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Antioxidant and antibacterial activities of *Coix lacryma-jobi* seed and root oil potential for meningitis treatment

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Abstract

Bacterial meningitis is a dreaded infectious disease in the human pulmonary system caused by bacterial invasion and promoting inflammation. Hanjeli (*Coix lacryma-jobi*) seed and root oil have antioxidant, anti-inflammatory, and antibacterial properties that can be used in the treatment of bacterial meningitis. The study aims to investigate the potential of oil from *C. lacryma-jobi* seed and root part for bacterial meningitis treatment. The phytochemical screening was performed using standard protocols. Antibacterial activity and minimum inhibitory concentration (MIC) were conducted by the disc diffusion and two-fold dilution method. The preliminary phytochemical screening of *C. lacryma-jobi* shows that essential oil of root and seed part contains steroids, carotenoids, tannins, alkaloid salts, reducing compounds, flavonoid, anthracenoid, coumarin derivatives, cardenoid, anthocyanins, and saponins. The determination of total phenolic, tannin contents, and antioxidant in essential oil from the root was significantly higher ($p < 0.05$) than the seed part. The *C. lacryma-jobi* oil from both parts expressed the highest antimicrobial activity against *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. Additionally, the lowest minimum inhibitory concentration (12.5–50 mg/mL) was observed against selected bacteria of meningitis. It is concluded that oil from *C. lacryma-jobi* root and seed parts contains compounds that might be used in the bacterial meningitis treatment.

Keywords: *Coix lacryma-jobi*; antioxidant; antibacterial; essential oil; bacterial meningitis; phytochemical; DPPH; minimum inhibitory concentration

1. Introduction

Hanjeli (*Coix lacryma-jobi*) is one of the Poaceae family (Chhabra and Gupta, 2015; Patel et al., 2017). Hanjeli may be indigenous and unfamiliar cereal grains grown in Sumatra, Indonesia. Hanjeli is reported to have higher carbohydrates, protein, fat and dietary fiber than rice and corn (Chhabra and Gupta, 2015; Rajesh, 2016). Additionally, other phytochemical components are also reported in Hanjeli, such as calcium, phosphorus, iron, niacin, thiamine, and riboflavin (Rajesh, 2016; Qu et al., 2014).

In addition, *Coix lacryma-jobi* is used as nourishing food in traditional Chinese medicine and has proven to reduce the risk of cancer (Kuo et al., 2002; Wang et al., 2016). In recent years, an adlay seed oil emulsion has been approved as an antineoplastic therapy by the Chinese Ministry of Public Health, in particular for lung cancer (Wang et al., 2016; Zhang et al., 2014).

The preliminary phytochemical screening of the Hanjeli essential oil indicated the presence of alkaloids, carbohydrates, saponins, glycosides, flavonoids, phenols, tannins, and steroids, whereas the seed and root essential oil contained glycosides, flavonoids, phenols, and steroids, which revealed higher antimicrobial activity (Al-Shuneigat et al., 2015; Diningrat et al., 2020; Zhang et al., 2014). However, the lowest minimum inhibitory concentration was observed against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pneumoniae,* and *Streptococcus feaecalis,* which were selected as bacteria of meningitis.

Bacterial meningitis is a severe infectious disease of the membranes lining the brain resulting in high mortality and morbidity throughout the world (El Bashir et al., 2003). An annual Islamic pilgrimage to Mecca, known as the Hajj and Umra, attracts more than a million pilgrims from many countries worldwide, including Indonesia, which is the biggest Moslem country, and this has been associated with the outbreak of bacterial meningitis disease (Al-Gosha'ah et al, 2014; De Gans et al., 2002; Yezli et al., 2016). The first reported international outbreak of bacterial meningitis following the Hajj occurred in 1987 (van de Beek et al., 2012; Yezli et al., 2016). This epidemic emphasized the potentially high risk of transmission during the pilgrimage and in their home countries (Al-Gosha'ah et al, 2014; van de Beek et al., 2012; van de Beek et al., 2016), such as Indonesia.

In the last decades, the epidemiology and treatment strategies for community-acquired bacterial meningitis

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have significantly changed (van de Beek et al., 2012; van de Beek et al., 2016). Firstly, the introduction of conjugate vaccines has substantially reduced the burden of bacterial meningitis (De Gans et al., 2002; van de Beek et al., 2016). As a result, community-acquired bacterial meningitis has become a disease that currently affects more adults than infants, with its specific complications and treatment options (van de Beek et al., 2012; Al-Lahham et al., 2018). The second important development is the increasing rate of reduced susceptibility to common antimicrobial agents among strains of *Streptococcus pneumoniae (pneumococcus)* and *Neisseria meningitidis (meningococcus)*. There is a large difference in the resistance rates, and the empiric antibiotic treatment needs to be adjusted according to regional epidemiology (Al-Lahham et al., 2018; De Gans et al., 2002). Finally, several adjunctive treatments have been tested in randomized controlled trials, often with conflicting results (van de Beek et al., 2016).

Therefore, this study aimed to determine phytochemical compounds contained in the essential oil extracted from the root and seed of *C. lacryma-jobi*, related to its antioxidant and antibacterial. This study also aimed to determine the antibacterial activity of essential oil from the seed and root of *Coix* against bacterial meningitis. This study suggests the potential of *C. lacryma-jobi* essential oil for bacterial meningitis treatment, which would be beneficial because meningitis affects many Hajj pilgrims from Indonesia.

2. Materials and Methods

2.1. Collection, identification, and processing of plant samples

The seeds and roots of *C. lacryma-jobi* were collected from Pamah Village Area, Semelir District, Langkat District North Sumatra 20773, Indonesia and submitted in the Herbarium Bandungense, Bandung Institute of Technology in Indonesia, Bandung (3749/II.CO2.2/PL/2019). Firstly, the seeds and roots were naturally dried for seven days to remove the water contents. After it was kept in an air-tight container with necessary markings for identification, it was stored in a cool, dark, and dry place for further investigation (Diningrat et al., 2020).

2.2. Oil extraction

Oil extraction of *C. lacryma-jobi* was performed by steam distillation. The essential oil from (seeds and root part of *C.lacryma-jobis*) was obtained by steam distillation using a Clevenger apparatus. Batches of 100 g of homogenized plant materials were mixed with 3 L of distilled water in a 5 L round-bottomed flask and heated using a heating mantle. The vapor of the essential oil was condensed and collected. Fractions of essential oil were collected every hour within 4 hours. The extracts were evaporated at room temperature to concentrate the samples, which were then stored in the refrigerator prior to the analysis (Al-Shuneigat et al., 2015; Benkeblia, 2004; Diningrat et al., 2020; Tepe et al., 2005).

2.3. Preliminary antioxidant screening

Comprehensive antioxidant screening of the essential oils was performed as preliminary phytochemical screening by following the procedures as described in the previous study (Aiyegoro and Okoh, 2010; Diningrat et al., 2020; Gowri and Vasantha, 2010; Igbinosa et al., 2009; Valgas et al., 2007).

2.3.1. Determination of total phenolic content

The reaction mixture consisted of 1 mL of essential oil, 1 mL of FCR (Folin Ciocalteu Reagent) 2N, and 3 mL of a 20% sodium carbonate solution. This mixture was placed at room temperature for 40 mins, and the absorbance was then measured (spectrophotometer Agilent 8453) at 760 nm. Distilled water was used as a blank in this measurement. A standard curve was plotted using tannic acid (1-5 µg/mL). Tests were performed in triplicate. The total phenolic content of the extract was calculated using the formula:

$$T_{PT} = \frac{C_{Tube}}{C_i} \times D$$

Where T_{PT} is the total phenolic content of the extract expressed as tannic acid equivalent (mg TAE)/g, C_{Tube} is the concentration (mg/mL) in the test tube, D is the dilution factor, and C_i is the concentration in mg/mL in the stock solution (Igbinosa et al., 2009; Tan and Lim, 2015).

2.3.2. Determination of tannin content

Polyvinyl polypyrrolidone (PVPP) can precipitate tannins by the formation of a complex; 100 mg of PVPP can complex 2 mg of total phenolics. 1 mL of essential oil (0.50 mg/mL) was added to the quantity of PVPP to complex the total phenolics present in the essential oil (and determined as described above). The mixture was vortexed, stored at 4°C for 15 min, and then centrifuged at 3000 g for 10 min. The supernatant contains phenolic compounds other than tannins (which have been precipitated by PVPP). A test tube was performed for total phenolics. After 40 minutes, it was centrifuged, and the absorbance of the supernatant was measured at 760 nm. The total phenolic content of the supernatant was calculated by the above formula. Tannin content was determined as the difference between the first value of total phenolics (containing tannins) and the second value of total phenolics (in the absence of tannins) (Valgas et al., 2007; Tan and Lim, 2015).

2.3.3. Determination of flavonoid content

100 µL of essential oil (10 mg/mL) in methanol was mixed with 100 µL of 20% antioxidant activity and adjusted to 5 mL with methanol. After 40 minutes, the absorbance was recorded at 415 nm (with a spectrophotometer Agilent 8453). Blank consisted of 100 µL of extract, a drop of acetic acid and adjusted to 5 mL with methanol. The absorbance of Quercetin (0.10 mg/mL), used as a reference compound, was measured under the same conditions. The tests were performed in triplicate. The flavonoids content expressed as quercetin equivalent (QE) was calculated using the following formula:

$$T_{Flav} = \frac{A \cdot m_0}{A_0 \cdot m}$$

T_{Flav} is the flavonoid content of essential oil in mg QE/mg; A is the absorbance of the extract; A_0 is the absorbance of Quercetin; m is the mass of the extract in mg, and m_0 is the mass of Quercetin in mg (Aiyegoro and Okoh, 2010; Tan and Lim, 2015).

2.3.4. Determination of flavonol content

The flavonol content was determined using Quercetin as a reference compound. The dosage was based on the formation of a complex with maximum absorption at 440 nm. 1 mL of essential oil was mixed with 1 mL of aluminum trichloride (20 mg/mL) and 3 mL of sodium acetate (50 mg/mL). The absorption was measured after 2 h 30 min. The absorbance of Quercetin (0.025 mg/mL in methanol) was measured under the same condition. The tests were performed in triplicate. The content of flavonols in the essential oil (expressed as quercetin equivalent, QE) was calculated using the same formula with the determination of flavonoids (above) (Aiyegoro and Okoh, 2010; Tan and Lim, 2015).

2.3.5. Antioxidant activities

The antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) test was evaluated using the method described by Kim et al. (2003). The essential oil was prepared with an initial concentration of 21 mg/mL in dimethyl sulfoxide (DMSO). The concentration range of extracts or standard (Quercetin) was prepared by successive dilution using a microplate technique. The absorbance of residual DPPH in each well was measured at 490 nm (with a spectrophotometer BIO-RAD Model 680) after incubation at 37°C for 30 min. The antioxidant activity of a sample (calculated by the following formula) was given as the percentage of reduced DPPH:

$$I\% = \frac{A_0 - A_s}{A_0} \times 100$$

Where I is the percentage of inhibition, A_0 is the absorbance of the control, A_s is the absorbance of the sample (Tan and Lim, 2015).

2.4. Antibacterial activity

The antimicrobial screening was performed by the disc diffusion method against selected bacterial meningitis (Table 1) collected as pure cultures from the Department of Microbiology, Adam Malik Hospital, Medan, Indonesia. Standard disc of Ciprofloxacin (5 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as the positive and negative control, respectively. The antimicrobial activity of the test agents was determined by measuring the diameter of the zone of inhibition expressed in mm (Al-Shuneigat et al., 2015; Diningrat et al., 2020; Restuati and Diningrat, 2018).

2.5. Determination of MIC

The MIC of the essential oil was determined by a two-fold dilution method. Subjected bacterial strains were grown in Nutrient Agar (HiMedia) until they reached the exponential phase. *C. lacryma-jobi* seed and root essential oil with different dilutions were given concentrations of 50, 25, 12.5, 6.25, 3.14, 1.56, and 0.78 mg/ mL, respectively. Thereafter, 0.5 mL essential of each concentration was added into separate test tubes containing 0.5 mL nutrient broth with the bacterial suspension at a final concentration of 1 mL in each tube and incubated at 37°C for 24 h. As a negative control, 7% of 0.5 mL chloroform was added with 0.5 mL liquid broth of bacteria. After incubation, 100 µL of culture from each tube was transferred and spread over the Mueller-Hinton agar (HiMedia) plate and incubated (Binder, Germany) at 37°C overnight for the bacterial count (Diningrat et al., 2020; Klančnik et al., 2020).

2.6. Statistical analysis

In the antioxidant and antibacterial activity test, the experimental data were calculated as mean ± SEM, evaluated by unpaired One-way ANOVA. Test values of $p < 0.01$ were considered statistically significant (Diningrat et al., 2020; Restuati and Diningrat, 2018).

3. Results

3.1. Preliminary phytochemical screening

The essential oil from the root and seed part of *C. lacryma-jobi* contained steroids, carotenoïds, tannins, alkaloid salts, reducing compounds, flavonoid, anthracenoid, coumarin derivatives, cardenoid, anthocyanins, saponins, while alkaloids and coumarins were absent (Table 1).

Table 1. Phytochemical composition of *C. lacryma-jobi* root and seed oil

Chemical groups	Essential oil	
	Root	Seed
Steroids	+	+
Carotenoïds	+	+
Alkaloid bases	-	-
Coumarins	-	-
Tannins	+	+
Alkaloid salts	+	+
Reducing compounds	+	+
Flavonoid	+	+
Anthracenoid	+	+
Coumarin derivatives	+	+
Cardenoid	+	+
Anthocyanins	+	+
Saponins	+	+

+: indicates the presence of phytochemical compounds; -: indicates the absence of a phytochemical compound

3.2. Antioxidant activity and phenolic content

DPPH scavenging activities of all the extracts depends on the concentration (Table 2). However, the highest DPPH scavenging activity was shown by the essential oil extract from seeds (9.58 ± 0.12 µg/mL, IC_{50}). Both essential oils showed a weak activity compared to those of Quercetin (4.60 ± 0.08 µg/mL, IC_{50}), which was a reference antioxidant. The total phenolic, tannin, flavonoid, and flavonol contents of the two essential oils were determined in this study (Table 2). The concentration of phenolic compounds, tannins, and flavonoids was higher in the essential oil from seeds than that of the roots. For the flavonol content, essential oil from the roots (0.28

± 0.01 mg QE/g) was richer than essential oil from the seeds (0.24 ± 0.01 mg QE/g).

Table 2. Phenolic content and scavenging activity by the DPPH test of *C. lacryma-jobi* root and seed oil

	Total phenolic mg TAE/g	Tannins mg TAE/g	Flavonoids mg QE/g	Flavonols mg QE/g	Antioxidant activity against DPPH IC50 (µg/ml)
Root essential oil	236.00 ±0.63[a]	124.80 ±0.75[a]	18.98 ±0.16 [a]	0.28 ±0.01[a]	33.81±0.57[a,b]
Seed essential oil	357.90 ±0.80	193.74 ±0.90	21.47 ±0.10	0.24 ±0.01	9.58±0.12[b]
Quercetin					4.50±0.12

TAE, Tannic Acid Equivalent; QE, Quercetin Equivalent; values are mean ±SEM (n = 3); a, p<0.05 against seed; b, p<0.05 against quercetin

3.3. Determination of antibacterial activity

The essential oil from the root of C. lacryma-jobi showed a wide range of antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pneumoniae, and Streptococcus feaecalis at a concentration of 800 µg/mL, whereas the range of inhibition zone was within 20–30 mm (Table 3).

However, when essential oil from the seeds of *C. lacryma-jobi* was subjected to antibacterial screening at 800 µg/disc, it showed antibacterial activity against *E. coli, P. aeruginosa, K. pneumoniae, S. pneumoniae,* and *S. feaecalis* with the zone of inhibition ranging from 20 to 32 mm, whereas the remaining tested microorganisms were found to be resistant at the same concentration (Table 3).

Table 3. Antibacterial activity by disc diffusion assay (mm)

Bacterial meningitis isolates		Zone of inhibition in diameter		
		Root Essential Oil	Seed Essential Oil	Ciprofloxacin
Gram-negative bacteria	*Escherichia coli*	21	21	40
	Pseudomonas aeruginosa	26	20	31
	Klebsiella pneumoniae	30	32	40
Gram-positive bacteria	*Streptococus pneumoniae*	28	21	34
	Streptococus feaecalis	24	20	31

3.4. Determination of MIC

The inhibition of microorganism's growth at the lowest concentration of plant extract is known as MIC. In the MIC test, the essential oil of *C. lacryma-jobi* root was capable of inhibiting all types of Gram-positive and Gram-negative (*E. coli*, *P. aeruginosa, K. pneumoniae, S. pneumoniae, S. feaecalis*) meningitis bacterials at the concentration of 12.5–50 mg/mL (Table 4). In contrast, *E. coli*, *P. aeruginosa, K. pneumoniae, S. pneumoniae, S. feaecalis* were inhibited at the concentration of 25–50 mg/mL in the essential oil from *C. lacryma-jobi* seed (Table 5).

Table 4. MIC of *C. lacryma-jobi* root essential oil against selected bacteria of meningitis.

Bacterial meningitis isolates		MIC of root essential oil (mg/mL)						
		50	25	12.5	6.25	3.14	1.56	0.78
Gram-negative bacteria	*Escherichia coli*	0	0	0	29	106	166	222
	Pseudomonas aeruginosa	0	0	0	8	23	46	102
	Klebsiella pneumoniae	0	0	0	4	7	16	46
Gram-positive bacteria	*Streptococus pneumoniae*	0	0	0	7	20	48	96
	Streptococus feaecalis	0	0	0	6	16	27	66

Values are represented as the number of colonies.

Table 5. MIC of *C. lacryma-jobi* seed essential oil against selected bacteria of meningitis.

Bacterial meningitis isolates		MIC of seed essential oil (mg/mL)						
		50	25	12.5	6.25	3.14	1.56	0.78
Gram-negative bacteria	*Escherichia coli*	0	0	13	35	87	137	202
	Pseudomonas aeruginosa	0	0	7	16	41	92	137
	Klebsiella pneumoniae	0	0	6	10	23	47	106
Gram-positive bacteria	*Streptococus pneumoniae*	0	0	11	25	53	85	136
	Streptococus feaecalis	0	0	6	19	63	89	136

Values are represented as the number of colonies.

4. Discussion

Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus feaecalis, and Escherichia coli are the leading causes of bacterial meningitis infections worldwide (Al-Gosha'ah et al, 2014; Paireau et al., 2016; van de Beek et al., 2016; Yezli et al., 2016). Bacterial meningitis causes inflammation of the meninges, which leads to a sudden onset of fever, headache, stiff neck, nausea, vomiting, and altered mental status, and can rapidly result in death (Paireau et al., 2016; van de Beek et al., 2016). The relative contribution of these pathogens to the incidence of bacterial meningitis varies over time, by location, and by characteristics such as the age of patients. Although vaccination programs have been implemented in many countries, including Indonesia, and have had a considerable impact on the disease, more than 1.2 million cases of bacterial meningitis are estimated to occur each year (El Bashir et al., 2003; Paireau et al., 2016; van de Beek et al., 2016; Yezli et al., 2016).

Phytochemical analysis of the *Coix lacryma-jobi* oil from root and seed part revealed the presence of steroids, carotenoids, tannins, alkaloid salts, reducing compounds, flavonoid, anthracenoid, coumarin derivatives, cardenoid, anthocyanins, saponins (Table 1). These phytochemical compounds are known to be biologically active, which enhances the antimicrobial activities of plants. In addition, alkaloids are also known to have antimicrobial, anti-inflammatory, anti-asthmatic, and anti-anaphylactic properties (Diningrat et al., 2018; Diningrat et al., 2020; Diningrat and Marwani, 2018).

The presence of flavonoids in *C. lacryma-jobi* oil is essential because they have been reported to exhibit antimicrobial, anti-inflammatory, anti-angiogenic, analgesic, anti-allergic, cytostatic, antioxidant, antitrypanosomal, and antileishmanial properties (Diningrat et al., 2020; Sari et al., 2018). Saponins and tannins were also reported in this study. Saponins were responsible for numerous pharmacological properties and were known to induce inhibitory effects on inflammation (Benkeblia, 2004; Tepe et al., 2005). Moreover, tannins exerted antimicrobial activities by iron deprivation, hydrogen bonding, or specific interactions with vital proteins, such as enzymes in microbial cells (Devi et al., 2015; Wang et al., 2016).

Steroidal compounds were also present in *Coix lacryma-jobi* oil, and they have drawn much interest due to their relationship with compounds such as sex hormones (Qu et al., 2014). Anthracenoid has a role as a free radical binding agent (Rajesh, 2016). Coumarin derivatives (4-hydroxycoumarin compounds) are oral anticoagulants (OA) that prevent vitamin K from acting as a cofactor in the hepatic synthesis (Chhabra and Gupta, 2015). Cardenoid, carotenoid, and anthocyanin might be responsible for antioxidants and anti-cancer (Rajesh, 2016; Qu et al., 2014). *Coix lacryma-jobi* oil was very potential as an agent that can be used for the treatment of bacterial meningitis based on its phytochemical compounds.

According to the observed antioxidant potential of *Coix lacryma-jobi* oil from roots and seeds and the potent DPPH radical, OH^- radical, $O2^-$ radical, NO^- scavenging activities, the results demonstrated that the oil possessed high phenol contents, antioxidant and nitric oxide scavenging abilities. In other words, the plants contained certain compounds that were potential antioxidants (Table 2). It is assumed that this oil is able to prevent lipid peroxidation, and it is further suggested that the extract was a potential therapeutic agent for the control of oxidative and non-oxidative damage caused by reactive oxygen and nitrogen species (Devi et al., 2015; Kuo et al., 2002; Wang et al., 2016). Since reactive oxygen species are thought to be associated with the pathogenesis of bacterial infections and inflammatory diseases (Rajesh, 2016), the observed inhibitory potential might partially explain the beneficial effects of *Coix lacryma-jobi* oil in treating bacterial meningitis disease.

Plant oil has been long used in pharmaceuticals, alternative medicine, and natural therapies (Benkeblia, 2004; Tepe et al., 2005). *Coix lacryma-jobi* oil is a potential source of novel antimicrobial compounds, especially against bacterial pathogens (Diningrat et al., 2020). In vitro studies in this study showed that the oil inhibited some bacterial meningitis growth, but their effectiveness was classified strong (Table 3). Several compounds, such as steroids, carotenoids, tannins, alkaloid salts, reducing compounds, flavonoid, anthracenoid, coumarin derivatives, cardenoid, anthocyanins, and saponins (Table 1) were identified in the composition of the obtained *Coix lacryma-jobi* oil. Many components of *Coix lacryma-jobi* oil were characterized by Diningrat et al. (2020) using GC-MS, and all of these compounds had antibacterial effects.

The results indicated that *Coix lacryma-jobi* oil with concentrations of 6.25 and 12.5 mg/ml prevented the growth of *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pneumoniae,* and *Streptococcus feaecalis.* Furthermore, a study by Diningrat et al. (2020) investigated *Coix lacryma-jobi* oil and reported a significant effect on the standard *E. coli, S. aureus,* and *B. subitlis*. In this study, the antimicrobial effect of *N. Sativa* oil extract was investigated in the laboratory against bacteria causing bacterial meningitis. The results showed that the antimicrobial effect of *Coix lacryma-jobi* oil from roots and seeds was comparable with antibiotics, such as Ciprofloxacin.

The activity of *Coix lacryma-jobi* oil from roots and seeds against both Gram-positive and Gram-negative selected bacteria of meningitis is an indication of the broad spectrum of activity, so it can be used as sources of antibiotic substances for drug development in the control of these bacterial meningitis infections (van de Beek et al., 2016; Al-Lahham et al., 2018; Diningrat et al., 2020). The essential oil of *C. lacryma-jobi* roots and seeds showed a wide range of antimicrobial activity against Gram-positive and Gram-negative bacterial meningitis. Finally, our results showed that *Coix lacryma-jobi* oil-induced antimicrobial activity against a broad range of bacteria in bacterial meningitis. From this study, it can be concluded that the *Coix lacryma-jobi* oil from roots and seeds possesses antibacterial activity for bacterial meningitis treatment. Furthermore, our results support the use of the plants in traditional medicine and suggest that *Coix lacryma-jobi* oil possesses compounds with good antibacterial properties. Thus, it can be used as an antibacterial supplement in developing countries towards the development of a new therapeutic agent. However, additional in vivo studies and clinical trials would be needed to justify and further evaluate its potentials as an antibacterial agent for topical or oral applications.

5. Conclusions

Based on the observation of the antioxidant and antibacterial potential of the investigated oil from *Coix lacryma-jobi* roots and seeds, it can be concluded that this oil is able to prevent bacterial meningitis and becomes a potential therapeutic agent for bacterial meningitis treatment. The results also suggest that oil extracted from *Coix lacryma-jobi* roots and seeds can be used in bacterial meningitis treatment as a natural antioxidant and antibacterial. The antioxidants and antibacterial present in the *Coix lacryma-jobi* oil may function by combining with the pharmaceutical components. However, it is important to further investigate the in vivo potentials of the *Coix lacryma-jobi* oil and also isolate the active components, which can ultimately lead to their application in the pharmaceutical formulations industry as antioxidant and antibacterial agents for bacterial meningitis treatment.

6. Conflict of interest statement

All authors stated that they have no conflict of interest in the results of this study.

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