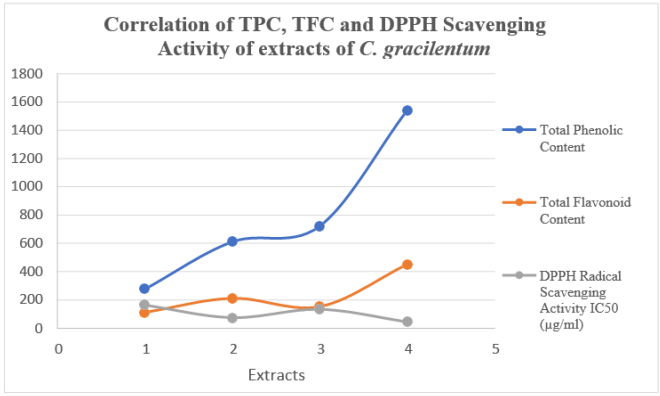


Figure 3. IC50 for DPPH Free Radical Scavenging Activity for Calophyllum gracilentum Crude extracts. And Correlation of TPC, TFC and DPPH Scavenging Activity of extracts of C. gracilentum. (Types of extract; 1= Hexane extract; 2 = Ethyl acetate extract; 3 = Chloroform extract; 4 = Methanolic extract)

Table 3. Total Phenolic Content, Total Flavonoid Content and DPPH Free Radical Scavenging Activity in the Crude Extracts of *Calophyllum gracilentum*. (CRH= *C. recurvatum* hexane extract, CREA = *C. recurvatum* ethyl acetate extract, CRC = *C. recurvatum* chloroform extract, CRM = *C. recurvatum* methanol extract, CGH =, *C. gracilentum* hexane extract, CGEA = *C. gracilentum* ethyl acetate extract, CGC = *C. gracilentum* chloroform extract, CGM = *C. gracilentum* methanol extract. The experiment was done in triplicate and the data expressed as mean ± SEM, with n = 3. Data within rows with a common superscript alphabet are not significantly different from others chemical at TPC ($p < 0.05$) and superscript number are not significantly different from others chemical at TFC ($p < 0.05$) (two-way ANOVA, followed by Tukey's test)).

Sample Extracts	Total Phenolic Content (mg GAE/ g extract)	Total Flavonoid Content (mg QE/ g extract)	DPPH Radical Scavenging Activity IC50 (µg/ml)
CGH	276.50 ± 0.0041^{a}	111.95 ± 0.0025^{1}	162.61± 2.24
CGEA	$610.80 \pm 0.0031^{b, d}$	211.40 ± 0.0044^{1}	132.66 ± 0.98
CGC	722.20 ± 0.0119^{d}	152.60 ± 0.0005^{1}	74.23 ± 0.85
CGM	1542.40 ± 0.0246^{e}	451.70 ± 0.0003^{3}	44.27 ± 1.20
Positive control	(Gallic acid) Y = 0.49X - 0.0256 R^2 = 0.979	(Quercitin) Y = 0.3591X - 0.0089 R^2 = 0.989	(Ascorbic Acid) 14.46 ± 2.88

diphenyl-1-picrylhydrazyl (DPPH) was added into a vial containing a mixture of 1 mL of sample solutions at different concentrations and 3 mL of ethanol. The solution mixture was allowed to react for 60 minutes. The analysis was carried out using UV-Vis's spectrophotometer Perkir-Elmer- Lambda 35 by measuring the absorbance with a detection of 517 nm with ethanol as blank, ascorbic acid as positive control and 1 mL ethanol plus 3 mL DPPH as a negative control. The radical scavenging activity is then described by the percentage of inhibition which is calculated using the following formula:

% inhibition= $[(Ab_C - Ab_S)/Ab_C]\times100\%$

Ab_C is the absorbance of negative control and Ab_S is the absorbance of samples. The results were expressed in IC_{50} value.

Statistical Analysis (regression)

The analysis of TPC, TFC, and DPPH scavenging activity was conducted using Microsoft Excel 365, and the data were presented as the mean standard deviation of three replicates. The significance of the differences between the data was ascertained using the two- way ANOVA and Tukey's comparison procedure. Significant p-values were defined as 0.05 or less.

Method validation

Detection and quantification limits (LOD and LOQ) are two fundamental elements of method validation. In this study, the LOD and LOQ was determined based on standard deviation of the response and the slope from the standard curve. Samples need to be taken in the range of the LOD and LOQ and not extrapolated into the range. Normally six or more determinations are made at five concentrations. The detection limits may be expressed as in the equation below:

LOD = 3.3 σ/Slope LOQ = 10

σ/Slope Where:

σ = the standard deviation of the response at low concentrations

Slope = the slope of the calibration curve.

The slope may be estimated from the calibration curve of the analyte. The estimate of σ is typically the root mean squared error (RMSE) or standard deviation of the residuals taken from the regression line. The slope is used to convert the variation in the response back to the scale of the theoretical concentration

Results

Extraction yield of *Calophyllum gracilentum*

This study proposed extraction by maceration techniques to obtain the crude extracts from stem bark parts of *C. gracilentum* . The percentage yield of extract is shown in Table 1.

The highest yield was obtained from methanol extract (200 g), followed by chloroform extract (150 g), n-hexane extract (100 g), and ethyl acetate extract (80 g) of dark and light yellowish viscous materials on solvent removal, respectively.

Standard Curve for Total Phenolic Content and Total Flavonoid Content

The total phenolic contents of *Calophyllum gracilentum* were estimated using the Folin–Ciocalteu method as described by (Wani et al., 2019). The crude extract's total phenolic content (TPC) was quantified based on the established calibration curve of the gallic acid as the standard ($y = 0.49x - 0.0256$, $R^2 = 0.979$) (Figure 1). The values obtained for the concentration of total phenolic contents are expressed as mg of GAE/g of extract. On the other hand, total flavonoid content (TFC), in the crude extract was quantified based on the calibration curve of the quercetin as the standard ($y = 0.3591x - 0.0089$, $R^2 = 0.989$) (Figure 1). The values obtained for the concentration of total phenolic contents are expressed as mg quercetin equivalents (QE)/g dry weight (DW) of extracts. All the samples were examined in triplicates and the results were tabulated in table.

Method Validation

Validation is the key factor in controlling the reliability of a method that is determined by validation results, where specificity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ), sensitivity and applicability are reported (Geetha et al., 2012; Ozkan, 2018). Validated analytical methods play a major role in achieving the quality and safety of the final product especially in pharmaceutical industry and in natural product analysis, environmental analysis, biomedical analysis, and life sciences, etc. In this study, the LOD and LOQ was determined based on standard deviation of the response and the slope from the standard curve and the calculation shown in Table 2.

Based on the results obtained, the total phenolic content (TPC) and total flavonoid content (TFC) were determined using spectrophotometric methods. The methanolic extracts for *C. gracilentum* exhibited the highest TPC with 1542.40 ± 0.0246 mg GAE/g extract Meanwhile, hexane extracts for *C. gracilentum* exhibited the lowest TPC with 276.50 ± 0.0041 mg GAE/g extract respectively. Compared to the ethyl acetate extracts and chloroform extracts, TPC ranged from 508.00 ± 0.0083 to 722.20 ± 0.0119 mg GAE/g extract. The results as tabulated in Table 3 and expressed in Figure 3.

Meanwhile, for the total flavonoid content (TFC) assay, the methanolic extract of *C. gracilentum* exhibited the highest TFC with 451.70 ± 0.0003 mg quercetin equivalents (QE)/g extract. In addition, hexane extract demonstrated TFC with 111.95 ± 0.0025 mg quercetin equivalents (QE)/g extract, as well as chloroform extract with 152.60 ± 0.0005 mg quercetin equivalents (QE)/g extract and ethyl acetate with 211.40 ±

0.0044 mg quercetin equivalents (QE)/g extract. The results as shown in Table 3 and Figure 4.

Antioxidant activity by Free Radical Scavenging activity using DPPH

The antioxidant potential of the test sample, which shows its effectiveness, prevention, interception, and repair mechanism against injury in a biological system, can also be determined by the scavenging of DPPH. This DPPH method was carried out according to Blois's (1958) method as described by Salleh et al. (2011). In this study, the antioxidant activity of different solvent crude extracts from the stem bark of *C. gracilentum* were evaluated in terms of inhibition concentration (IC_{50}). Ascorbic acid was used as positive control where the IC_{50} value was 14.46 ± 2.88 µg/mL and the experiment was repeated in triplicates. Based on the results, all the extracts revealed IC_{50} values ranging from 44.17 ± 1.26 µg/mL to 162.61± 2.24 µg/mL (Table 2). IC_{50} is inversely proportional to the antioxidant activity of a compound, which means that a lower IC_{50} value shows higher antioxidant activity.

The methanolic extract for *Calophyllum gracilentum* showed the highest antioxidant activity with an IC_{50} value of 44.27 ± 1.20 µg/mL. In addition, ethyl acetate and chloroform extracts showed IC_{50} values of 132.66 ± 0.98 µg/mL and 74.23 ± 0.85 µg/mL. Lastly, the hexane extract exhibited the lowest activity with an IC_{50} value of 162.61± 2.24 µg/mL for the evaluation of antioxidant activity for *Calophyllum gracilentum*.

However, all IC_{50} values obtained by the analysis of the DPPH activity for crude extracts showed potential antioxidant activity as the values are ranging for DPPH assay (IC_{50}>250 µg mL^{-1} (inactive); >100–250 µg mL^{-1} (weakly active); >50–100 µg mL^{-1} (moderately active); 10–50 µg mL^{-1} (strongly active) and <10 µg mL^{-1}, very strongly active (Jadid et al., 2017; Phongpaichit et al., 2007). The results were tabulated in Table 2, Figure 5, and Figure 6.

Discussion

Antioxidants are the body's natural first line of defense against dangerous chemicals identified as free radicals, and they must respond quickly to free radicals to stop biomolecules from getting damaged. With greater exposure to free radicals, the requirement for antioxidants becomes even more crucial. Free radicals and reactive oxygen species (ROS) are formed during normal oxidative metabolism and pathological conditions in addition to exogenous factors. The antioxidant is a substance that can delay a substrate's oxidation by inhibiting the propagation of oxidizing caused by free radicals. It plays an important role in preventing fats and oils from becoming rancid and protecting the human body from the detrimental effects of free radicals and ROS such as inflammation and cancer. Thus, consuming dietary antioxidants could be an important aspect of the body's defense mechanism to protect against free radicals and ROS.

Total antioxidant capacity refers to the overall ability of a food or its components to scavenge and neutralize free radicals or reactive oxygen species (ROS) in the body (El-Lateef et al., 2023). Meanwhile, total phenolic content (TPC) is a measure of the number of phenolic compounds, a large group of secondary metabolites found in plants, known for their antioxidant properties and potential health benefits. TPC is commonly used as an indicator of the antioxidant capacity of plant extracts, as phenolic compounds are known for their ability to scavenge free radicals and protect against oxidative stress.

In this present study, the findings indicated that *C. gracilentum* contain significant amounts of phenolic and flavonoid compounds, which highlighted the methanolic extracts showed the highest values for TPC, TFC and IC_{50} for DPPH assays. This result was supported by previous study reported on the methanol extract of *C. ferrugineum* showed the highest TPC value at 122.08 mg GAE/g and the lowest DPPH IC_{50} value at 11.80 µg/mL (Aminudin et al., 2019). Meanwhile, the recent study on the phytochemical screening on *Calophyllum innophyllum* showed that significant TPC (289.12 mg GAE/g) and TFC (410.4 mg QE/g) as well (Hapsari et al., 2022).

These data contribute to their antioxidant and potential medicinal properties. The TPC and TFC values of different plant species can vary widely depending on factors such as plant genetics, environmental conditions, and extraction methods, so it is important to consider these factors when comparing different studies.

Therefore, according to (Rabeta & Faraniza, 2013) high total phenolic content gives high antioxidant capacity due to the linear correlation between the two parameters. Polyphenolics exhibit a wide range of biological effects which have been attributed to their free radical scavenging activity and antioxidant activity (Tung et al., 2007).

Previously, there was no report on the antioxidant activity of *Calophyllum gracilentum*. Thus, from this study showed that this *Calophyllum* species are potent for further investigation development in producing antioxidant products since the test for DPPH radical scavenging activity gave good positive results. In considering the facts that phenolics possess a broad spectrum of interesting biological activities including cytotoxic, anti-inflammatory, antioxidant, anti-bacterial, neuroprotective, anti-HIV, enzyme inhibition, hypoglycemic and others. This was due to the radical scavenging ability of these molecules' which is responsible for most of the added therapeutic values (Salman et al., 2019).

Conclusion

This study involved testing crude extracts *C. gracilentum* species for their biological activities tested included the total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The results showed that *C.gracilentum* extracts contain ranging from moderate to high levels of phenolic and flavonoid compounds, which are known for their antioxidant properties. The DPPH free radical scavenging assay showed that the compounds exhibited significant antioxidant activity. Overall, these preliminary findings will provide beneficial reference for future research especially in phytochemistry study and on their potential medicinal value highlighting the importance of further investigation and development for therapeutic applications.

Author Contributions

Contributed to writing—original draft preparation resources, N.M.U.S; Conceptualization writing, supervision, and funding acquisition, V.Y.M.J.; Conceptualization writing and supervision, N.H.Z. and T.K.; Review and editing, L.F.K., W.T.Z. and C.T.H. All authors have read and agreed to the published version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

References

Amarowicz, R., & Pegg, R. B. (2019). Chapter One - Natural antioxidants of plant origin. In I. C. F. R. Ferreira & L. Barros (Eds.), Advances in Food and Nutrition Research (Vol. 90, pp. 1-81). Academic Press. https://doi.org/https://doi.org/10.1016/bs.afnr.2019.02.011

Aminudin, N. I., Ahmad, F., & Taher, M. (2019). Antibacterial and antioxidant activities of extracts from Calophyllum ferrugineum and Calophyllum incrassatum. Malaysian Journal of Analytical Sciences, 23(4), 637-647.

Aminudin, N. I., Norazhar, N. A., & Ahmad, F. (2021). Development of a HPLC Method for Quantification of Amentoflavone in Leaf Extracts of Three Calophyllum Species. Malaysian Journal of Chemistry, 23(2), 33-39.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181(4617), 1199-1200.

Chukwumah, Y. C., Walker, L. T., Verghese, M., & Ogutu, S. (2009). Effect of frequency and duration of ultrasonication on the extraction efficiency of selected isoflavones and trans-resveratrol from peanuts (Arachis hypogaea). Ultrasonics sonochemistry, 16(2), 293-299.

Dragusha, S., Qazimi, B., & Ejupi, V. (2023). Assessment of Total Phenolic and Flavonoid Content of Medicinal Plant Extracts from Kosovo. Journal of Medicinal plants and By-product, 12(2), 175-180. https://doi.org/10.22034/jmpb.2023.361637.1538

Dweck, A., & Meadows, T. (2002). Tamanu (Calophyllum inophyllum)-the African, Asian, polynesian and pacific panacea. International Journal of Cosmetic Science, 24(6), 341-348.

El-Lateef, H. M. A., El-Dabea, T., Khalaf, M. M., & Abu-Dief, A. M. (2023). Recent Overview of Potent Antioxidant Activity of Coordination Compounds. Antioxidants, 12(2), 213.

Geetha, G., Raju, K. N. G., Kumar, B. V., & Raja, M. G. (2012). Analytical method validation: an updated. Review. Int. J. Pharm. Biol. Sci, 1, 64-71.

Gupta, S., & Gupta, P. (2020). The Genus Calophyllum: Review of Ethnomedicinal Uses, Phytochemistry and Pharmacology. In (pp. 215-242). Springer Singapore. https://doi.org/10.1007/978-981-15-1394-7_5

Hapsari, S., Yohed, I., Kristianita, R. A., Jadid, N., Aparamarta, H. W., & Gunawan, S. (2022). Phenolic and flavonoid compounds extraction from Calophyllum inophyllum leaves. Arabian Journal of Chemistry, 15(3), 103666. https://doi.org/https://doi.org/10.1016/j.arabjc.2021.103666

Hien, H. M., Heng, P. W., Van Thi, N., Cu, N. K. Q., & Hue, V. T. B. (2011). Development and validation of a GC-MS method for rapid determination of calophyllolide in Calophyllum inophyllum L.: A quality control approach. Chinese Journal of Natural Medicines, 9(6), 429-434.

Jadid, N., Hidayati, D., Hartanti, S. R., Arraniry, B. A., Rachman, R. Y., & Wikanta, W. (2017). Antioxidant activities of different solvent extracts of Piper retrofractum Vahl. using DPPH assay. AIP conference proceedings,

Jong, V. Y. M. (2007). Chemical constituents from bintangor (Calophyllum inophyllum) and geronggang (Cratoxylum arborescens) and their biological activities Universiti Putra Malaysia].

Karunakaran, T., Firouz, N. S., Santhanam, R., & Jong, V. Y. M. (2022). Phytochemicals from Calophyllum macrocarpum Hook. f. and its cytotoxic activities. Natural Product Research, 36(2), 654-659.

Lizazman, M. A., Karunakaran, T., & Jong, V. Y. M. (2022). Trapezifolixanthone as a common constituent in the genus Calophyllum: An insight Review. Biocatalysis and Agricultural Biotechnology, 44, 102471. https://doi.org/https://doi.org/10.1016/j.bcab.2022.102471

Mokhtar, N., Karunakaran, T., Santhanam, R., Abu Bakar, M. H., & Jong, V. Y. M. Phenolics and triterpenoids from stem bark of Calophyllum lanigerum var. austrocoriaceum (Whitmore) P. F. Stevens and their cytotoxic activities. Natural Product Research, 1-6. https://doi.org/10.1080/14786419.2023.2196075

Ozkan, S. A. (2018). Analytical method validation: the importance for pharmaceutical analysis. Pharm Sci, 24(1), 1-2.

Phongpaichit, S., Nikom, J., Rungjindamai, N., Sakayaroj, J., Hutadilok-Towatana, N., Rukachaisirikul, V., & Kirtikara, K. (2007). Biological activities of extracts from endophytic fungi isolated from Garcinia plants. FEMS Immunology & Medical Microbiology, 51(3), 517-525. https://doi.org/10.1111/j.1574-695X.2007.00331.x

Rabeta, M., & Faraniza, R. N. (2013). Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of Garcinia atrovirdis and Cynometra cauliflora. International Food Research Journal, 20(4), 1691.

Salleh, W. M. N. H. W., Ahmad, F., Yen, K. H., & Sirat, H. M. (2011). Chemical compositions, antioxidant and antimicrobial activities of essential oils of Piper caninum Blume. International Journal of Molecular Sciences, 12(11), 7720-7731.

Salman, Z., Yu-Qing, J., Bin, L., Cai-Yun, P., Iqbal, C. M., Atta-ur, R., & Wei, W. (2019). Antioxidant nature adds further therapeutic value: an updated review on natural xanthones and their glycosides. Digital Chinese Medicine, 2(3), 166-192.

Shanmugapriya, n., Chen, Y., Kanwar, J. R., & Sasidharan, S. (2017). Anticancer Activity and Molecular Mechanism of Polyphenol Rich Calophyllum inophyllum Fruit Extract in MCF-7 Breast Cancer Cells. Nutrition and Cancer, 69(8), 1308-1324. https://doi.org/10.1080/01635581.2017.1367944

Tung, Y.-T., Wu, J.-H., Kuo, Y.-H., & Chang, S.-T. (2007). Antioxidant activities of natural phenolic compounds from Acacia confusa bark. Bioresource technology, 98(5), 1120-1123.

Wani, M. A., Prasad, S., & Prakash, O. (2019). Qualitative phytochemical analysis of various parts of bamboo (Bambusa balcooa) for possible therapeutic usages in bovine reproductive disorders. Journal of Pharmacognosy and Phytochemistry, 8(1), 217-221.

Zailan, A. A. D., Karunakaran, T., Abu Bakar, M. H., & Mian, V. J. Y. (2022). The Malaysian genus Calophyllum (Calophyllaceae): a review on its phytochemistry and pharmacological activities. Natural Product Research, 36(17), 4569-4579.

Zamakshshari, N. H., Ee, G. C. L., Ismail, I. S., Ibrahim, Z., & Mah, S. H. (2019). Cytotoxic xanthones isolated from Calophyllum depressinervosum and Calophyllum buxifolium with antioxidant and cytotoxic activities. Food and Chemical Toxicology, 133, 110800. https://doi.org/10.1016/j.fct.2019.110800