

# Phytochemical Screening and Antibacterial Activity *Coix lacryma-jobi* Oil

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Received: 27 January 2020 / Revised: 17 March 2020 / Accepted: 17 March 2020  
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**Abstract** *Coix lacryma-jobi* (Hanjeli) is known to possess anti-microbial properties. Therefore, phytochemical compounds of *C. lacryma-jobi* have been studied to produce novel anti-microbial agents as treatments against antibiotic-resistant bacteria. The objective of this study was to determine the phytochemical composition and antibacterial activity of the *C. lacryma-jobi* oil against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. The phytochemical composition of the oil was determined via gas chromatography mass spectrophotometry (GC-MS). Moreover, agar disk and agar well diffusion were employed to screen the antibacterial activity of the oil. An agar well diffusion test was implemented to determine MIC's (minimum inhibitory concentrations). Dodecanoic acid, tetradecanoic acid, 2,3-dihydroxypropylester, 1,3-dioctanoin, N-methoxy-N-methyl-3,4-dihydro-2H-thiopyran-6-carboxamide, propanamide, 5-Amino-1-(quinolin-8-yl)-1,2,3-triazole-4-carboxamide, and pyridine were identified in the *C. lacryma-jobi* oil. The MIC value of the oil was 0.031 g/L and the MBC of the oil was 0.125 g/L effective in all test bacteria. Dodecanoic acid displayed inhibitory activity against gram-positive and gram-negative bacteria. Therefore, our research demonstrated *C. lacryma-jobi* (Hanjeli) oil exhibited antibacterial activity against *E. coli*, *S. aureus*, and *B. subtilis*.

These research suggest that *C. lacryma-jobi* root oil could be used for medicinal purposes; however clinical and in vivo tests must be performed to evaluate its potential as an antibacterial agent.

**Keywords** Phytochemical, antibacterial, essential oil, GCMS, MIC (Minimum Inhibitory Concentrations)

## Introduction

Some species of plants are invaluable resources useful in routine life as an alternative food, food additives, herbs, aroma, color or used directly in pharmaceuticals (Azwanida 2015; Duke 2017). These plant species have content phytochemical properties that have the potential to prevent or treat diseases caused by bacteria (Azwanida 2015; Elisha et al. 2017). The uses of plant extracts and oils in dealing of human diseases have long been firm (Daniel et al. 2015; Mandal and Mandal 2015). Almost plant extracts have been proven to have antimicrobial property agents that are active against microorganisms in in vitro conditions (Balouiri et al. 2016; Calo et al. 2015; Restuati et al. 2016).

Medicinal plant species used in traditional medicine are effective in dealing with diseases caused by bacteria or oxidant stress (Baydoun et al. 2015; Singh et al. 2017; Restuati and Diningrat 2018). The phenolic phytocomponents from plants play an important role as antimicrobial agents (Diningrat et al 2018; Smeriglio et al. 2017). These antimicrobial agents work by destroying microorganisms through decaying the protein components of the cell wall, disrupting the work of enzymes and also DNA and RNA replication (Bakal et al. 2017).

Essential oils produced from plants are used in industry as food seasonings, pharmaceuticals and medicines (Silou et al. 2017). These oils are designed as a new source of antimicrobial drugs, especially against antibiotic-resistant

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bacteria (Baydoun et al. 2015; Silou et al. 2017). The effectiveness of essential oils as antibacterial has been divided into adequate, moderate or poor quality (Knezevic et al. 2016). These essential oils are also produced by plants as a armament product against several natural enemies. In addition, these essential oils are used to continue their natural growth and development. Even essential oils can contain a number of secondary metabolites or phytochemical compounds in response to some external stress (Knezevic et al. 2016; Silou et al. 2017).

*Coix lacryma-jobi* is a family of poaceae in the form of panicoid grass, also called Adlay or in some parts of Indonesia called Hanjeli. This annual plant in Indonesia grows wild and is cultivated in some areas which are used as popular nutritional supplements in Chinese traditional medicine. Traditionally, Hanjeli has been used to treat bumps on the skin, bumpy skin, neuralgia, and rheumatism. Modern research shows that Hanjeli seeds have shown bio-activity as anti-inflammatory, anti-mutagenic, anti-cancer, anti-allergic, anti-obesity, anti-microbial, and anti-oxidant properties (Bon et al. 2017). There is no information yet on essential oils from *C. lacryma-jobi* root having antimicrobial activity. The importance of this study aims to evaluate the in vitro antibacterial activity of *C. lacryma-jobi* root oil in several bacteria *E. coli*, *S. aureus*, and *B. subtilis*.

## Materials and Methods

### Plant sample collection and oil extraction

Hanjeli collected from Pamah Village Area, Semelir District, Langkat District North Sumatra 20773, Indonesia. This research project was conducted from February 2019 to June 2019. Sample of hanjeli roots are washed from impurities and other contaminants.

Distillation of *C. lacryma-jobi* essential oil was carried out with Steam Distillation: This distillation method draws aromatic molecules from plant roots using heat steam. The power of hot steam could open a storage area where oil was stored in plant material. The essential oil molecules are released from the root of the material *C. lacryma-jobi* and then evaporate into steam. Steam temperature needs to be controlled so that it can force the roots of *C. lacryma-jobi* to release oil, but excessive heat to burn the roots of *C. lacryma-jobi* and damage the oil. The resulting essential oil is passed through a cooling system to condense vapors. Dew is a liquid consisting of essential oils and water that must be separated. The vapors produced in this system come

from pressures greater than the atmosphere so that they boil above 100 degrees Celsius which facilitates the removal of oil from plant material at a faster rate, and this condition prevents damage to the oil (Duke 2017; Mandal and Mandal 2015).

### Phytochemical Screening

To identify tannins, terpenoids, flavonoids, alkaloids, phenols, phytosterols and saponins in *C. lacryma-jobi* root oil used standard phytochemical analysis methods according to the Balouiri et al. (2016) and Mandal and Mandal (2015).

### Gas chromatography mass spectrometry (GC-MS)

To analyze the phytochemical compounds of *C. lacryma-jobi* oil GC-MS was used. DB-5 column silica diffusion film was used as thick as 0.25  $\mu\text{m}$ . The oven temperature setting is at 500°C for 5 minutes. Programming the temperature from 50-2800°C for 40 minutes. The helium flow rate is set in such a way that is maintained at a speed of 2 ml / min, with a split ratio of 1: 3. 1  $\mu\text{l}$  of sample is injected with an ionization voltage which is run by the EI technique at 70eV for MS analysis. The volatile essential oil constituents of the MS analyst were identified by matching databases on the engine. The retention index used is the Spectra standard which is matched with the fragmentation patterns that exist in the Mass Spectra29 database. The NIST standard reference database (AMDIS version 2.70) is used to interpret mass spectral data generated from machine readings (Diningrat et al. 2018; Hussein et al. 2016)

### Microorganism sources

The antibacterial activity of *C. lacryma-jobi* oil was assessed against three bacteria species: *E. coli* (ATCC No. 25922), *S. aureus* (ATCC No. 25923) and *B. subtilis* (ATCC No. 21332), maintained in Laboratory of Department Biology Universitas Negeri Medan, Indonesia at -20°C. Each bacterial strain used in this study was activated in the Nutrient broth (NB) medium at 37°C for 18 hours. 60  $\mu\text{l}$  of bacteria cultured in NB was subcultured to Nutrient agar (NA) and then incubated at 37°C for 24 hours. The concentration of bacterial suspension used for the study was then adjusted to obtain a final concentration of around 10<sup>8</sup> cfu / ml using NB dilution.

### Culture media

Nutrient Broth (NB) and Nutrient Agar (NA) used in this

study are microbiological growth media commonly used for testing antibiotic sensitivity regulated according to company instructions (Sigma Inc.). The medium is autoclaved and poured as much as 20 ml per plate in a 12 cm Petri dish. The NB to be used is incubated overnight to ensure medium sterility before use.

#### Evaluation of antimicrobial activities

To evaluate the antibacterial properties of *C. lacryma-jobi* oil the standard protocol of the disc agar and well diffusion agar method were used. 1 g/L of *C. lacryma-jobi* essential oil resulting from steam distillation is prepared in six serial dilutions (v / v). Each of the 60 µl dilution results are poured in each disk in sequence. As a positive control this study used Kanamycin, while DMSO was used as a negative control. After 24 hours incubation, the diameter of the zone of inhibition against bacterial growth around the disk and well was measured. Using this protocol can determine two values for antibacterial activity, Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC). For determining the MIC and MBC values, a microbroth dilution method is also applied. Determination of MBC can be determined by pouring 60 µl MIC tubes and six dilutions before filling the agar plate, after the incubation period. The lowest concentration that makes no microbial growth is determined as MBC (Balouiri et al. 2016; Restuati et al. 2016; Restuati and Diningrat 2018).

#### Statistical Analysis

The notation for antibacterial activity is expressed in terms of the mean value ± standard error. The resulting data were

analyzed by One-way variance analysis (ANOVA) using SPSS 22 software. Data with P value lower than 0.05 ( $p < 0.05$ ) were considered to be significantly different (Balouiri et al. 2016; Restuati et al. 2016; Restuati and Diningrat 2018).

## Results and Discussion

#### Chemical composition

The investigation of phytochemical screening was The oil of *C. lacryma-jobi* root. The result revealed that the *C. lacryma-jobi* root oil recorded the presence of alkaloid, flavonoid, tannins, Reducing compounds, Flavonoid Anthracenoid, steroids, and Carotenoids (Table 1).

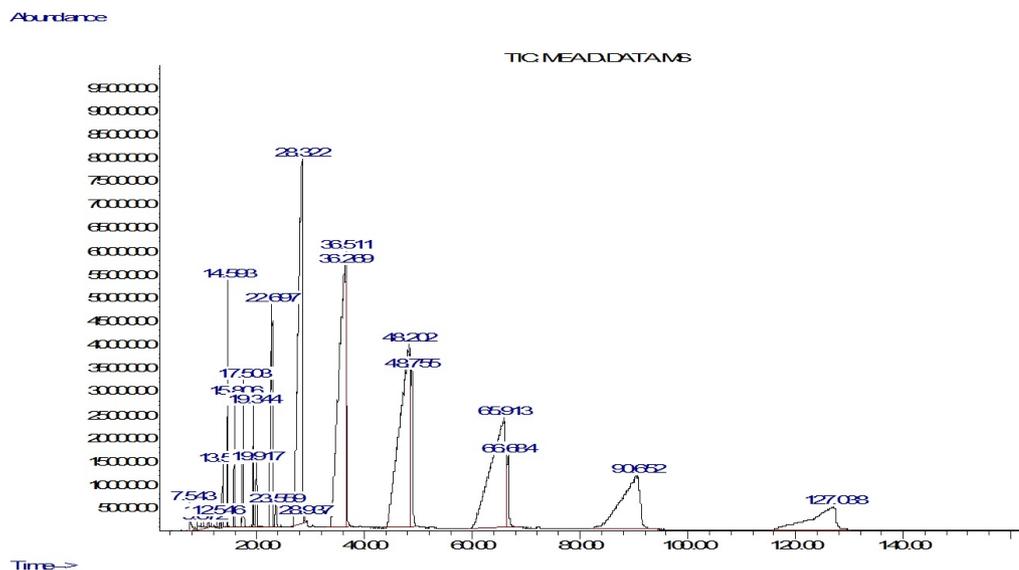
Table 2 and Fig. 1 show the results of GC-MS analysis on the *Coix lacryma-jobi* root essential oil. The analysis showed the presence of anti-microbial compounds. Data shown from the results of GC-MS analysis are active fundamental data in the form of Retention Time (RT),

**Table 1** Preliminary phytochemical screening of *Coix lacryma-jobi* root oil

Chemical groups	Root essential oil
Tannins	+
Alkaloid salts	+
Reducing compounds	+
Flavonoid	+
Anthracenoid	+
steroids	+
Carotenoids	+

**Table 2** GC-MS analysis of phytocompounds identified in *Coix lacryma-jobi* root oil

PEAK	R.T	AREA %	COMPOUND NAME	Activity
1	7.541	0.30	Dodecanoic acid	Displaying inhibitory activity against gram-positive and/or gram-negative organisms
2	9.670	0.16	Tetradecanoic acid	Antitumor activity
3	11.046	0.04	2,3-dihydroxypropyl ester	Pheromone system
4	12.542	0.04	1,3-Dioctanoin	Antiobesity
5	13.585	0.19	N-Methoxy-N-methyl-3,4-dihydro-2H-thiopyran-6-carboxamide	Antioxidant and neuroprotective
6	14.594	1.13	Propanamide	React in many different organic processes to form other useful compounds for synthesis.
7	15.808	0.79	5-Amino-1-(quinolin-8-yl)-1,2,3-triazole-4-carboxamide	Antiviral, antibacterial, antifungal, antituberculosis, anticonvulsant, antidepressant, anti-inflammatory, anticancer
8	17.500	1.50	Pyridine	Vitamins, food flavorings, paints, dyes, rubber products, adhesives, insecticides, and herbicides



**Fig. 1** GC-MS chromatogram for *Coix lacryma-jobi* root oil

**Table 3** Diameters of growth inhibition zones in agar disk diffusion test in different dilutions of *Coix lacryma-jobi* root oil

Dilution (g/L) Microorganism	Inhibition zone (mm) in disk diffusion		
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>
Positive control	22	26	22
0.125	21	29	24
0.062	15	17	15
0.031	9	10	9
0.015	4	4	3
0.007	0	0	0
0.003	0	0	0
Negative control	0	0	0

Molecular Formula (MF), Molecular Weight (MW), and Concentration (peak area%). The oil of *C. lacryma-jobi* roots were subjected to GC-MS analysis. Where the data for means the oil of *Coix lacryma-jobi* roots, some of the important compounds detected in the ethanol fraction include which Dodecanoic acid, Tetradecanoic acid, 2,3-dihydroxy-propylester, 1,3-Dioctanoin, N-Methoxy-N-methyl-3,4-dihydro-2H-thiopyran-6-carboxamide, Propanamide, 5-Amino-1-(quinolin-8-yl)-1,2,3-triazole-4-carboxamide, Pyridine. Based on Pub-chem software analysis results on GC-MS results, it is obtained that the bioactivity of these compounds as Antitumor, Antibesities, Antioxidant, Neuroprotective, Antiviral, Antibacterial, Anti-fungal, Antituberculosis, Anticonvulsant, Antidepressant, Anti-inflammatory, Antioxidant, Neuroprotective, Antiviral, Antibacterial, Anti-fungal, Antituberculosis, Anticonvulsant, Antidepressant, Anti-inflammatory, Antioxidant, Neuroprotective, Antiviral, Antibacterial, Anti-fungal, Antituberculosis, Anticonvulsant, Antidepressant, Anti-inflammatory, Anti-oxi-

dant, Anti-cancer, Vitamin, food seasoning, adhesive, insecticides, and herbicides.

#### Agar disk diffusion test

The results of testing the antibacterial activity of essential oils showed that *E. coli*, *S. aureus* and *B. subtilis* were sensitive to the un-diluted *C. lacryma-jobi* root oil. The results inhibition zones based on different dilutions are listed in Table 3. The test results also showed that the DMSO treatment did not have inhibitory zones.

#### Agar well diffusion test

Table 4 shows that the widest of the diameter inhibition zone in *S. aureus* (29 mm) growth was seen at a concentration of 0.125 g / L of *Coix lacryma-jobi* root oil from steam distillation. At a concentration of 0.007 g / L there was

**Table 4** Diameters of growth inhibition zones in agar well diffusion test in different dilutions of *Coix lacryma-jobi* root oil

Dilution (g/ml)	Inhibition zone (mm) in well diffusion		
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>
Microorganism			
Positive control	22	25	23
0.125	20	29	21
0.062	14	14	14
0.031	8	9	8
0.015	0	8	0
0.007	0	0	0
0.003	0	0	0
Negative control	0	0	0

**Table 5** MIC and MBC of the oil of *Coix lacryma-jobi* roots

Microorganism	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>
MIC	0.031	0.031	0.031
MBC	0.125	0.125	0.125

no inhibition of bacterial growth.

#### MIC and MBC ascertaining

The lowest MIC value that can be read from observations is at a concentration of 0.031 g / ml for *E. coli* / *S. aureus* *B. subtilis*. While the MBC value of the *Coix lacryma-jobi* root essential oil was 0.125 g / ml for *E. coli*, *S. aureus* and *B. subtilis* (Table 5).

Table 5 shows, the oils of *C. lacryma-jobi* roots have prevented the growth of *E. coli*, *S. aureus* and *B. subtilis*. The result also showed, by increasing the concentrations of the oil of *Coix lacryma-jobi* roots, the inhibition zone increased. The most sensitivity was observed in *S. aureus*.

Table 5 shows that the *C. lacryma-jobi* root essential oil has prevented the growth of *E. coli*, *S. aureus* and *B. subtilis*. This result also shows that the more the concentration of *Coix lacryma-jobi* root essential oil increases, the inhibition zone increases. The best sensitivity of the *C. lacryma-jobi* essential oil is most sensitive to *S. aureus*. The results of statistical analysis on the results of this study indicate that the sensitivity of *C. lacryma-jobi* root essential oil to all bacteria tested, there is a significant difference ( $p \leq 0.005$ ).

## Discussion

*Coix lacryma-jobi*, is an annual flowering plant with white flower is native to Asia include Indonesia. Grains from hanjeli plants are commonly used to cook traditional dishes,

such as porridge (Bon et al. 2017). *Coix lacryma-jobi* has also been used by thousands of people to improve their natural healing abilities (Hussein et al. 2016). Regarding the essential oil distillation method used aims to prevent damage at high temperatures and to reduce the level of damage to effective herbal compounds contained in the oil.

Plant oils have been used in pharmaceuticals, alternative medicine and natural therapies for many thousands of years. Phytochemical compounds contained in essential oils such as tannins, flavonoids and some aromatic compounds or secondary metabolites function as armament in the face of predation either by organisms or many microorganisms. These substances function molecularly as plant defenses against predation by microorganisms, insects, and herbivores. Furthermore, these compounds include plant odors (terpenoids), pigmentation (tannins and quinine), and taste (capsacin) (Knezevic et al. 2016; Silou et al. 2017). The GC-MS analysis results from compounds identified from the essential oil of *C. lacryma-jobi* root have bioactivity as antimicrobial, anti-inflammatory, neuroprotective, and strong antioxidant activity. This is in line with the study of the structure and kinetics of n-hexedeconic acid (palmitic acid) which states that this acid is an inhibitor of phospholipase compounds and acts as an antimicrobial (Hussein et al. 2016; Knezevic et al. 2016).

Therefore initial screening tests were useful in detecting bioactive principle compounds which can further lead to research on drug discovery and development. *Coix lacryma-jobi* essential oil has potential as a new antimicrobial source against bacterial pathogens. In vitro studies in this research indicate that *C. lacryma-jobi* essential oil has an effective

ability to inhibit pathogens bacterial growth. Earlier reports stated that the antimicrobial activity of many essential oils that had been reviewed was classified as average or medium (Balouiri et al. 2016). However, Coix essential oil is classified in the high classification.

All of these phytochemicals contained in oil had antibacterial effects. The results showed that the *C. lacryma-jobi* root essential oil with a concentration of about 0.031 g / L had inhibited the growth of *E. coli*, *S. aureus* and *B. subtilis*. Thus, this study shows that *C. lacryma-jobi* essential oil is medically antibacterial in gram-negative and gram-positive pathogenic bacteria. A number of authors have mentioned the antimicrobial activity of *C. lacryma-jobi* extract but not oil. The essential oil of *C. lacryma-jobi* roots showed antibacterial activity against *E. coli*, *B. cereus* and *S. aureus*, our results are in agreement with others who showed that *C. lacryma-jobi* roots oil produce antimicrobial activity against a broad range of microbes.

## Conclusion

Based on the results of this study it can be concluded that the essential oil of *C. lacryma-jobi* root has antibacterial activity with inhibition classified as good. Significant statements from this research are that: Essential oil of *C. lacryma-jobi* root can be used as an antibacterial supplement that potential for the development of new therapeutic agents. Additional in vivo and clinical tests are important to justify and further evaluate the potential of *C. lacryma-jobi* root essential oil as an antibacterial agent.

## Conflict of interest statement

We are all authors of this study stated that we have no conflict of interest in the results of this study.

## Acknowledgments

We, the authors wish to thank Biology Department FMIPA Universitas Negeri Medan, LPPM Universitas Negeri Medan, DRPM Kemenristekdikti Republic of Indonesia (Grant No. 36/UN33.8/PL.DRPM/2019).

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