

Subacute Toxicity Yellow Flower Extract (*Cananga Odorata*) on Histopathology of White Rat Heart (*Rattus Norvegicus*)

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Abstract.

Introduction: The inflammatory process is influenced by chemical mediators such as nitric oxide (NO), cytokines, and arachidonic acid metabolites. Currently, herbal treatment techniques are preferred by the public because they generally do not cause side effects. The therapeutic effect of herbal plants comes from the content of phytochemicals that have strong antioxidant activity. **Objective:** Objective of the study was to examine the phytochemical content and anti-inflammatory effects of *Cananga odorata*. **Methods:** This research is a two-stage research, namely *in vitro* and *in vivo* tests. Many previous studies have proven that anti-inflammatory activity is not only related to antioxidant activity but also its ability to suppress the production of nitric oxide and pro-inflammatory cytokines. This effect is still being developed using a variety of herbal plants. One of the plants whose effects have not been studied in the field of health-medicine is ylang flower. *Cananga odorata* (Annonaceae) or ylang flower plant is a tropical plant that has fragrant petals and thrives without requiring special care. **Results:** Results of the study were total phenol 1,06 %w/w and antioxidant IC50 DPPH by spectrophotometric examination was 808.86 ppm. These results do not show good antioxidant activity of ylang flower extract. Research in rats showed a decrease in blood glucose levels but not in lipid profiles. Furthermore, the toxicity test did not show any toxicity in experimental rats. Histological appearances showed no differences in cardiovascular disease model mice with standard-feeding mice. **Conclusions:** *Cananga odorata* ethanol extract did not showed any toxicity on histopatology on white rat heart.

Keywords: Antioxidant; Flower; Inflammation; Phytochemicals; Remembrance; Rat

Received: May 5, 2023 | Revised: May 12, 2023 | Accepted: May 14, 2023

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1. Introduction

Ylang (*Cananga odorata*) is the name for a type of flower and the tree that produces it. There are two main groups of ylang cultivated, namely the *Cananga* group (*Forma marcophylla* Steeins) and Ylang-ylang (*Forma genuina* Steeins). *Cananga* has branches perpendicular to the stem while Ylang-ylang has drooping branches and smaller leaves. In addition, the *cananga* shrub (*Cananga odorata fruticosa*) is also known, which is widely grown as an ornamental plant in the yard. Ylang is a typical flora for India, Indonesia, and Malaysia especially in North Sumatera as folk medicine. It also has the potential to be studied, especially for herbal medicines with anti-inflammatory effects.¹⁻⁴

Inflammation is a complex response of the host to injury that results in the release of leukocytes and extravasation of plasma proteins. Some of the diseases or sport injury associated with inflammation, some of diseases such as cardiovascular disease, metabolic disorders, neurological disorders, rheumatoid arthritis, and pulmonary disease. Sports injury also produce inflammation reactions and pro-oxidant. The inflammatory process is influenced by chemical mediators such as nitric oxide (NO), cytokines, and arachidonic acid metabolites. Treatment of this inflammatory disorder usually uses non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids for a long time. The mechanism of action of this synthetic drug is the inhibition of enzymes that produce arachidonic acid to inhibit the expression of cytokines. This synthetic drug has proven to be effective in treating symptoms of inflammation, but its therapeutic action can cause unwanted effects such as gastrointestinal damage.⁵

Currently, herbal treatment techniques are preferred by the public because they generally do not cause side effects. The therapeutic effect of herbal plants comes from the content of phytochemicals that have strong antioxidant activity. Many previous studies have proven that anti-inflammatory activity is not only related to antioxidant activity but also its ability to suppress the production of nitric oxide and pro-inflammatory cytokines. This effect is still being developed using a variety of herbal plants. One of the plants whose effects have not been studied in the field of health-medicine is ylang flower.⁶⁻⁹

Cananga odorata (*Annonaceae*) or ylang flower plant is a tropical plant that has fragrant petals and thrives without requiring special care. Today ylang flower extract is produced in large quantities as ylang-ylang essential oil due to its freshness and fragrance. Its role in the perfume industry has grown rapidly, but information about the potential of ylang flower in medicine and dentistry has not been widely investigated.²⁻⁴ This research will investigate the antioxidant activity of ylang.

2. Methods

This research method is observational research, cross sectional. The methodology of this research is that this research in vitro tests which include the detection total phenolic, antioxidant IC50-DPPH, and heart histopathologic features.

Medicinal materials that are used in humans need to be researched first for their effectiveness and safety, but including human subjects should only be done if the tested materials have passed the testing in the laboratory completely, followed by using experimental animals for feasibility and safety. In general, the human response to drug exposure is qualitatively similar to the animal response. One of the advantages of using experimental animals is that they can use one or several relatively high doses that induce signs of toxicity.

Rats (*Rattus norvegicus*) are animals that are commonly used in this study. *Rattus norvegicus* has a weight of 150-600 grams, a blunt nose and a body length of 18-25 cm, has a head and body that is shorter than its tail, and relatively small ears with a long body 20-23 mm.

The interaction between toxicants and cells can result in the emergence of effects called toxicity. Toxicity test aims to determine the effects caused by the interaction of the toxicant and the cells, this test is based on toxicant exposure divided into acute toxicity, subacute toxicity, and subchronic toxicity.

Oral subacute toxicity test is a test to detect toxic effects that appear after administration of the test preparation with repeated doses given orally to test animals for part of the animal's life, but not more than 10% of the entire life of the animal. Test animals were given the preparation every day and observed every day to determine the presence of toxicity. The purpose of the oral subacute toxicity test is to obtain information on the presence of toxic effects on substances that are not detected in the acute toxicity test which is only carried out once the test preparation is administered. obtain information on doses that do not cause toxic effects or NOAEL (No Observed Adverse Effect Level), and study the cumulative effect and reversibility of the tested substance.

Observations of body weight were also carried out to determine the presence of toxic effects that occurred in white rats. Histopathological observations were carried out to determine the changes in the structure of the cells in the observed organs due to the toxicity of a compound. If the administration of the test material exceeds 7 days, then an observation is made on the organ weight, the toxic effect of a compound can be observed through the organ weight if the administration of the test material is carried out for a minimum of 7 days.

The relative weight of the organs is an important index to see the physiological and pathological state of the experimental animal's organs after exposure to the test preparations, the relative weight of the organs is the basis for diagnosing the presence or absence of organ damage due to metabolic reactions of toxic substances. The heart and lungs as the observed organs were weighed first to obtain absolute organ weights. Relative organ weight was obtained by dividing absolute organ weight by body weight.

The total number of samples used were 30 white rats. The repetition was done 4 times. Determination of the minimum number of samples using the experimental sample size formula from Federer. The number of rats used was 5 for each study group (4 treatment groups, 1 positive control group, and 1 negative control group). There were 6 treatments with each treatment carried out 4 times, so that the samples used in this study amounted to a total of 30 white rats.

About 30 white rats (*Rattus norvegicus*) were acclimatized for 1 week before being used in the study, during which the rats were fed and drank according to the dose. To avoid bias towards body weight in rats, white rats (*Rattus norvegicus*) were weighed before receiving treatment, then randomly divided into 6 groups, each consisting of 5 males. The dose used in this study was obtained based on the smallest dose from the acute test of the *Cananga odorata* extract of 50 mg and the dose limit of 1000 mg/KgBW, the dose used was 100 mg; 300 mg; 500 mg/KgBW. This study has received approval from the Universitas Sumatera Utara Ethics Committee no. 1380/KEPK/USU/2021, date December 29th, 2021.

3. Results

The results of the examination of the total phenol of flower memories showed very low antioxidant activity based on the examination of total phenol and the IC₅₀ DPPH antioxidant test using a spectrophotometric device. The results of the spectrophotometric examination found that the total phenol was 1.06% (w/w) and the antioxidant IC₅₀-DPPH was 808.86 ppm (Figure 1).

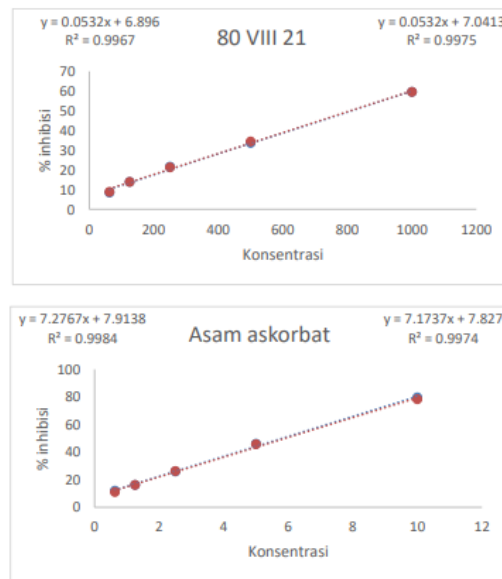


Figure 1. Antioxidant Curve

Research on the subacute toxicity of the ethanolic extract of *C. odorata* on cardiac histopathology (*Rattus norvegicus*) with the aim of obtaining information on the presence of toxic effects of substances that were not detected in acute toxicity tests and toxic effects after repeated exposure of the test preparations within a certain period of time through observation of toxicity symptoms, weight, body weight, relative organ weight, morphology and histopathology of the heart and lungs of white rats. This study used 30 male white rats (*Rattus norvegicus*) wistar strain which were divided into 6 groups, with 5 rats in each treatment group. The results of the study are as follows.

Results of Observation of Toxicity Symptoms in Rats

Observation of toxicity symptoms was carried out every day after extract induction for 28 days. Symptoms of toxicity that occurred in rats during subacute toxicity tests are presented in Table 1.

Table 1. Observation of Toxicity Symptoms

Treatment	Score	Category
C (-)	1	No indication of pain and no stress
C (+)	1	No indication of pain and no stress
T1	1	No indication of pain and no stress
T2	1	No indication of pain and no stress
T3	1	No indication of pain and no stress

C (-) : Negative control with standard diet

C (+) : Positive control with high fat diet

T1 : Treatment 1 (*Cananga odorata* extract with 50 mg/KgBW weight)

T2 : Treatment 2 (*Cananga odorata* extract with 300 mg/KgBW weight)

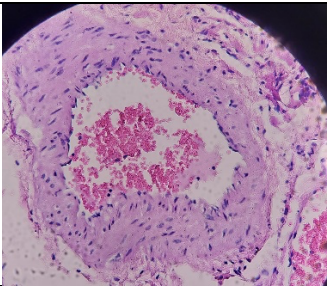
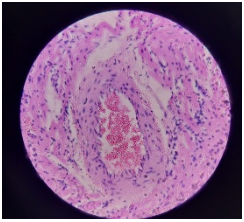
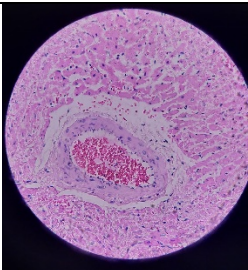
T3 : Treatment 3 (*Cananga odorata* extract with 500 mg/KgBW weight)

The characteristic of normal heart muscle is that each heart muscle has only one (or two) pale nuclei located in the middle. Surrounding the muscle cells is a connective tissue endomysium that contains a wide network of capillaries. The presence of transverse dark lines that cross the heart cells at irregular intervals is called the intercalated disc. The intercalated disc is a junctional complex located at the junction of adjacent cardiac muscle cells, has many desmosomes and adherentes fascia which functions to tightly bind cardiac cells to prevent these cells from being

separated due to constant contraction activity. The longitudinal portion of each disc has many gap junctions that function for continuous ion exchange in adjacent cells.

The results showed that the nucleus is located in the middle, oval in shape, branching which is characteristic of the heart muscle, and intercalated discs are also seen. Histopathological changes that occurred in the form of hydropic degeneration in the control, treatment, and satellite groups. There is a nucleus that is larger and swollen than the normal nucleus, hydrophilic degeneration has the characteristics of swollen cells.

Table 2. Histopatology features

No	No Preparat	Name of treatment	Enlargement	Microscopic picture	Microscopic
1	321	Control Negative M1-1	400x		Plaque : Negative (-) Thrombus : Negative (-) Calcification: Negative(-)
	327	Control Positif M2 -2	400x		Plaque : Negative (-) Thrombus : Negative (-) Calcification: Negative(-)
	331	M3-1	400x		Plaque : Positif (-) Thrombus : Negative (-) Calcification: Negative(-)

4. Discussions

Organic drugs have advantages over inorganic drugs, including relatively few side effects, synergistic effects in their content, and more suitable for metabolic and generative diseases. The use of organic drugs has been accepted in almost all countries, the factors for the increase in the use of organic drugs in developed countries are a longer life expectancy when the prevalence of chronic diseases increases, the failure to use inorganic drugs for certain diseases including cancer and wider access to information about drugs. organic worldwide. However, its use must be appropriate, which includes the correctness of the ingredients, the accuracy of the dose, the time of use, the accuracy of the method of use, the accuracy of reviewing the information, and without the abuse of the organic drug itself.^{1,2,10,11}

The use of organic medicine or commonly called traditional medicine namely *C. odorata* as an anti-inflammatory must go through a series of tests so that the results obtained are in line with expectations and can be ensured of safety, one of which is preclinical testing. The subacute toxicity test is one of the preclinical tests that aims to see the toxic effects caused by the administration of *C. odorata* for a long period of time.^{10,12}

The selection of male white rats was caused by the nature of male rats which was not affected by the reproductive cycle. The presence of an estrus cycle in female rats will affect kidney function and nitric oxide activity. The estrus cycle in virgin female rats lasts for 4-5 days consisting of proestrus, estrus, and diestrus. When proestrus estrogen reaches its maximum level, blood vessels will narrow during the process of reduced circulating estradiol due to aromatase inhibition which causes an increase in total nitric oxide production in female rats. According to nitric oxide, it will initiate an acute inflammatory response in the lungs, if there are free radical compounds in the form of nitric oxide into the lung tissue, it will cause an increase in the number of macrophages and/or neutrophils that function to phagocytize foreign particles. This will bias the histopathological and physiological changes of white rats if female white rats are used as experimental animals.^{5,10,12}

Giving the extract for 28 days did not cause death in white rats, indicating that the test preparation in the form of *C. odorata* extract was not toxic. Rats are also not in a state of stress and pain. In addition to appetite, the characteristics of the condition of rats, whether under stress or not, can be seen from the observation of toxicity symptoms.^{8,9,13}

Symptoms of toxicity are parameters of the presence of toxic effects that are easily observed. Stressed rats can show symptoms of very coarse hair, sunken eyes, slow or unresponsive movements, slouching, large abdominal mass, and aggressive behavior. While mice that are not stressed and indicate no pain will be seen in well-groomed hair, in a state of calm or sleep, normal appetite, spinal vertebrae and dorsal pelvis are not prominent. The results showed that the rats showed no stress and did not experience pain in all treatment groups. This indicated that the administration of *C. odorata* extract had no effect on white rats indicated by the absence of toxicity symptoms that appeared in the control, treatment, and satellite groups.

5. Conclusions

Cananga odorata ethanol extract did not showed any toxicity on histopatology on white rat heart.

Ethics approval: Sumatera Medical Journal (SUMEJ) is a peer-reviewed electronic international journal. This statement below clarifies ethical behavior of all parties involved in the act of publishing an article in Sumatera Medical Journal (SUMEJ), including the authors, the chief editor, the Editorial Board, the peer-reviewer and the publisher (TALENTA Publisher Universitas Sumatera Utara). This statement is based on COPE's Best Practice Guidelines for Journal Editors.

Authors contributions: The authors gratefully acknowledge that the present research was supported by the Ministry of Research and Technology and the Higher Education Republic of Indonesia, Research and Community Service, Universitas Sumatera Utara. The support is under the research TALENTA Universitas Sumatera Utara Skema Penelitian Dasar 2021 year 2021, number 345/UN5.2.3.1/PPM/SPP-TALENTA USU/2021. The authors also thanks to cooperation between Universitas Sumatera Utara (USU), Lovely Profesional University (LPU), and Universiti Kuala Lumpur Royal College of Medicine (UniKL). Lovely Profesional providing data analysis and a visit in LPU Botanical Garden in 2022, Universitas Sumatera Utara provide in laboratory assessment, and Uni KL providing discussion for this paper.

Funding: No funding.

Disclosure: Authors declares no conflict of interest.

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