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RESEARCH ARTICLE

Antiasthmatic effect of *Curcuma aeruginosa* extract on isolated organ of the trachea

[version 1; peer review: 1 approved with reservations, 2 not approved]

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Abstract

Background: Asthma is a major health problem worldwide. Antiasthma drugs have side effects and can be expensive. It is important to develop antiasthma drugs from medicinal plants that have fewer side effects and are cheaper. One of the medicinal plants used for antiasthma treatment comes from *Curcuma aeruginosa* (*Zingiberaceae* family). The aim of the research is to examine spasmolytic activity of ethanol extract of *C. aeruginosa* on isolated guinea pig tracheas to determine the antiasthma effects.
Methods: The spasmolytic activity of *C. aeruginosa* extracts was tested in separated organs of guinea pig trachea. Guinea pig was sacrificed and its trachea rings were suspended in L-shaped wire loops in organ baths containing the Krebs solution aerated with carbogen. Isometric contractions of tracheal rings were measured by the transducer coupled to the amplifier. The trachea rings were exposed to DMSO as negative control, aminophylline as positive control and *C. aeruginosa* extracts. The single concentration-relaxation curve was obtained in every preparation.
Results: The result showed that the decrease of the spasmolytic activity in the guinea pig tracheal tone due to *C. aeruginosa* extract was significantly better (p=0.022) when compared to the negative control. Meanwhile, the EC_{50} value of aminophylline (0.019 ± 0.05) was not significantly different (p=0.454) with *C. aeruginosa* (0.024 ± 0.05).
Conclusion: It could be concluded that *C. aeruginosa* extracts have the potency to be further developed as a new natural source of the antiasthma agents.

Keywords

antiasthma, Curcuma aeruginosa, spasmolytic

Open Peer Review

Approval Status ✗ ? ✗

	1	2	3
version 1 15 Nov 2018	✗ view	? view	✗ view

1. **Ian Sinha**, University of Liverpool, Liverpool, UK
2. **Waras Nurcholis**, IPB University, Bogor, Indonesia
3. **Sven-Erik Dahlén**, Karolinska Institute (KI), Stockholm, Sweden

Any reports and responses or comments on the article can be found at the end of the article.



This article is included in the ICTROPS 2018 collection.

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Author roles: Paramita S: Conceptualization, Project Administration, Supervision, Writing – Review & Editing; **Moerad EB**: Formal Analysis, Funding Acquisition, Resources; **Ismail S**: Data Curation, Methodology, Software, Writing – Original Draft Preparation; **Marliana E**: Investigation, Validation, Visualization

Competing interests: No competing interests were disclosed.

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Introduction

Asthma is an inflammatory airway disease characterized by the occurrence of an respiratory airway hyper response and reversible narrowing of the airway[1]. Asthma is one of the major non-communicable diseases in the world. About 235 million people worldwide suffer from asthma, particularly children. The strongest risk factors for developing asthma are a combination of genetic susceptibility to certain inhalable allergens and environmental exposure to them[2]. Asthma medications are given to manage asthma sufferers[3]. Herbal preparations are one of the most popular complementary treatments used by asthmatic patients. Many important asthma drugs such as B2-agonists, anticholinergics, methylxanthines, and cromones have herbal origins[4]. Some medicinal plants have the effect of reducing smooth muscle stiffness, similar to the mechanism of asthma drugs, especially the anticholinergic drugs[5]. Research has also shown that some medicinal plants have the anti-inflammatory effects, following the same mechanism of corticosteroid drug used in asthma treatment[6].

The genus *Curcuma* (family *Zingiberaceae*) consisting of more than 100 species is used widely as food a and in traditional medicines[7]. Indonesia is home to many species of *Curcuma*. The various species of *Curcuma* often used are *C. longa* (turmeric), *C. xanthorrhiza, C. heyneana*, *C. aeruginosa*, *C. mangga*, and *C. zedoaria.* Turmeric is the most frequently used plant for traditional medicine in Indonesia. *C. aeruginosa* considered as indigenous *Curcuma* species in Indonesia are currently not extensively studied, yet[8].

Important medicinal plants from the genus *Curcuma* with anti-asthmatic potential include *C. longa*[9]. Other rhizomes of *Curcuma* species are traditionally used in the treatment of asthma, i.e. *C. aeruginosa*[10], *C. mangga*[11], *C. caesia*[12], and *C. zedoaria*[13]. The antiasthma effects of *C. aeruginosa* are currently known, therefore, the objective of this study was to establish the tracheospasmolytic activity of *C. aeruginosa* applied on isolated tracheas of guinea pigs.

Method

Plant materials

The sampling of medicinal plants was conducted in Kutai Kartanegara District, East Kalimantan (0°59'51.1"S 116°58'33.1"E). Plants were then identified in the Faculty of Mathematics and Natural Sciences, Mulawarman University by comparing to the university herbarium collection.

Plant extractions

The rhizomes of *C. aeruginosa* were sliced and dried at room temperature for 3 days, crushed and transferred into a glass container. Approximately 1 kg of crushed rhizomes was soaked in 1 L of absolute ethanol (9401-03 Alcohol, Anhydrous, Reagent, J.T. Baker) for 5 days. The mixture was shaken occasionally with a shaker (3525 Incubator Orbital Shaker, Lab-Line, US). After 5 days, the materials were filtered (Whatman Filter Paper 11µm, Sigma-Aldrich) and evaporated using a rotary evaporator (RV06-ML Rotary Evaporator, IKA, Germany). The dried extracts were obtained and stored at 4°C in a dark bottle until use.

Experimental model

One male guinea pig (*Cavia porcellus*) (6 months old, 485 g) was obtained from Animal House Faculty of Medicine (Mulawarman University). They were treated in a controlled room temperature of 25°C, with a 12-hour light/dark cycle, and access to food pellets and filtered water *ad libitum*. The guinea pig was anesthetized intraperitoneally with a ketamine injection (Hameln Pharmaceuticals, Germany) at a dose of 60 mg/kg before the trachea was taken. After anesthetized, animals were euthanized by cervical dislocation. The trachea was quickly dissected by adhering fat and connective tissue of guinea pig.

Spasmolytic activity

The trachea rings were suspended in L-shaped wire loops in 10 ml organ baths (PL3508B6 Panlab Organ Bath System, ADInstruments), containing the Krebs solution (K3753 Krebs-Henseleit Buffer, Sigma-Aldrich) aerated with carbogen by maintaining the temperature at 37°C. Isometric contractions of tracheal rings were measured by the transducer (7004 Isometric Force Transducers, Ugo Basile) coupled to the amplifier (FE 221 BridgeAmp, ADInstruments) connected to PC running LabChart V5 software. An equilibration period of 90 minutes was done in Krebs solution. At the end of the equilibration period, the tracheal rings were stimulated with histamine in order to establish viability. After equilibration, the tracheal rings were exposed to DMSO (W387520 Methyl sulfoxide, Sigma-Aldrich) as the negative control, aminophylline (A1755 Aminophylline, Sigma-Aldrich) as positive control drugs and extract of *C. aeruginosa* according to the experimental protocol by Janbaz *et al.*[14]. The dosage for DMSO, aminophylline and plant extract were 0.0001, 0.0003, 0.001, 0.003, 0.01, and 0.03 mg/ml given 700, 750, 800, 850, 900, and 950 seconds after equilibration on the organ baths. The dose-response curve for trachea relaxation activity was obtained in every preparation.

Data analysis

Trachea relaxation activity is tabulated in the mean ± SD curve of the dose-response curve. The value of EC_{50} was calculated with Microsoft Excel 2016 as shown in Dataset 1. Data were analyzed using the Mann-Whitney because not normally distributed. All statistical analysis was performed using SPSS version 16.0 for Windows. A p-value of ≤ 0.05 was considered to be significant.

Ethical considerations

All protocols used in this experiment received approval from the Ethical Animal Care from the Medical and Health Research Ethics Commission, Faculty of Medicine, Mulawarman University No. 72/KEPK-FK/V/2018. All efforts were made to ameliorate any suffering of animals used in this research.

Results

The results of trachea relaxation between negative control, aminophylline, and *C. aeruginosa* extract presented in Figure 1. The result showed that the decrease of spasmolytic activity of *C. aeruginosa* extract was significantly better (p=0.000) than that in negative control. Meanwhile, the EC_{50} value of aminophylline (0.019 ± 0.05) was not significantly different (p=0.454) with *C. aeruginosa* (0.024 ± 0.05), as shown in Figure 2.

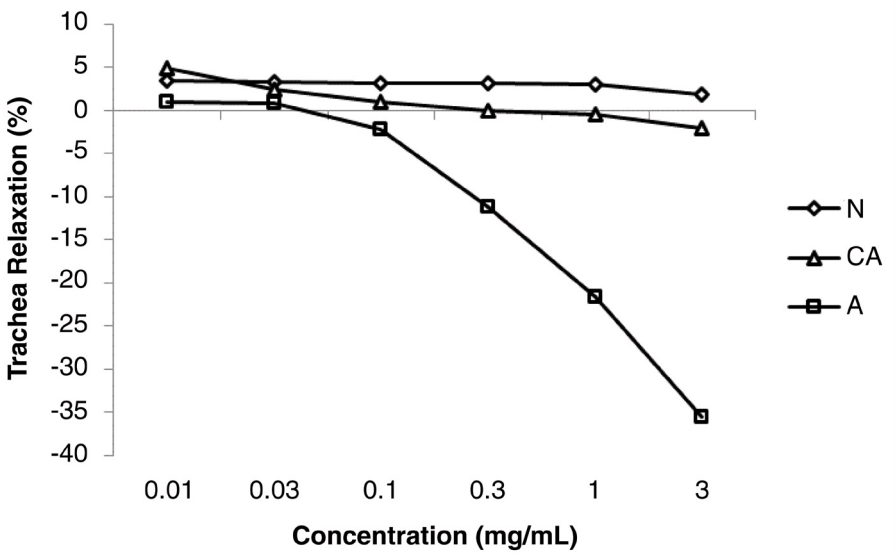


Figure 1. Graph of trachea relaxation differences between C. aeruginosa (CA), aminophylline (A) as the positive control and negative control (N) in isolated trachea of guinea pig.

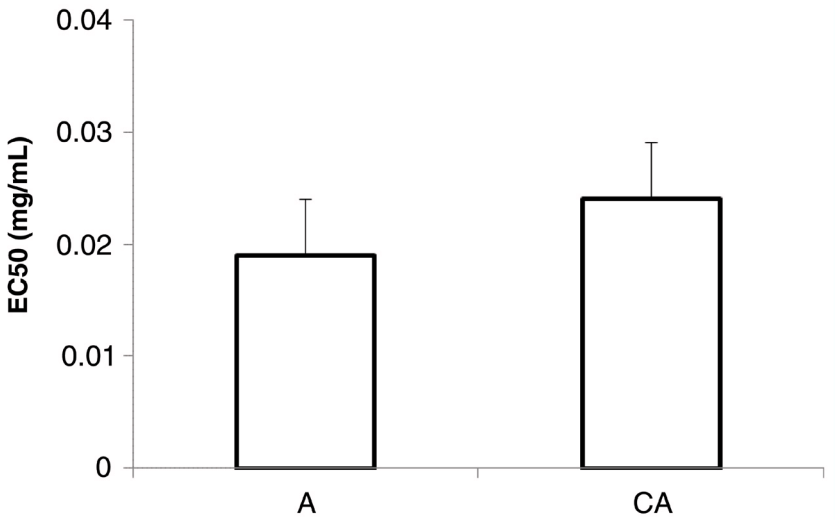


Figure 2. The EC_{50} result on trachea relaxation between C. aeruginosa (CA) and aminophylline (A) as a positive control.

Dataset 1. Trachea relaxation between C. aeruginosa (CA), aminophylline (A) and negative control (N) and EC50 result on trachea relaxation between C. aeruginosa (CA) and aminophylline (A)

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Discussion

C. aeruginosa (Supplementary File 1) is known in Indonesia as *temu ireng* or "pink and blue ginger" in English[15]. The color of fresh the rhizome can be yellows or greenish blue in color and mildly aromatic with a ginger-like aroma[16]. *C. aeruginosa* has been used as a traditional medicine in South and Southeast Asia[17]. The rhizomes have been used for gastrointestinal and uterine disorders, as well as parasitic and fungal infection[18].

Other pharmacological activities of *C. aeruginosa* that have been reported include inhibition of HIV, anti-cancer activity, hepatoprotective, antiandrogenic, estrogenic properties, antimicrobial, antioxidant, antiplatelet-activating factor-like, antipyretic, antinociceptive, and anti-inflammatory[19]. Germacrone, zedoarone, dehydrocurdione, curcumenol, zedoarondiol, and isocurcumenol were chemical constituents from sesquiterpenes isolated from rhizomes of *C. aeruginosa*[20]. In this study, the examination of

antiasthma effects of *C. aeruginosa* has been reported. Further research is needed to identify the chemical compounds from *C. aeruginosa* that could convey antiasthma activity.

Conclusion

The results of this study indicate that ethanol extract of *C. aeruginosa* has an antiasthma effect based on the tracheospasmolytic activity. Therefore, *C. aeruginosa* can be developed as a possible source of new antiasthma drugs.

Data availability

F1000Research: Dataset 1. Trachea relaxation between C. aeruginosa (CA), aminophylline (A) and negative control (N) and EC50 result on trachea relaxation between C. aeruginosa (CA) and aminophylline (A)., 10.5256/f1000research.16416.d221690[21]

Grant information

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supplementary material

Supplementary File 1: Picture of rhizome of *Curcuma aeruginosa* Roxb.

Click here to access the data

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http://www.doi.org/10.5256/f1000research.16416.d221690

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Current Peer Review Status: ✗ ? ✗

Version 1

Reviewer Report 08 November 2019

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✗ **Sven-Erik Dahlén**
Institute of Environmental Medicine (IMM), Karolinska Institute (KI), Stockholm, Sweden

The authors attempt to evaluate if an ethanolic extract of the plant Curcuma Aeruginosa possesses bronchodilatory actions in the guinea pig trachea. Figure 1 shows that the influence of the extract is no different from a presumed negative control (DMSO). The data therefore do not support the author's conclusion that the extract has anti-asthmatic effects. The title of the manuscript is therefore wrong.

Specific major concerns:

1. Although figure 1 displays data without indications of the variability, the curves for the extract and the negative control are almost superimposed, whereas the curve for aminophylline shows the expected relaxation.

2. The calculations for Figure 2 are impossible to understand, and as far as I can see incorrect. EC 50 values are often calculated as 50% of the maximal response to e.g. a supramaximal dose of aminophylline. Here, it seems that the value causing 50% of the response to the highest dose tested (3 mg/mL) has been used for aminophylline, but this dose causes only 35% relaxation of the preparation. It is even more difficult to understand how an EC 50 value can be calculated for the extract because figure 1 indicates it causes no relaxation. In addition, the weight of an extract containing many undefined ingredients cannot be compared for potency with a pure pharmacologic agonist. The only comparison that would be relevant would be the qualitative documentation that the extract has bioactivity. The study has failed to show that.

3. The method description is unclear. Was the extract and the controls tested in a histamine-precontracted preparation, or only in a preparation kept at baseline tension?

Minor comments:

1. Why was DMSO the negative control? Was really the lyophilized extract dissolved in DMSO for the tests?

2. The dosage of DMSO is given in mg/mL and seems to be quite high.

3. The statement in the introduction suggesting that herbal preparations are popular for treatment of asthma is new to me. This needs to be referenced.

4. The description of the experimental protocol is incomplete, eg. dose of histamine not stated, the isometric load not defined, etc.

5. In the discussion, the authors report a number of pharmacologic activities of the extract. This would seem to preclude the use of the extract as many of the effects eg on endocrine regulation would be expected to be associated with adverse reactions.

6. The language of the manuscript needs language review. The wording is often imprecise and incorrect.

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Airway pharmacology; Translational asthma research

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 03 October 2019

https://doi.org/10.5256/f1000research.17935.r53981

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?

Waras Nurcholis
Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, Indonesia

This manuscript mainly describes the antiasthmatic activity from ethanol extract of *Curcuma aeruginosa* rhizome. The work purpose is quite interesting but the organization is weak. So, the major decision was required for the following reasons.

1. In title, please give information about your extract (ethanolic extract) and part used in this study (rhizome). I recommend changing the title to “Antiasthmatic activity from ethanol extract of *Curcuma aeruginosa* Roxb. rhizome”.

2. Please check and re-write some sentences in Abstract.

3. Introduction
   - Paragraph 2. “The genus *Curcuma* … and in traditional medicines”. Please write the sentence with an appropriate meaning.
   - Please add a reference in “Turmeric is the most…in Indonesia”.
   - Please review in your introduction for several pharmacological activities from *C. aeruginosa* rhizome such as cytotoxicity[1], antioxidant[2] etc.
   - Please create important novelty in your work in this article.

4. Method
   - In spasmolytic activity:
     1. Please give information, how to calculate for trachea relaxation? Please give a calculation formula in your method.
     2. Please give information for negative control? Are you sure DMSO? Please explain more. Your data and in method must be in line.
   - In plant extraction: Please give information for extract yield? Please give information for certificate analysis in your ethanolic extract or standardize your extract?

5. Results
   - In Figure 1 and Figure 2, please show the value of significantly? Also, note in your figure. Analysis data with the results must be appropriate.

6. Discussion is poor of information. Please discuss more about your data. Please study more about antiasthmatic activity from medicinal plant extract and metabolite?

7. Conclusion: Please give information value for potency your sample in antiasthma activity?

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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biochemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 12 September 2019

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Ian Sinha
Institute of Translational Medicine, University of Liverpool, Liverpool, UK

This is an interesting paper. Cumin has been shown to have potential anti-inflammatory effects in

other papers, and I think you should do a proper literature review around this - you will need to incorporate this in both your background and discussion which are both focussed on cumin but not asthma.

I am not sure why you chose aminophylline rather than a better bronchodilator such as salbutamol, and you will need to explain the limitation of looking at the trachea in an animal model, rather than being able to evaluate the small airways.

Nonetheless, this is an interesting paper.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Paediatric asthma.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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