Original Article

Pharmaceutical Sciences and Research (PSR), 11(1), 2024, 34 - 40

Antioxidant Activities and Protective Effects of *Shorea macrophylla* Leaf and Bark Extracts

Salfarina Ramli[1,2], Nik Nor Sabrina Sukri[2], Nur Adhwa' Faqihah Zulkifli[2], Donna Maretta Ariestanti[3], Chee Yan Choo[2], Hasseri Halim[1,2], Nurhuda Manshoor[2], Richard Johari James[1,2*]

[1]Integrative Pharmacogenomics Institute, Universiti Teknologi MARA Cawangan Selangor, Puncak Alam, Selangor, Malaysia
[2]Faculty of Pharmacy, Universiti Teknologi MARA Cawangan Selangor, Puncak Alam, Selangor, Malaysia
[3]Faculty of Pharmacy, Universitas Indonesia, Depok, West Java, Indonesia

ABSTRACT

Shorea macrophylla, also known as the '*Engkabang*' tree or Light Red *Meranti*, is renowned for its role in reforestation efforts. Despite lacking records of traditional use, the reported biological activities of other species within the Shorea genus spark curiosity about the potential biological activities of this plant. Therefore, this study aims to explore the antioxidant capabilities of *S. macrophylla* leaf and bark extracts, along with their protective effects against oxidative stress using a brine shrimp model. In the evaluation of antioxidant potential, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays were employed. Extracts were incubated in DPPH and FRAP solutions, and absorbances were measured at 517 and 593 nm, respectively. The brine shrimp lethality assay (BSLT) involved exposing brine shrimps (*Artemia salina*) to various concentrations of the extract, with LC_{50} values determined through probit regression analysis. Oxidative stress protection assays entailed treating brine shrimps with safe doses of *S. macrophylla* extracts before exposure to H_2O_2, with subsequent observation of survival rates. The DPPH assay unveiled IC_{50} values of 1.025 and 0.693 mg/mL for *S. macrophylla* leaf and bark extracts, respectively, while FRAP values exhibited a concentration-dependent relationship. BSLT demonstrated concentration-dependent mortality, with LC_{50} values of 0.93 and 0.6455 mg/mL for *S. macrophylla* leaf and bark extracts, respectively. Pre-treatment with *S. macrophylla* extracts significantly increased brine shrimp survival against H_2O_2-induced oxidative stress. In conclusion, both *S. macrophylla* leaf and bark water extracts demonstrated noteworthy antioxidant activities and exhibited protective effects against oxidative stress in brine shrimps. These findings provide insights into the antioxidant activities and protective effects of *S. macrophylla* Leaf and Bark extracts.

ARTICLE HISTORY

Received: February 2024
Revised: April 2024
Accepted: April 2024

Keywords: *Shorea macrophylla; antioxidant activities; brine shrimp model; oxidative stress protection*

**corresponding author*
Email: richard@uitm.edu.my

INTRODUCTION

Shorea macrophylla de Vriese, known as the '*Engkabang*' tree and Light Red *Meranti*, respectively, are prominent members of the Shorea genus within the Dipterocarpaceae family (Fajri et al., 2020). It thrives in the tropical rainforests of Borneo, Malaysia, Indonesia, and the Philippines, offering local communities a valuable resource for timber and fat-rich illipe nuts (Chew et al., 2022; Fajri et al., 2020). The ripe illipe nuts are widely utilized as a replacement for cocoa butter fat and are a favoured food source for wildlife. Its edible fat is highly valued for its moisturizing properties and is used in the treatment of skin conditions (Kamal et al., 2010). The nuts are characterized by their high oil content, predominantly comprising of beneficial unsaturated triglycerides (Chew et al., 2022). *S. macrophylla* is not only used as a reforestation plant due to its rapid growth, but also contributes to the sustenance of wildlife, such as *Tor tambroides* which is a species of mahseer fish native to Southeast Asia (Chew et al., 2022).

Being an endemic species, previous studies on *S. macrophylla* have concentrated on its critical ecological aspects such as sustaining its growth, facilitating reforestation activities, and fostering canopy closure. However, the presence of stilbene resveratrol oligomers in various Shorea species and their antioxidant properties (Rammohan et al., 2020) has been reported. Furthermore, stilbenes, particularly resveratrol, have gained prominence due to their diverse health-promoting properties, spanning anticancer, anti-inflammatory, anti-aging, antioxidant, antimicrobial, and anti-atherogenic effects (Teka et al., 2022). Although, the investigations into the traditional medicinal properties of *S. macrophylla* have remained conspicuously sparse, it hints promise for uncovering valuable therapeutic benefits that could lead to new pharmaceuticals of natural products.

Therefore, this study aims to explore the antioxidant activities of *S. macrophylla* leaf and bark extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays.

Copyright 2024 by Authors. Published by Universitas Indonesia. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original author, and source are properly cited.

Additionally, this study investigates the protective effects of these extracts against oxidative stress using brine shrimp embryos as a model organism. The brine shrimp lethality test (BSLT) serves as a valuable bioassay for monitoring the biological activities of *S. macrophylla*, offering insights into intrinsic toxicity and acute overdose effects (Hamidi et al., 2014; Waghulde et al., 2019). The choice of using a crustacean model, the brine shrimp, not only provides a convenient system but also avoids the ethical complications associated with vertebrate toxicity experiments (Chan et al., 2021; Lima et al., 2022). This exploration seeks to explore the biological activities of *S. macrophylla* leaf and bark extracts.

METHODS

Plant Material Preparation

The dried leaves and bark of *S. macrophylla* were provided by Forest Research Institute Malaysia (FRIM). The plant species was authenticated by Dr. Rasadah Mat Ali, a certified phytochemist. Voucher specimens (FR2861) were deposited in FRIM herbarium. All samples were kept in dry and cold storage room with limited light penetration.

Shorea macrophylla Leaf and Bark Extracts

Freshly harvested bark (0.6 kg) and leaf (2.6 kg) materials of *S. macrophylla* were chopped into thin flakes, and dried in an oven at 60°C until a constant weight was achieved. The dried samples were then reduced into coarse powder using a commercial grinder. Prior to the actual extraction using water/acetone mixture, the plant materials were pre-treated to remove unwanted component in the sample. The leaf powder was extracted with petroleum ether to remove chlorophyll and other pigments, while the bark was extracted with *n*-hexane overnight at room temperature to remove nonpolar constituents. The extraction processes were performed by maceration with respective solvents, in three cycles (1.0 L) of one day each. The treated plant materials were air-dried, and later exhaustedly extracted with a water/acetone mixture (70:30) by maceration for 20 hours and lixiviation with fresh solvent for 4 hours. The extracts for all maceration and lixiviation processes were combined and filtered using Whatman® no.1 filter paper. The filtered solution was reduced under pressure at 40°C until evaporated to dryness. The process yielded dried extracts of bark (97 g) and leaf (45 g).

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity

The free radical scavenging activity of *S. macrophylla* leaf and bark extracts were determined using DPPH assays described by Izzati et al. (2020) with slight modifications. The DPPH powder was dissolved in ethanol to produce 2.0 mM DPPH solution. Five different concentrations of *S. macrophylla* leaf and bark extracts (0.4, 0.8, 1.2, 1.6, 2.0) mg/mL were utilised as sample concentrations. Subsequently, five different concentrations of ascorbic acid (0.4, 0.8, 1.2, 1.6, 2.0) mg/mL and distilled water were prepared and used as positive control and blank solution, respectively. Each sample were mixed with DPPH solution and the mixtures were shaken vigorously and left incubated for 30 minutes in the dark at room temperature. Then its absorbance was measured at 517 nm using a microplate reader. The DPPH radical scavenging activity were expressed as the percentage of inhibition of the DPPH according to expression:

$$I\,\% = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$$

Where, is the absorbance of control and is the absorbance of the extract. The IC_{50} value which is the concentration of the sample required to inhibit 50% of the radical were calculated to determine the radical scavenger activity (Jadid et al., 2017). The IC_{50} value for each sample were calculated using linear regression analysis. The DPPH assays were performed in triplicates.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay were carried out following the method described by Ahmouda et al. (2022) with slight modifications. The FRAP solution was freshly prepared using a 10:1:1:1 ratio mixture of acetate buffer, 2,4,6-Tripyridyl-S-triazine (TPTZ), ferric chloride solution and distilled water. Five different concentrations (0.4, 0.8, 1.2, 1.6, 2.0) mg/mL of *S. macrophylla* leaf and bark extracts and ascorbic acid were prepared via serial dilution. A total of 3 µL of each concentration of *S. macrophylla* leaf and bark extracts and ascorbic acid were transferred into a well plate. Then, 100 µL of FRAP solution was added into the well plate. After 30 minutes of incubation in the dark at room temperature, the absorbance of each well plate was measured at 593 nm using a microplate reader. The mean FRAP values were calculated for each concentration of *S. macrophylla* leaf and bark extracts using ascorbic acid equivalent (µg of AAE/ µg of *S. macrophylla* leaf or bark extracts), which described the reducing power of *S. macrophylla* leaf and bark extracts. The FRAP assay was conducted in triplicate.

Brine Shrimp Lethality Test

The brine shrimp lethality test was conducted to assess the toxicity of *S. macrophylla* extracts following the methodology outlined in James et al. (2023) with slight modifications. Five different concentrations of the extracts (0.03125, 0.0625, 0.125, 0.5, and 1.0 mg/mL) were prepared in triplicate using serial dilutions with saltwater. Each well of a 24-well plate contained 10 nauplii and 1 mL of the corresponding extract. Negative

control wells included 10 nauplii and 1 mL of saltwater, while positive control wells contained 10 nauplii and 1 mL of 0.2 mg/mL potassium dichromate. Following exposure to the extracts, the plate was incubated at 28°C for 24 hours. Mortality was determined by counting the number of dead nauplii in each well after incubation. This triplicate experiment ensured the reliability and reproducibility of the obtained data.

Oxidative Stress Protection Assay

The oxidative stress protection assay was conducted according to methodology outlined in James et al. (2023) with slight modifications. In brief, nauplii were obtained similarly to the brine shrimp lethality test, and five safe concentrations of the *S. macrophylla* extracts were chosen based on the lethality test results. The *S. macrophylla* extracts were dissolved in salt water, and ten nauplii were exposed to 1 mL of the solution in each well. The control group received 1 mL of salt water. The well plate was then incubated at 28°C for one hour. Following the 1-hour exposure to *S. macrophylla* extracts, the nauplii were subjected to a solution of H_2O_2 diluted in salt water for 24 hours, with the concentration of H_2O_2 selected based on the LC_{50} value in brine shrimps. The well plate was then incubated at 28°C, and the survival of the nauplii was observed under a microscope after the 24-hour incubation period. The experiment was performed in triplicate.

Statistical Analysis

One-way ANOVA and t-tests were employed to assess mean differences between groups using GraphPad Prism 9. A significance level (α) of 0.05 was used, meaning p-values lower than 0.05 were considered statistically significant. Additionally, probit regression analysis was performed in Microsoft Excel using Finney's table to convert mortality percentages to probit values. The IC_{50} and LC_{50} were determined by analysing the best-fit line from the regression analysis (Hamidi et al., 2014).

RESULTS AND DISCUSSION

Antioxidant Activity of *Shorea macrophylla* Leaf and Bark Extracts

The DPPH assay absorbance for each well-plate was measured at 517 nm to assess the DPPH free radical scavenging potential of *S. macrophylla* extracts. In this assay, an antioxidant donates a hydrogen atom to the DPPH radical (DPPH•), causing a color change from violet to pale yellow (Chen et al., 2020). Figure 1 demonstrates a positive correlation between *S. macrophylla* extract/ascorbic acid concentration and DPPH inhibition. Results were expressed as the percentage of inhibition, and the calculated IC_{50} values were 1.02 mg/mL, 0.68 mg/mL, and 0.70 mg/mL for *S. macrophylla* leaf extract, *S. macrophylla* bark extract, and ascorbic acid, respectively. A lower IC_{50} value indicates higher antioxidant potency (Olugbami et al., 2015). Interestingly, the *S. macrophylla* bark extract's slightly lower IC_{50} value suggests a slightly greater antioxidant capacity compared to ascorbic acid. This finding could be attributed to the high phenolic content and free radical-scavenging activity observed in stem bark extracts of *Shorea* spp. (Subramanian et al., 2013), along with the high flavonoid content found in *S. macrophylla* water bark extract (Munteanu & Apetrei, 2021).

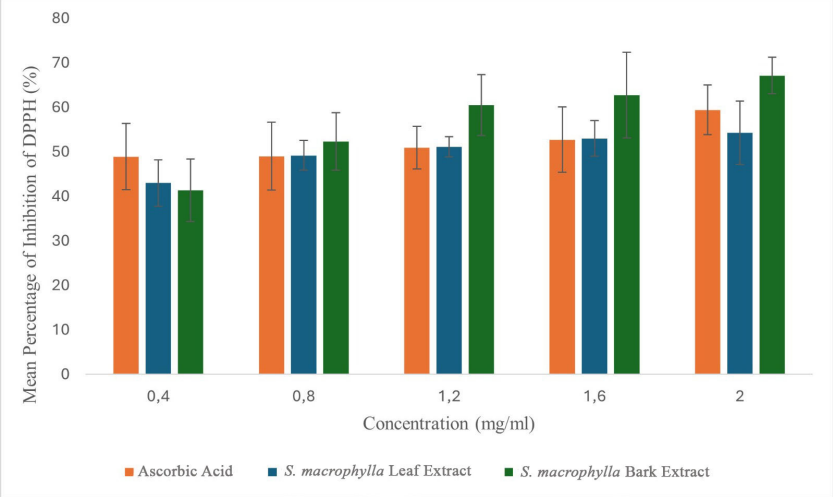

Figure 1. Antioxidant activities of ascorbic acid and *S. macrophylla* leaf and bark extracts in several concentrations measured by DPPH assay

There were no significant differences between the *S. macrophylla* extracts and ascorbic acid of the same concentration ($p>0.05$).

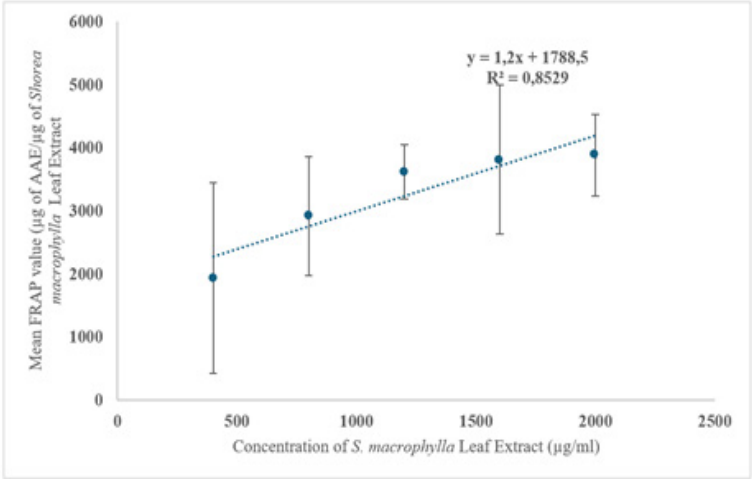

Figure 2. Antioxidant activities of *S. macrophylla* leaf extract in several concentrations measured by FRAP assay

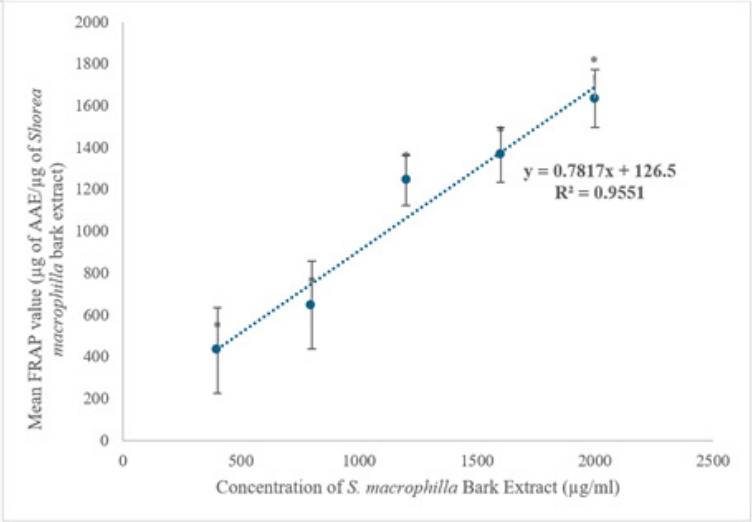

Figure 3. Antioxidant activities of *S. macrophylla* bark extract in several concentrations measured by FRAP assay

In FRAP assays, the antioxidant activity of *S. macrophylla* extracts was assessed by its ability to neutralize ferric ions. Figure 2 displays that the FRAP values had a linear relationship with the *S. macrophylla* extract concentration. The *S. macrophylla* leaf and bark extracts showed higher reduction capacity compared to ascorbic acid, as illustrated in Figures 2 and 3, respectively. The relationship between the concentration of *S. macrophylla* extracts and FRAP values observed in this study suggests that the extract possesses a high potential to reduce ferric ions, making it an effective antioxidant agent. According to Ahmouda et al. (2022), the antioxidant's FRAP power in the aqueous extract acts as a reductant of Fe^{3+} to Fe^{2+} cations. This leads to the conclusion that FRAP of plant extract affects the amount of Fe^{3+} reduced to Fe^{2+}. An increase in FRAP causes an increase in the amount of Fe^{2+} reduced from Fe^{3+}. The reducing power of a compound may be a good predictor of its potential antioxidant activity (Subramanian et al., 2013). The similar observation can be seen from previous study where the presence of antioxidants in the methanolic and acetone extracts of *Shorea roxburghii* stem bark results in the reduction of Fe^{3+} complex to its form. Subramanian et al. (2013) also reported that the increase of the phenolic content of the extract as well as the extract concentrations increased the reducing power. This implies that these extracts have a significant ability to react with free radicals in order to convert them into more stable nonreactive species and disrupt the chain reaction (Subramanian et al., 2013). Similarly, Vashisht et al. (2016) also reported that with increasing dosage, the reducing power of methanol extract of *Shorea robusta* resin increased. All doses exhibited significantly higher activities than the control, indicating that *Shorea robusta* contains hydrophilic polyphenolic compounds that cause the higher reducing power (Vashisht et al., 2016).

Oxidative Stress Protection in Brine Shrimps

The toxicity of *S. macrophylla* extracts was assessed using BSLT. In the BSLT assay, it was observed that the *S. macrophylla* extracts exhibited toxic effects in a dose-dependent manner in which the mortality of *Artemia salina* was directly proportional to the increasing concentration of the extracts (Figure 4). The LC_{50} value obtained were 0.93 mg/mL and 0.64 mg/mL for the leaf and bark extracts, respectively. In general, the lower the LC_{50}, the higher the toxicity of the analysed element. The results indicate that the *S. macrophylla* extracts are weakly toxic according to Clarkson's toxicity indices. Referring to the Clarkson classification, substances with an $LC_{50} > 1$ mg/mL are considered non-toxic, 0.5 mg/mL $< LC_{50} < 1$ mg/mL are considered weakly toxic, and an $LC_{50} < 0.1$ mg/mL are extremely toxic (Konan et al., 2022). Other toxicity studies on the Shorea genus, specifically on *Shorea robusta* leaf extracts (both aqueous and methanol), have been conducted on various groups of mice and male Wistar rats. These studies revealed that the extract was non-toxic to rats when administered orally (0-2500 mg/kg) or by intraperitoneal injection (0-1000 mg/kg). Over 14 days, treated animals showed no signs of toxic effects or mortality. This suggests a strong safety profile for both aqueous and methanol extracts (Debprasad et al., 2012). The LD_{50} of the orally methanolic and aqueous extracts of *Shorea robusta* was determined as 2.4 gm/kg and 2.7 gm/kg, while it was 1.2 gm/kg and 1.4 gm/kg in intraperitoneal, respectively. Hence the extract was classified as nontoxic. Moreover, further *in vivo* toxicological study for 21 days with aqueous extract up to 1200 mg/kg orally did not induce mortality or clinical toxicity or reveal any histopathological changes in kidney, liver and spleen (Debprasad et al., 2012). Cytotoxic activity was observed in other *Shorea* genus which were *Shorea macroptera* Dyer. In the study, four resveratrol oligomers isolated from the acetone extract of *Shorea macroptera* namely ε-viniferin, laevifonol, davidiol, stenophyllol B, and hemsleyanol D were assayed against three cell lines namely HeLa, HL-60, MCF-7 using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium Bromide (MTT) assay. It was found that ε-viniferin and davidiol displayed strong cytotoxic effects against HL-60 cell line with the IC_{50} 9.0 and 9.1 µg/mL respectively (Atikah et al., 2012). Previous studies highlight the crucial role of solvent type during extraction. Different solvents yield varying quantities and qualities of compounds within crude extracts. This explains why extracts may share similar compounds but exhibit different toxicity levels due to variations in the proportions and types of those compounds (Konan et al., 2022).

The oxidative stress protection assay (Figure 5) demonstrates a positive correlation between extract concentration and *Artemia salina* survival. This suggests a dose-dependent protective effect of *S. macrophylla* extracts against hydrogen peroxide-induced oxidative stress. Compared to the control (treated with saltwater and H_2O_2), both *S. macrophylla* leaf and bark extracts led to statistically significantly lower mortality at 0.06 mg/mL and 0.12 mg/mL. This protective effect could be attributed to flavonoids and phenolic compounds present in the extracts. Previous research on *Shorea tumbuggaia* bark found a linear relationship between increased antioxidant activity and higher phenolic content (Rammohan et al., 2020). Importantly, solvent choice plays a significant role in extracting polyphenolics that contribute to the protective effect and antioxidant activity (as measured by DPPH and FRAP assays). Methanol and acetone, for example, tend to extract different polyphenolics, with methanol favouring flavonoids and terpenoids, and acetone favouring stilbene derivatives.

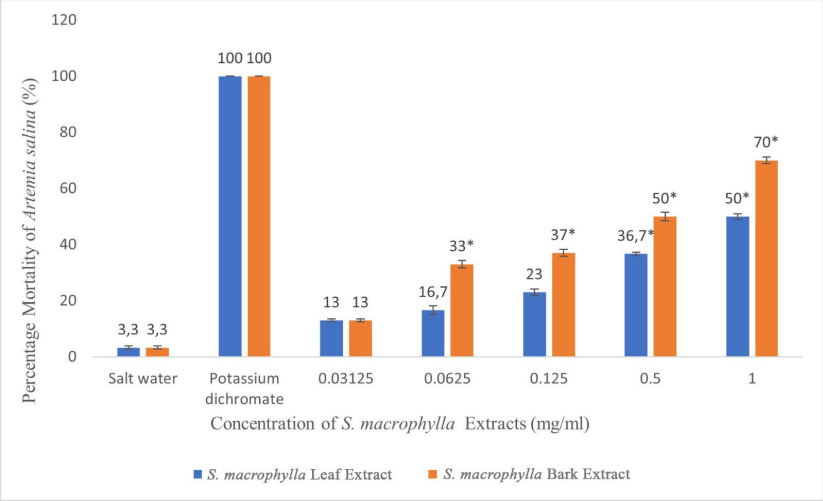

Figure 4. The toxicity assay of *S. macrophylla* leaf and bark extract using brine shrimp (*Artemia salina*)

*indicates statistically significantly difference ($p<0.05$) compared to salt water.

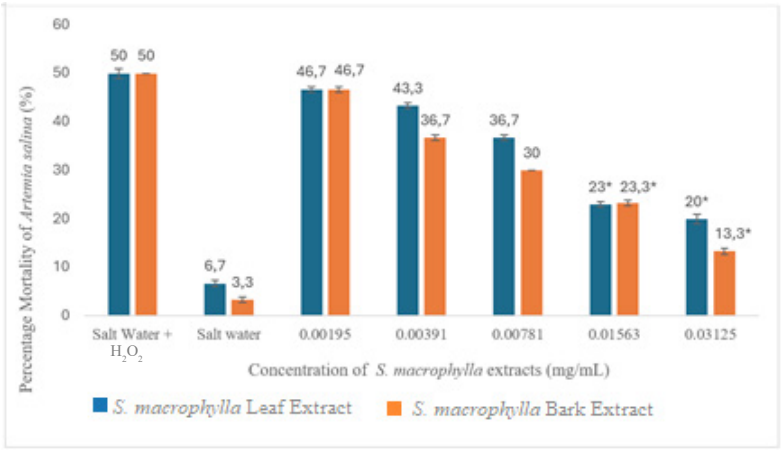

Figure 5. The oxidative stress protection assay of *S. macrophylla* leaf and bark extract using brine shrimp (*Artemia salina*)

*indicates statistically significantly difference ($p<0.05$) compared to the group of salt water with addition of H_2O_2

CONCLUSION

In conclusion, both *S. macrophylla* leaf and bark extracts demonstrate antioxidant activity and protective effects against oxidative stress in brine shrimps. The *S. macrophylla* bark extract, while effective in this regard, exhibited moderate toxicity towards the test organisms. Further research is necessary to identify the specific mechanisms underlying the observed antioxidant and protective actions of both *S. macrophylla* leaf and bark extracts. These findings support the potential use of *S. macrophylla* extracts as natural antioxidant agents in medicine and traditional treatments, pending additional studies to confirm mechanisms and safety.

ACKNOWLEDGMENT

The research was financially supported by Universiti Teknologi MARA through the Supervisory Research Grant (600-RMC/GIP 5/3 (128/2023)).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ahmouda, K., Benhaoua, B., & Eddine, S. (2022). Plant extract FRAP effect on cation vacancies formation in greenly synthesized wüstite (Fe x O) nanoparticles : A new contribution. *Sustainable Chemistry and Pharmacy*, *25*(August 2021), 100563. https://doi.org/10.1016/j.scp.2021.100563

Atikah, N. A., Nazri, M., Ahmat, N., Abdullah, M., Sidik, N. J., Tajudin, S. A., & Johari, T. (2012). Antioxidant, antimicrobial and cytotoxic activities of resveratrol oligomers of *Shorea macroptera* Dyer. *Australian Journal of Basic and Applied Sciences*, *6*(8), 431–436.

Chan, W., Shaughnessy, A. E. P., van den Berg, C. P., Garson, M. J., & Cheney, K. L. (2021). The validity of brine shrimp (*Artemia* Sp.) toxicity assays to assess the ecological function of marine natural products. *Journal of Chemical Ecology*, *47*(10–11), 834–846. https://doi.org/10.1007/s10886-021-01264-z

Chen, X., Liang, L., & Han, C. (2020). LWT - Food Science and Technology Borate suppresses the scavenging activity of gallic acid and plant polyphenol extracts on DPPH radical: A potential interference to DPPH assay. *LWT - Food Science and Technology*, *131*(February), 109769. https://doi.org/10.1016/j.lwt.2020.109769

Chew, I. Y. Y., Chung, H. H., Lau, M. M. L., Wee, B. S., & Sim, S. F. (2022). *Shorea macrophylla*: Overview of Illipe Nut Producing Tree. *Pertanika Journal of Tropical Agricultural Science*, *45*(3), 663–675. https://doi.org/10.47836/pjtas.45.3.08

Debprasad, C., Hemanta, M., Paromita, B., Durbadal, O., Kumar, K. A., Shanta, D., Kumar, H. P., Tapan, C., Ashoke, S., & Sekhar, C. (2012). Inhibition of NO 2, PGE 2, TNF-α, and iNOS EXpression by *Shorea robusta* L.: An ethnomedicine used for anti-inflammatory and analgesic activity. *Evidence-Based Complementary and Alternative Medicine*, *2012*. https://doi.org/10.1155/2012/254849

Fajri, M., Pratiwi, & Ruslim, Y. (2020). The characteristics of *Shorea macrophylla*'s habitat in Tane' Olen, Malinau District, North Kalimantan Province, Indonesia. *Biodiversitas*, *21*(8), 3454–3462. https://doi.org/10.13057/biodiv/d210806

Hamidi, M. R., Jovanova, B., & Panovska, K. (2014). Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina* L.) model. In *Macedonian pharmaceutical bulletin* (Vol. 60, Issue 1).

Izzati, N., Zakaria, A. B., & Ramli, S. B. (2020). Determination of total phenolic content, antioxidant and monophenolase inhibition activities of *Carica papaya* peel extracts in solvents with different polarity. *International Journal of Pharmaceuticals, Nutraceuticals and Cosmetic Science*, *1*(March), 16–25.

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*, *1854*. https://doi.org/10.1063/1.4985410

James, R. J., Halim, H., Mursyidul, M., Nasir, A., & Huda, F. (2023). Protective effect of *Carica papaya* leaves against oxidative stress in brine shrimps. *International Journal of Public Health Science*, *12*(3), 1070–1077. https://doi.org/10.11591/ijphs.v12i3.22494

Konan, A. M. L., Golly, K. J., Kra, A. K. M., Adima, A. A., & Lohoues, E. E. C. (2022). Phytochemical screening and toxicity assessment of *Imperata cylindrica* (L.) P. Beauv. (Poaceae) raw extracts with brine shrimp (*Artemia salina*) lethality assay. *Journal of Biosciences and Medicines*, *10*(08), 153–171. https://doi.org/10.4236/jbm.2022.108014

Kamal, I., Masseat, K., Poh, K. M., Eng, T. Y., Basir, S., Puteh, N., ... & Noran, N. (2010). Physical and mechanical assessments of fire retardant-treated *Shorea macrophylla* and *Acacia mangium* particleboards. *Modern Applied Science*, 4(7), 3. https://doi.org/10.5539/mas.v4n7p3

Lima, W. G., Santos, L. B., Nizer, W. S. da C., Castilho, R. O., & Brito, J. C. M. (2022). Brine shrimp (*Artemia salina Leach*) as an alternative model for assessing the *in vivo* antioxidant activity of rutin. *Brazilian Journal of Health and Pharmacy*, *4*(1), 39–44. https://doi.org/10.29327/226760.4.1-4

Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. In *International Journal of Molecular Sciences* (Vol. 22, Issue 7). https://doi.org/10.3390/ijms22073380

Olugbami, J., Gbadegesin, M., & Odunola, O. (2015). *In vitro* evaluation of the antioxidant potential, phenolic and flavonoid contents of the stem bark ethanol extract of *Anogeissus leiocarpus*. *43*(Suppl 1), 101–109.

Rammohan, A., Bhaskar, B. V., Camilo, A., Gunasekar, D., Gu, W., & Zyryanov, G. V. (2020). *In silico*, *in vitro* antioxidant and density functional theory based structure activity relationship studies of plant polyphenolics as prominent natural antioxidants. *Arabian Journal of Chemistry*, *13*(2), 3690–3701. https://doi.org/10.1016/j.arabjc.2019.12.017

Subramanian, R., Subbramaniyan, P., & Raj, V. (2013). Antioxidant activity of the stem bark of *Shorea roxburghii* and its silver reducing power. *SpringerPlus*, *2*(1), 28. https://doi.org/10.1186/2193-1801-2-28

Teka, T., Zhang, L., Ge, X., Li, Y., Han, L., & Yan, X. (2022). Stilbenes: Source plants, chemistry, biosynthesis, pharmacology, application, and problems related to their clinical application-A comprehensive review. *Phytochemistry*, *197*, 113128. https://doi.org/10.1016/j.phytochem.2022.113128

Vashisht, S., Singh, M. P., & Chawla, V. (2016). *In-vitro* antioxidant and antibacterial activity of methanolic extract of *Shorea robusta* Gaertn. F. Resin. *International Journal of Pharmaceutical and Phytopharmacological Research*, *6*(4), 68–71. https://doi.org/10.24896/eijppr.2016641

Waghulde, S., Kale, M. K., & Patil, VijayR. (2019). Brine shrimp lethality assay of the aqueous and ethanolic extracts of the selected species of medicinal plants. *Proceedings*, *41*(1), 47. https://doi.org/10.3390/ecsoc-23-06703