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Tracheospasmodic and anti-inflammatory activity of indigenous *Curcuma* species as traditional antiasthmatic medicines

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Abstract. Paramita S, Moerad EB, Ismail S, Marlina E. 2018. Tracheospasmodic and anti-inflammatory activity of indigenous *Curcuma* species as traditional antiasthmatic medicines. *Nusantara Bioscience* 10: 105-110. Asthma is still a major health problem in the world. Antiasthma drugs raise the problem of side effects and medical expenses. Thus, it is necessary to develop medicinal plants based antiasthma drugs with fewer side effects and more affordable costs. The potential antiasthma medicinal plant came from the genus of *Curcuma* (Zingiberaceae family). Turmeric (*Curcuma longa* L.) is the most prominent species in this genus and has been extensively studied for the treatment of asthma. However, there are other *Curcuma* indigenous species that are also traditionally used for asthma, namely *Curcuma aeruginosa* Roxb. and *Curcuma mangga* Val. Zipp. Both indigenous species have not been studied pharmacological activity as an antiasthma. This research was conducted to know the tracheospasmodic activity of ethanol extract of *C. aeruginosa* and *C. mangga* on the separate organ of guinea pig trachea to determine the antiasthma effect. The research was also conducted to know anti-inflammatory activity with membrane stabilization test and inhibition of lipoxigenase. Results showed that *C. aeruginosa* and *C. mangga* had lower tracheospasmodic activity when they were compared with aminophylline as a positive control. However, the EC₅₀ value of *C. aeruginosa* (0.020 ± 0.004) and *C. mangga* (0.039 ± 0.005) were not significantly different with aminophylline (0.016 ± 0.005) as positive controls. The result of membrane stabilization test showed that the EC₅₀ value of *C. aeruginosa* (47.76 ± 1.57) and *C. mangga* (67.50 ± 0.97) were high when they were compared with indomethacin (26.39 ± 2.91) as the positive control. Results of lipoxigenase inhibition test showed that the EC₅₀ value of *C. aeruginosa* (-111.11 ± 2.30) and *C. mangga* (-101.98 ± 1.32) were high than that of indomethacin (-282.84 ± 7.41) as the positive control. These results show the potential use of *C. aeruginosa* and *C. mangga* as antiasthma via tracheospasmodic activity in separate organs of guinea pig. They also had anti-inflammatory effect based on membrane stabilization and inhibition of lipoxigenase test.

Keywords: Anti-asthma, anti-inflammatory, *Curcuma aeruginosa*, *Curcuma mangga*, tracheospasmodic

INTRODUCTION

Asthma is a chronic inflammatory airway disease characterized by the occurrence of respiratory airway hyper response and reversible narrow airway (Kasper et al. 2015). Asthma is one of the major non-communicable diseases in the world. About 235 million people worldwide suffer from asthma, especially in children. The main risk factor for the occurrence of asthma is a combination of genetic susceptibility to environmental exposures such as air pollution (WHO 2018). Asthma medications are given to manage asthma sufferers (Papadakis and McPhee 2013). Herbal preparations are one of the most popular complementary treatment used by asthmatic patients. Almost 40% of people with asthma use herbal medicines. Many important asthma drugs such as B₂-agonists, anticholinergics, methylxanthines, and cromones have herbal origins (Karaman et al. 2012). Research also shows that some medicinal plants have the effect of reducing

smooth muscle stiffness. The mechanism of those medicinal plants is similar to the mechanism of asthma drugs, especially the anticholinergic drug (Amit and Vandana 2013). Research also shows that some medicinal plants have the anti-inflammatory effect, which is the same with the mechanism of corticosteroid drug in asthma drugs (Paramita et al. 2017).

The genus *Curcuma* (Family Zingiberaceae) comprising of more than 100 species, have been recognized widely as food and traditional medicines. *C. longa* (turmeric) is the most studied species of all *Curcuma* species (Akarchariya et al. 2017). Indonesia is home to many species of *Curcuma* and several species of these have been commonly used as traditional medicines. The various species of *Curcuma* often used are *C. longa* (turmeric), *C. xanthorrhiza*, *C. heyneana*, *C. aeruginosa*, *C. mangga*, and *C. zedoaria*. Turmeric appears to be the species, which is most frequently used as raw materials for traditional medicine in Indonesia, followed by *C. xanthorrhiza*. The

other species, including *C. aeruginosa* and *C. mangga*, are considered as indigenous *Curcuma* species, which are not intensively studied, yet (Rahayu et al. 2011). The rhizomes of other *Curcuma* species are also pharmacologically important but several of these species are not exploited commercially (Shakeri et al. 2017).

Important medicinal plants from genus *Curcuma* having anti-asthmatic potential is *C. longa*. Other rhizomes of *Curcuma* species are traditionally used in the treatment of asthma, i.e., *C. aeruginosa* (Rajamma et al. 2012), *C. mangga* (Dewangan et al. 2014), *C. caesia* (Pathan et al. 2016a; Pakkirisamy et al. 2017), and *C. zedoaria* (Pathan et al. 2015). The examination of antiasthma effects of two indigenous *Curcuma* species, i.e., *C. aeruginosa*, and *C. Mangga* is not studied, yet. Therefore, the objective of this study was to know the tracheospasmodic and anti-inflammatory activity of *C. aeruginosa* and *C. mangga* applied on isolated trachea of guinea pig.

MATERIALS AND METHODS

Sampling and sample identification

The sampling of medicinal plants was conducted at the Kutai Kartanegara District, East Kalimantan. Plants were then identified in the Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University to certify the plant legality. This study has obtained the ethical clearance of the Medical and Health Research Ethics Commission, Faculty of Medicine, Mulawarman University. This study was conducted in the Pharmacology Laboratory of the Faculty of Medicine, Mulawarman University.

Plant extractions

The extraction of medicinal plant followed the guidelines from the Indonesian Herbal Pharmacopoeia (MoH RI 2008). The rhizomes of *C. aeruginosa* and *C. mangga* were dried and then crushed into simplicia. The

simplicia were then macerated using absolute ethanol solvent in a ratio of 1 part of simplicia to 10 parts of solvent. The mixture was soaked for 6 hours followed by stirring occasionally with an orbital shaker at room temperature in each interval hours, which were then stood for 18 hours. The mixture was separated using filter paper, followed by the evaporation using a rotary evaporator at 50°C. The obtained viscous extract was dried to obtain a dry extract. The dried extracts were then stored in a refrigerator of -20°C for further study.

Tracheospasmodic activity

Tracheospasmodic activity of medicinal plant extracts was tested in separated organs of tracheal guinea pigs via histamine injection. Guinea pigs were sacrificed and the trachea was quickly dissected by adhering fat and connective tissue. Trachea was placed in the petri dish containing Krebs Physiological Salt Solution (PSS). The rings were suspended in L-shaped wire loops in 10 ml organ baths containing the Krebs PSS aerated with carbogen by maintaining the temperature at 37°C. Isometric contractions of tracheal rings were measured by the transducer (Force Transducers) coupled to the amplifier (BridgeAmp) connected to PC running ChartV5 software, all from ADInstruments (Dunedin, New Zealand). An equilibration period of 90 minutes was allowed in Krebs PSS, at the end of the period, the tracheal rings were stimulated with histamine in order to establish viability. After equilibration, the tracheal rings were exposed to aminophylline as positive control drugs and medicinal plants extract of *C. aeruginosa* and *C. mangga* according to the experimental protocol. In each preparation, a single concentration-relaxation curve was obtained. The relaxant effect induced by positive control drug and medicinal plant extracts was expressed as the reverse percentage of initial contraction force, which was elicited by histamine in tracheal rings (Ozolua et al. 2010; Janbaz et al. 2011; Lima et al. 2011).



Figure 1. A. Rhizome of *Curcuma aeruginosa* Roxb., B. Rhizome of *Curcuma mangga* Val. Zijp

Anti-inflammatory activity

Anti-inflammatory activity of medicinal plant extracts was measured by membrane stabilization and lipoxygenase inhibition test. For membrane stabilization test, blood samples were collected into an anticoagulant and centrifuged at 3000 rpm for 10 minutes at room temperature. The supernatants (plasma and leucocytes) were carefully removed while the packed red blood cell was washed in fresh normal saline. The process of washing and centrifugation was repeated five times until supernatants were clear. The membrane stabilizing activity assay was carried out using erythrocyte suspension while Indomethacin was used as drug standard. The mixtures consisted of hyposaline sodium chloride, sodium phosphate buffer, erythrocyte suspension, drug standard, plant extracts and final reaction mixtures were mixed to produce isosaline. Drugs were mixed in the blood control, while the drug control did not contain the blood suspension. The reaction mixtures were incubated at 56°C for 30 min on a water bath. The tube was cooled under running water followed by centrifugation at 5000 rpm for 10 min at room temperature. The supernatant was collected. The absorbance of the released hemoglobin from the supernatant was measured at 560 nm (Omale and Okafor 2008; Oyedapo et al. 2010).

For lipoxygenase inhibition test, a mixture of a solution of sodium borate buffer and lipoxygenase enzyme was incubated with plant extract sample at room temperature for 5 minutes. The reaction was started by the addition of linolic acid substrate. The absorbance of the resulting mixture was measured at 234 nm as a function of time at a rate of one measurement/minutes (3 readings). Indomethacin was used as a positive standard (Khasawneh et al. 2011; Singh et al. 2012).

Data analysis

Tracheospasmodic activity is tabulated in the mean \pm SE curve of the dose-response. The value of EC_{50} was calculated. The homogeneity and variance analysis were conducted when the data showed the normal distribution and variance followed by t-test analysis. The significant difference was performed when $p < 0.05$. Membrane stabilization activity and lipoxygenase inhibition were tabulated in mean \pm SD form, in order to calculate EC_{50} values. The difference between the control was performed based on t-test analysis with the significance of $p < 0.05$.

RESULTS AND DISCUSSION

Tracheospasmodic activity

The results of spasmodic activity of ethanol extract of *C. aeruginosa* on separated organ of trachea guinea pig at 0.01, 0.03, 0.1, 0.3, 1 and 3 mg/ml concentrations were as follows: (4.90 \pm 2.40), (2.44 \pm 1.86), (1.02 \pm 3.08), (0.01 \pm 3.51), (-0.48 \pm 3.69), (-2.02 \pm 4.33); *C. mangga* (3.39 \pm 2.77), (3.25 \pm 2.70), (3.13 \pm 2.57), (3.11 \pm 2.58), (2.98 \pm 2.47), (1.90 \pm 2.23); and on control (solvent extract) obtained results (2.31 \pm 0.61), (3.68 \pm 0.42), (5.54 \pm 0.97),

(6.00 \pm 0.47), (5.85 \pm 0.57), (4.80 \pm 0.86); whereas in aminophylline obtained results (0.82 \pm 1.01), (0.68 \pm 1.32), (-3.94 \pm 3.19), (-23.78 \pm 5.53), (-57.78 \pm 5.14), (-93.68 \pm 3.01). The result of trachea relaxation between negative control, aminophylline and group with plant extracts is presented in Figure 1. The result showed that the decrease of spasmodic activity of *C. aeruginosa* and *C. mangga* in the guinea pig tracheal tone was significantly better ($p < 0.05$) than that in negative control.

The EC_{50} value of *C. aeruginosa* (0.020 \pm 0.004) and *C. mangga* (0.039 \pm 0.005) was higher than aminophylline (0.016 \pm 0.005) as a positive control. This shows that the effect of the plant extract was not as strong as aminophylline, as shown in Figure 2. However, the *C. aeruginosa* had indifferent value from that of aminophylline as a positive control. This suggests that *C. aeruginosa* has a better efficacy than *C. mangga* as spasmodic when they were compared with aminophylline.

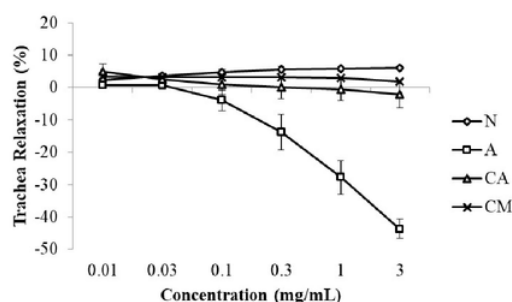


Figure 1. Graph of trachea relaxation differences between *C. aeruginosa* (CA), *C. mangga* (CM), aminophylline (A) as the positive control and negative control (N) in trachea isolates organ of guinea pig

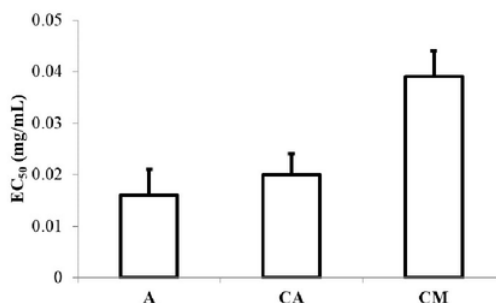


Figure 2. The EC_{50} result on tracheospasmodic activity between *C. aeruginosa* (CA), *C. mangga* (CM) and aminophylline (A) as a positive control ($p < 0.05$).

Anti-inflammatory activity

The result of membrane stabilization test showed that the EC₅₀ value of *C. aeruginosa* (47.76 ± 1.57) mg/mL and *C. mangga* (67.50 ± 0.97) mg/mL were higher compared with indomethacin (26.39 ± 2.91) mg/mL as the positive control, as shown in Figure 3. The results of lipoxygenase inhibition test showed that the EC₅₀ value of *C. aeruginosa* (-111.11 ± 2.30) µg/mL and *C. mangga* (-101.98 ± 1.32) µg/mL were also higher than indomethacin (-282.84 ± 7.41) µg/mL as the positive control (Figure 4). The smaller EC₅₀ concentration determines the better anti-inflammatory activity based on membrane stabilization test and lipoxygenase inhibition. The results showed that indomethacin as a positive control had a better anti-inflammatory activity than that in *C. aeruginosa* and *C. mangga*, based on membrane stabilization test and lipoxygenase inhibition.

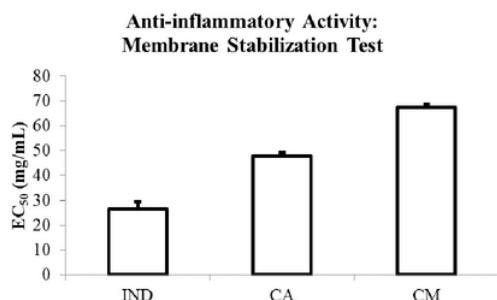


Figure 3. The EC₅₀ results on membrane stability test between *C. aeruginosa* (CA) and *C. mangga* (CM) with indomethacin (IND) as the positive control ($p < 0.05$)

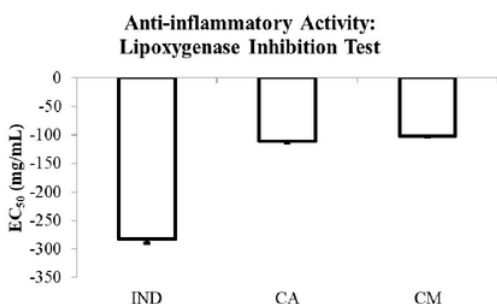


Figure 4. The EC₅₀ results on lipoxygenase inhibition test between *C. aeruginosa* (CA) and *C. mangga* (CM) with indomethacin (IND) as the positive control ($p < 0.05$)

Discussion

Curcuma longa from genus *Curcuma* is the most important medicinal plants, which is traditionally used as antiasthma for the treatment of respiratory disorders such as asthma, bronchitis and allergic reactions in the respiratory tract (Gonuguntla et al. 2013). Anti-asthmatic and antioxidant property of *C. longa* has been tested in the experimental animal model of asthma (Prasad et al. 2009). Bronchodilatory effect of *C. longa* may be due to the mast cell stabilizing activity, suppression of immunoglobulin E, and inhibition of inflammatory mediators (Gohil and Mehta 2011; Dongre et al. 2015).

Curcuma caesia, *C. zedoaria*, *C. aeruginosa*, and *C. mangga* are another *Curcuma* species traditionally used as asthma treatment. *C. caesia* tested antiasthma for its relaxant effect in guinea pig trachea has been successfully reported as powerful protection against histamine-induced bronchospasm (Paliwal et al. 2011). *C. zedoaria* significantly decreased milk induced leukocytosis and showed anti-inflammatory activities (Pathan et al. 2016b). In this study, the examination of antiasthma effects of *C. aeruginosa* and *C. mangga* has been firstly reported.

Curcuma aeruginosa is known in Indonesia as “temu ireng” and “pink and blue ginger” in English (Nurcholis et al. 2012; Simoh and Zainal 2015). *C. aeruginosa* has been used as traditional medicine in South and Southeast Asia for gastrointestinal and uterine disorders, parasitic and fungal infection (Jose and Thomas 2014; Hossain et al. 2015; Theanphong et al. 2015). Sesquiterpenes have been found as common chemical constituents of *C. aeruginosa* (Suphrom et al. 2012).

Curcuma mangga was known as “temu mangga” in Indonesia, meaning mango-like turmeric (Kaewkroek et al. 2010). The rhizomes of *C. mangga* are used as traditional treatment for stomach and chest pain, fever, debility, bronchitis, aphrodisiac, postpartum care and cancer-related diseases (Wan-Ibrahim et al. 2010; Malek et al. 2011). The composition of *C. mangga* is dominated by sesquiterpenes and monoterpenes (Kamazeri et al. 2012).

It is suggested that curcumin with other chemical compounds from *C. aeruginosa* and *C. mangga* could show antiasthma activity. Anti-asthmatic and antioxidant property of curcumin has been tested in the experimental animal model of asthma (Houssen et al. 2010). Curcumin is acted to inhibit histamine release (Kale et al. 2012). Curcumin alleviates the pathological changes of chronic asthma, by reducing lung and airway inflammation in asthma induced animal (Chong et al. 2014; Yang et al. 2017). Curcumin improves the airway obstruction by inhibiting mast cell degranulation so that it is safely used as therapy for asthma patients (Subhashini et al. 2013; Abidi et al. 2014; Chauhan et al. 2014).

The results of this study indicate that ethanol extract of *C. aeruginosa* and *C. mangga* has an antiasthma effect based on the tracheospasmolytic and anti-inflammatory activity. Therefore, *C. aeruginosa* and *C. mangga* can be developed as a source of the new antiasthma drug.

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