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Phytochemical analysis, *in vitro* screening of antioxidant and antibacterial potential of Cajuput (*Melaleuca cajuputi*) extract against pathogenic *Vibrio* spp.

Sunanta Khongsai *, Chakhriya Chalad, Preeda Phumee

Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Sikao, Trang, 92150, Thailand

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ABSTRACT

Cajuput (*Melaleuca cajuputi*) has been used as a form of herbal treatment. This study is aimed at evaluating the phytochemical constituents as well as the antioxidant and antibacterial activities of ethanol extract (MLE) and aqueous extract (MLW) of *M. cajuputi* leaves. Preliminary phytochemical screening was performed using colour-forming standard methods. The total phenolic content (TPC), total flavonoid content (TFC), and total condensed tannins (TCT) were determined using the Folin-Ciocalteu, aluminum chloride, and vanillin methods, respectively. The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays. The inhibition zone of antibacterial activity using the disc diffusion method with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts were performed using the microdilution method. The results showed that the TPC, TFC, and TCT of MLW were higher than those of MLE ($p < 0.05$). The MLW also showed higher antioxidant activity than MLE ($p < 0.05$), with the IC_{50} values of DPPH and ABTS radical scavenging activity at 0.078 mg/mL and 0.193 mg/mL, respectively. For antibacterial activity, MLW exhibited a higher zone of inhibition against *Vibrio harveyi* (7.94 mm) and *Vibrio parahaemolyticus* (7.65 mm) than MLE. All extracts were effective against both types of pathogenic bacteria, with MIC and MBC of 6.25 mg/mL and 25 mg/mL, respectively. The study concludes that the aqueous extract of *M. cajuputi* possesses better antioxidant activity than the ethanol extract and can be used to control the pathogenic *Vibrio* spp.

Keywords: *Melaleuca cajuputi*, Secondary metabolite, Polyphenols, Free radical scavenging, Antimicrobial activity

Introduction

Many medicinal plants are important sources of medicine because they contain numerous important substances, each of which may have a wide range of pharmacological effects. Accordingly, nature-derived compounds have played a critical role in new medication research and development.[1,2,3,4,5,6] Large number of plants have been investigated for their antioxidant properties and potential to inhibit bacteria growth.[7,8,9] Natural antioxidants in the form of raw extracts and their chemical constituents are very effective in preventing the destructive processes caused by oxidative stress.[10] Mostly, the presence of antioxidants in medicinal plants depends on secondary metabolites and phytochemical compounds such as phenolics, flavonoids, and tannins.[11] Nowadays, the use of plants as a source of medicinal properties is beneficial in the prevention and treatment of many diseases. The causes and mechanisms of some diseases including aging are attributable to oxidative reactions with side effects caused by free radicals in the body. *Melaleuca cajuputi* is a perennial plant that belongs to the Myrtaceae family and bears the common names of cajuput, or paper bark tree.

*Corresponding author. E mail: Sunanta.k@rmutsv.ac.th
Tel: + 66 8127396 64

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It is widespread in swamp forests and hot climate zones. The plants of the Myrtaceae family possess strong chemical and biological properties and have been widely used in a variety of industries due to some of the useful medicinal properties such as antioxidant bioactivity, especially in the case of their essential oils. Likewise, they have an interesting chemical and medicinal composition that relates to their biological activity.[9,12] Quinones, flavonoids, phenols, alkaloids, glycosides, cineol, pinene, linalool, caryophyllene, nerolidol, and terpenoids are among the major bioactive compounds found in *M. cajuputi*, and several studies have shown an excellent antimicrobial effect of *M. cajuputi* extracts against viruses, bacteria, protozoa, and fungal species.[13,14] The high flavonoid and phenolic contents of *M. cajuputi* flowers and leaves in Malaysia are identified for their antioxidant and antibacterial properties. These chemicals include significant terpenoids, aromatic, and fatty acid compounds.[15] *M. cajuputi* flowers and leaves are rich in phytochemicals with low toxicity and heavy metal concentrations that have insecticidal effects against *Aedes aegypti*, the yellow fever mosquito, and *Aedes albopictus*, the forest mosquito.[16,17] Herbal extracts from plants belonging to the Myrtaceae family, for instance, show antibacterial activity against common shrimp diseases such as those caused by the *Vibrio* species, which can cause major economic losses in the business.[18,19] In recent years, there has been an increase in interest in researching the potential applications of *M. cajuputi* extract in aquaculture, particularly in shrimp farming, to improve health and immunity. In addition, the aqueous extracts of *M. cajuputi* had no toxic effects with an LC_{50} value of 1062 µg/mL according to Meyer's toxicity index, which specifies that the extract is deemed harmful when LC_{50} is less than 1000 µg/mL. However, the mortality index varies in different models. For example, Clarkson's mortality test classifies an LC_{50} of more than 1000 µg/mL in brine shrimp as harmful.[17] Several investigations have been conducted to assess the positive effects of *M. cajuputi* extract on various aspects of shrimp immunity and health. For example, the possibility of *M. cajuputi* leaf extract as a diet supplement has been studied to determine whether the extract can enhance the growth, immune responses, and resistance

of giant river prawns (*Macrobrachium rosenbergii*) against *Aeromonas hydrophila*.[20] The present study was designed to investigate the phytochemical analysis that could be responsible for biological activity, to evaluate the antioxidant and antibacterial potential of ethanol and aqueous extracts of *M. cajuputi* against pathogenic *Vibrio* spp., and to identify any correlation between phytochemical constituents and biological activities to further explore the potential medicinal benefits of *M. cajuputi*.

Materials and Methods

Chemicals and reagents

Folin-Ciocalteu reagent was purchased from LobaChemie (Mumbai, India). Gallic acid, rutin, catechin, vanillin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, United States).

Plant collection and identification

The fresh leaves of *M. cajuputi* were collected from Sikao district, Trang province, Thailand (7.524009, 99.340293), in March 2021. The plant materials were authenticated at Bangkok Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Thailand, with herbarium number BKF 194868.

Plant preparation and Extraction

The leaves of *M. cajuputi* were washed, oven-dried, and powdered using an electric blender. The extracts were prepared using a method with slight modifications.[21] For the ethanol extract, a maceration was prepared by immersing 1 kg of powdered leaves in 5 L of 95% ethanol at room temperature for 7 days. For the aqueous extract, a decoction was prepared by boiling 1 kg of powdered leaves in 4 L of distilled water for 2 h. Then the mixture was filtered and concentrated using a rotary evaporator (Laborota 4000 efficient rotary evaporator; Heidolph Instruments GmbH & Co. KG, Germany) under reduced pressure at 40 °C to obtain ethanol extract (MLE) and aqueous extract (MLW) yielding 13.0% and 15.1%, respectively. Both extracts were stored at 4 °C until further use for the experiment.

Phytochemical analysis

The phytochemical analysis was conducted to identify the medicinal compounds including anthraquinones, terpenoids, flavonoids, saponins, tannins, alkaloids, and cardiac glycosides. The procedure was performed based on colour-forming or sedimentation reactions.[22,23]

Total phenolic, flavonoid contents, and condensed tannin

The Folin-Ciocalteu reaction was used to determine the total phenolic content (TPC) of the MLE and MLW extracts. This method is based on electron transfer, which measures the reductive capacity of an antioxidant, resulting in a blue coloured solution. The total content of these compounds was determined by a protocol with some modifications,[24] whereby 20 µL of extract (10 mg/mL in distilled water), 1.58 mL of distilled water, 100 µL of Folin-Ciocalteu reagent, and 300 µL of saturated sodium bicarbonate were added into the reaction vessel. After keeping the reaction vessels in the dark for 2 h at room temperature, the absorbance of the mixture was measured at 765 nm using UV-vis spectroscopy (U-1800 Spectrophotometer, Hitachi High-Tech Science Corp., Tokyo, Japan) while distilled water was used as a blank. Gallic acid (0-0.1 mg/mL) was prepared and the calibration curve was created. The experiment was measured in triplicate and expressed as means ± standard deviation in milligrams of gallic acid equivalent per milligram of extract (mg GAE/mg) using the following equation (1):

$$C[mg\ GAE/mg] = C_i[mg/mL] * (V_1[mL]/V_2[mL]) * (V_3[mL]/m[mg]) \quad \ldots (1)$$

where C_i[mg/mL] is the concentration from the calibration curve, V_1 is the total volume of the reaction vessel, V_2 is the volume of extract/standard added to the reaction, V_3 is the volume in which the extract was dissolved, and m is the mass of the extract dissolved in V_3 in order to prepare a stock sample of extract. The aluminum chloride method was used to determine the total flavonoid content (TFC) of the MLE and MLW extracts. This method is based on the formation of a complex between the aluminium ion (Al^{3+}) and the carbonyl and hydroxyl groups of flavones and flavonols, resulting in a yellow coloured solution. The total content of these compounds was determined by a protocol with some modifications,[24] whereby 200 µL of extract (10 mg/mL in methanol), 800 µL of methanol, and 60 µL of 5% $NaNO_2$ were added into the reaction vessel, and then placed in the dark for 5 min. After that, 60 µL of 10% $AlCl_3$ was added and placed in the dark for 5 min. Afterwards, 0.4 mL of 1 M NaOH was added, followed by 0.28 mL of methanol. Then, the mixture was placed in a dark for another 15 min. The absorbance of the mixture was measured at 430 nm using UV-vis spectroscopy (U-1800 Spectrophotometer, Hitachi High-Tech Science Corp., Tokyo, Japan) while methanol was used as a blank. Rutin (0-0.15 mg/mL) was prepared as the sample for the calibration curve. The experiment was measured in triplicate and expressed as means ± standard deviation in milligrams of rutin equivalent per milligram of extract (mg RE/mg) using the following equation (2):

$$C[mg\ RE/mg] = C_i[mg/mL] * (V_1[mL]/V_2[mL]) * (V_3[mL]/m[mg]) \quad \ldots (2)$$

where C_i[mg/mL] is the concentration from the calibration curve, V_1 is the total volume of the reaction vessel, V_2 is the volume of extract/standard added to the reaction, V_3 is the volume in which the extract was dissolved, and m is the mass of the extract dissolved in V_3 in order to prepare a stock sample of the extracts.

The vanillin method was used to determine the total condensed tannin (TCT) of the MLE and MLW extracts. This method is based on the reaction of the vanillin monomer of the condensed tannins, resulting in a red coloured solution. The total content of these compounds was determined by a protocol with slight modifications.[25] Then 1 mL of extract (10 mg/mL in methanol) was added into the reaction vessel and incubated at 30 °C. Then, 5 mL of the vanillin reagent was added and left in the water bath for 20 min. The absorbance of the mixture was measured at 500 nm using UV-vis spectroscopy (U-1800 Spectrophotometer, Hitachi High-Tech Science Corp., Tokyo, Japan) while 4% HCl was used as a blank. Catechin (0-0.3 mg/mL) was prepared as the sample for the calibration curve. The experiment was measured in triplicate and expressed as means ± standard deviation in milligrams of catechin equivalent per milligram of extract (mg CE/mg) using the following equation (3):

$$C[mg\ CE/mg] = C_i[mg/mL] * (V_1[mL]/V_2[mL]) * (V_3[mL]/m[mg]) \quad \ldots (3)$$

where C_i[mg/mL] is the concentration from the calibration curve, V_1 is the total volume of the reaction vessel, V_2 is the volume of extract/standard added to the reaction, V_3 is the volume in which the extract was dissolved, and m is the mass of the extract dissolved in V_3 in order to prepare a stock sample of extract.

Antioxidant activity

DPPH free radical scavenging

The DPPH assay was used for the determination of the antioxidant activity of the MLE and MLW extracts. This method is based on the reduction of the violet DPPH radical via a hydrogen atom transfer mechanism to cause a change in the colour to stable pale-yellow DPPH molecules. The DPPH-free radical scavenging activity was determined by a protocol with slight modifications.[21] The stock solution of 0.1 mM DPPH was prepared in absolute ethanol, while 500 µL of extract (0-0.1 mg/mL in absolute ethanol) and synthetic antioxidant, ascorbic acid, were placed in different test tubes. Then, 500 µL of 0.1 mM DPPH solution was added in each tube. The tubes were allowed to stand in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using UV-vis spectroscopy (U-1800 Spectrophotometer, Hitachi High-Tech Science Corp., Tokyo, Japan). Ethanol and DPPH in ethanol without extract were used as a blank and

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control, respectively. Ascorbic acid (0-0.005 mg/mL) was used as the positive control. The following formula (4) was used to determine the DPPH radical scavenging activity as a percentage of inhibition:

$$DPPH\ Inhibition\ (\%) = [(A_{control} - A_{sample})/A_{control}]\ x\ 100 \quad \ldots (4)$$

where A $_{control}$ is the absorbance of the control, and A $_{sample}$ is the absorbance of the sample. The experiment was measured in triplicate and expressed as means ± standard deviation of the concentration of the sample scavenging 50% of free radicals (IC_{50}).

ABTS free radical scavenging

The ABTS assay was used to determine of antioxidant activity of the MLE and MLW extracts. This method is based on the reduction of the ABTS radical via an electron transfer mechanism to reduce the ABTS radical and lose its bluish-green colour. The ABTS-free radical scavenging was determined by a protocol with slight modifications.[21] The stock solution of ABTS radical cations ($ABTS^+$) was prepared by mixing 7 mM ABTS and 2.45 mM $K_2S_2O_8$ in a ratio of 1: 0.5. The mixture was allowed to react for 16 h in the dark at room temperature. The $ABTS^+$ working solution was prepared by diluting the stock solution with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm measured using UV-vis spectroscopy (U-1800 Spectrophotometer, Hitachi High-Tech Science Corp., Tokyo, Japan). Then, 100 µL of extract (0-0.25 mg/mL) and synthetic antioxidant, ascorbic acid, were placed in different test tubes, whereupon 10 mL of $ABTS^+$ working solution was added to each tube. The reaction mixture was shaken and left to incubate for 6 min at room temperature. The absorbance of the mixture was measured at 743 nm. Ethanol and ABTS in ethanol without extract were used as a blank and control, respectively. Ascorbic acid (0-0.03 mg/mL) was used as the positive control. The following formula (5) was used to determine the ABTS radical scavenging activity as a percentage of inhibition:

$$ABTS\ Inhibition\ (\%) = [(A_{control} - A_{sample})/A_{control}]\ x\ 100 \quad \ldots (5)$$

where A $_{control}$ is the absorbance of the control, and A $_{sample}$ is the absorbance of the sample. The experiment was measured in triplicate and expressed as means ± standard deviation of the concentration of the sample scavenging 50% of free radicals (IC_{50}).

Antimicrobial activity

Inoculum preparation

The test organisms were *Vibrio parahaemolyticus* obtained from Songkhla Aquatic Animal Health Center, Thailand, and *Vibrio harveyi* VHAQ001 obtained from Kasetsart University, Thailand. All tested bacterial inoculum was prepared to a concentration of 1.5×10^8 CFU/mL (OD_{625} = 0.08-0.10) and adjusted with sterile sodium chloride (0.85% NaCl). The antibacterial assay was performed by the disc diffusion method with some modifications.[26] Whatman filter paper discs of 6 mm diameter were impregnated with 10 µL of 100 mg/mL extract in dimethyl sulfoxide (DMSO). The antibiotic tetracycline (30 µg/disc) was used as a positive control while DMSO was used as a negative control. Agar plates were incubated at 37 °C for 24 h and the clear zone of inhibition in mm was taken as the degree of antimicrobial sensitivity. The experiment was performed in triplicate and expressed as means ± standard deviation.

Minimum inhibitory concentration and minimum bactericidal concentration

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts were determined using the microdilution method with some modifications.[26] The cultures of *V. parahaemolyticus* and *V. harveyi* were diluted with nutrient broth (NB) in 2% NaCl to obtain 1×10^8 CFU/mL. The extracts were two-fold diluted to a concentration between 100 mg/mL and 0.098 mg/mL. After 24 h of incubation, 50 µL of 0.18% resazurin indicator was added. Readings were taken after 3 hours of reaction. The growth was indicated by colour changes from purple to pink. The lowest concentration at which the colour change occurred was taken as the MIC value. The well showing the complete absence of growth was considered as the MBC. The experiment was performed in triplicate and results expressed as means ± standard deviation.

Statistical analysis

The results were expressed as means ± standard deviation from triplicate observations by using MS Excel 2010 statistical software. The comparative effectiveness of TPC, TFC, and TCT among different types of solvents was analyzed using a paired t-test. The antioxidant and antibacterial activities were evaluated by one-way analysis of variance (ANOVA), followed by Duncan's comparison with a statistically significant level set at $p < 0.05$ by using the Statistical Package for Social Sciences (SPSS) version 22 software for Windows. The correlations between TPC, TFC, TCT, antioxidant activities, and antibacterial activities were evaluated using Pearson's method.

Results and Discussion

The phytochemical analysis of *M. cajuputi* extracts was performed using quantitative and qualitative analyses. The preliminary screening of phytochemical constituents was based on the appearance of colour or precipitation by sedimentation reactions. The results of preliminary screening are reported in Table 1. The phytochemical components revealed the presence of anthraquinones, terpenoids, flavonoids, saponins, tannins, and alkaloids in the MLE sample, while the same phytochemical groups, except for terpenoids, were also found in MLW sample. Cardiac glycosides were not found in either extract. The extraction of active biochemicals from plants depended upon the polarity of the solvents. Polar solvents will extract polar molecules and non-polar solvents will extract non-polar molecules. In this study, ethanol (polarity index of 5.2) and water (polarity index of 10.2) were used to investigate the phytochemical constituents of *M. cajuputi* extract.[27] Moderate phytochemical results were reported in ethanol solvents with polarity indices ranging from lower to moderate. These results are consistent with a prior study that evaluated the polarity of solvent performance in bioassays based on biochemical extraction employing solvents with moderate polarity indices, which produced positive results in a few bioassays.[28,29] Phytochemicals have been reported to have bioactive properties through *in vitro* studies. The reported phytochemicals such as phenolic and flavonoid groups could be responsible for the biological activities of *M. cajuputi*.[13] Naturally, many polyphenols are herbal-derived and are often produced in response to stress caused by any action. These substances are able to scavenge free radicals and possess major antioxidant activity and antibacterial effects against both gram-negative and gram-positive bacteria.[15,29,30] The current study focused primarily on phenolic, flavonoid, and tannin contents because they are known to be abundant in polyphenols.[18,31]

Table 1: Phytochemical screening of *M. cajuputi* extracts

Phytochemical group	Sample	
	MLE	MLW
Anthraquinones	+	+
Terpenoids	+	-
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Alkaloids	+	+
Cardiac glycosides	-	-

MLE, ethanol extract; MLW, aqueous extract.
+ indicates presence of component, - indicates absence of component

The TPC, TFC, and TCT are illustrated in Table 2. The MLW had higher TPC, TFC, and TCT than MLE ($p < 0.05$). These quantities dramatically increased as polarity increased, suggesting that the plants

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contained a variety of biological compounds with a range of polarity.[32,33] As a result, the type and polarity of the extraction solvent can have a substantial influence on the amount of polyphenols extracted. The polarity of the polyphenols ranges from polar to non-polar. Polar solvents are typically used for the best extraction of polyphenols because they have a higher effectiveness of solvation from interactions between the polar sites of antioxidant compounds and the solvent than is the case for non-polar solvents.[34] The antioxidant activity of *M. cajuputi* extracts are illustrated in Table 3. The MLW also showed higher antioxidant activity than MLE ($p < 0.05$). The ability of certain phytochemical extracts to inhibit the oxidation of other molecules by suppressing the initiation or propagation of oxidizing chain reactions has made them active alternatives in complementary medicine. These naturally occurring antioxidant chemicals have been reported to be composed of phenolic compounds. Phytochemical extracts containing constituents such as flavonoids, polyphenols, and phenolic compounds have been reported to exhibit antioxidant activities.[15,35] Solvent polarity has a substantial impact on the extract yield and antioxidant activity of phenolic compounds in plant material. Both the DPPH and ABTS assays showed that the hydrophilic extract had a higher antioxidant capacity than the hydrophobic portion. Flavonoids are secondary metabolites comprising antioxidant activity and the potency of this metabolite relies on the amount and location of free OH groups.[35,36]

Table 2: Total phenolic content (TPC), total flavonoid content (TFC), and total condensed tannin (TCT) of *M. cajuputi* extract

Sample	TPC (mg GAE/mg)	TFC (mg RE/mg)	TCT (mg CE/mg)
MLE	2.14 ± 0.05^{b}	0.38 ± 0.01^{b}	0.31 ± 0.04^{b}
MLW	4.37 ± 0.14^{a}	0.47 ± 0.01^{a}	0.59 ± 0.02^{a}

GAE, gallic acid equivalent; RE, rutin equivalent; CE, catechin equivalent; MLE, ethanol extract; MLW, aqueous extract.
Means ± SD in the same column superscripted with distinct lowercase letters are significantly different ($p < 0.05$).

Table 3: Antioxidant activity of *M. cajuputi* extract

Sample	IC_{50} (mg/mL)	
	DPPH	ABTS
MLE	0.088 ± 0.001^{b}	0.226 ± 0.001^{c}
MLW	0.078 ± 0.001^{b}	0.193 ± 0.002^{b}
Ascorbic acid	0.005 ± 0.001^{a}	0.022 ± 0.000^{a}

IC_{50}, the inhibitory concentration at 50%; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; MLE, ethanol extract; MLW, aqueous extract.
Means ± SD in the same column superscripted with distinct lowercase letters are significantly different ($p < 0.05$).

The present study showed that solvents with different polarities had a significant effect on phytochemical presence, TPC and TFC, as well as TCT, and the trend indicated a relatively greater yield of these phytochemical groups in high polar solvents. The high antioxidant activity of polar extracts in the current study supports a linear relationship between TPC, TFC, TCT, and antioxidant capacity, as previously observed.[13,28,29,31,36] These findings may be explained by a potential synergy between phenolic chemicals and other phytochemical substances produced in polar liquids. In addition, many polyphenols have demonstrated bactericidal effects against both gram-negative and gram-positive bacteria.[37] The potency of inhibiting bacterial growth is indicated by the zone diameter obtained from antimicrobial activity tests (very strong: more than 21 mm, strong: 10-20 mm, moderate: 5-10 mm, and weak: less than 5 mm).[38,39] The zone diameter observed from the aqueous extract strongly showed inhibition of *V. harveyi* and *V. parahaemolyticus* at a dose of 1 mg/disc. Table 4 indicates that at concentrations of 6.25 and 25 mg/mL, respectively, the MIC and MBC of the extract could suppress and show bactericidal activity against tested pathogenic *Vibrio* spp. These results suggest that *M. cajuputi* extract may be a naturally occurring antibiotic against *Vibrio* spp.

Since phenolic compounds can act on the cell membrane and cell wall of the microorganisms, they can interact with the membrane proteins of bacteria utilizing hydrogen bonding through their hydroxyl groups which can result in changes in membrane permeability and cause cell destruction. They can also penetrate bacterial cells and coagulate cell content. Therefore, it is logical that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms.[6] This study found that *M. cajuputi* extract was slightly moderately effective against the two gram-negative *V. harveyi*, and *V. parahaemolyticus* due to membrane disruption by lipophilic compounds and high permeability. The compounds in *M. cajuputi* extract could not penetrate the cell walls of *V. harveyi* and *V. parahaemolyticus*, which have thicker cell walls and fewer lipids and peptidoglycan. This causes more difficulty for antibacterial compounds to enter the cells so the antibacterial activity in gram-negative bacteria is relatively low.[39,40,41,42] Similarly, *M. cajuputi* leaves extract is capable of inhibiting *Bacillus* sp., *Bacillus subtilis*, *Bacillus licheniformis*, *Enterococcus faecalis*, and *Staphylococcus aureus* which are gram-positive bacteria, as well as the gram-negative bacteria *Vibrio cholerae* and *Shigella dysentriae*.[39] However, they had no effects on some gram-negative organisms such as *Escherichia coli* and *Salmonella typhimurium.*[15] Plant extracts have different mechanisms of activity against bacteria based on their composition and the quantity of chemical substances. Potential modes of activity include inhibiting metabolic processes, interference with protein biosynthesis, metabolism in bacteria, increasing membrane permeability, and disrupting membranes with lipophilic chemicals.[11,43]

Among the serious pathogens, *V. parahaemolyticus* are the main threatening pathogenic bacteria affecting some cultured shrimp species and cause acute hepatopancreatic necrosis diseases with high mortality and severe economic losses.[18] Differences in bioactive compounds may be varied by plant parts, extraction processes, ecological conditions, extraction times, geomorphological traits, sources, seasonal variation, taxonomy, and soil type.[9,17] The findings of this study revealed that the aqueous extract of *M. cajuputi* has sufficient scavenging properties in all radical scavenging tests. It is reported that TPC, TFC, and TCT are natural products that have been shown to possess various biological properties related to antioxidant mechanisms and showed the capacity to scavenge different free radicals, indicating that *M. cajuputi* may be useful as a therapeutic agent for treating radical-related pathological damage.[44] The study also suggests that phytochemical compounds extracted in polar solvents are pharmaceutically more important due to comparatively higher values of free radical scavenging activity, and are important contributors to the antimicrobial activity of extracts against microorganisms.[17,36] The MIC and MBC values of the extracts against *V. harveyi* and *V. parahaemolyticus* allow a similar evaluation of the antibacterial effect of bioactive compounds. The substance is bactericidal when the ratio of MBC/MIC ≤ 4, and bacteriostatic if the ratio of MBC/MIC > 4.[40,45] Based on these data, the *M. cajuputi* extracts exert bactericidal effects against *V. harveyi* and *V. parahaemolyticus* (MBC/MIC = 4).

Pearson's correlation was considered for the relationship between TPC, TFC, TCT, DPPH inhibition, ABTS inhibition, *VH* inhibition, and *VP* inhibition in ethanol and aqueous extracts investigated in the current study. The correlations between TPC, TFC, and TCT including the antimicrobial *VH* and *VP* were insignificant, while the negative correlation between the antioxidant DPPH and ABTS was significant as shown in Table 5. It can be concluded that TPC, TFC, and TCT play an important role in the antioxidant and antibacterial capacity of extracts.

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Table 4: Diameter averages of inhibition zone, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of *M. cajuputi* extract

Sample	*Vibrio harveyi*			*Vibrio parahaemolyticus*		
	Inhibition zone (mm)	**MIC (mg/mL)**	**MBC (mg/mL)**	**Inhibition zone (mm)**	**MIC (mg/mL)**	**MBC (mg/mL)**
MLE	4.99 ± 0.74^{c}	6.25	25	5.32 ± 0.59^{c}	6.25	25
MLW	7.94 ± 0.64^{b}	6.25	25	7.65 ± 0.44^{b}	6.25	25
Tetracycline	13.58 ± 1.04^{a}			13.58 ± 1.04^{a}		

MLE, ethanol extract; MLW, aqueous extract.
Means ± SD in the same column superscripted with distinct lowercase letters are significantly different ($p < 0.05$).

Table 5: Correlation coefficient (r) between total phenolic content (TPC), total flavonoid content (TFC), total condensed tannin (TCT), free radical inhibition, and antibacterial activity

Assays	TPC	TFC	TCT	free radical inhibition (IC_{50})		Inhibition zone (mm)	
				DPPH	**ABTS**	***VH***	***VP***
TPC	1	.981**	.985**	-.997**	-.985**	.940**	.956**
TFC		1	.970**	-.980**	-.978**	.939**	.914*
TCT			1	-.987**	-.946**	.977**	.897*
DPPH				1	.984**	-.934**	-.940**
ABTS					1	-.876*	-.963**
VH						1	.830*
VP							1

IC_{50}, inhibitory concentration at 50%; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; *VH*, *Vibrio harveyi*; *VP*, *Vibrio parahaemolyticus*
*, ** Correlations are significant at 0.05 and 0.01 levels (2-tailed), respectively.

Conclusion

The extracts from *Melaleuca cajuputi* showed a difference of *in vitro* biological effects. The abundance of phytochemicals with antioxidant properties, such as phenolics, flavonoids, and condensed tannins may be held responsible for the bioactive activity of *M. cajuputi*. The antimicrobial and antioxidant activities of *M. cajuputi* signified that the plant could be an excellent source of particular pharmacological active compounds. The selection of solvents used depends on the purpose of either yielding high content or for the specific extraction of phytochemical compounds that would be useful for its medicinal properties. These results indicate that *M. cajuputi* leaf extract can be used as a natural herb to control pathogenic bacteria in aquaculture. Further studies on bioassay-guided fractionation to obtain the pure compounds of *M. cajuputi* extracts and the investigation of their antimicrobial mechanisms are necessary to reveal the potential of the plant as a good source of novel pharmaceutical compounds for disease remedies for aquatic animals.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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