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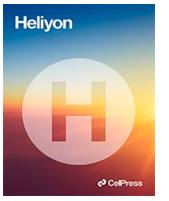

Research article

Inhibition of nitric oxide production in RAW 264.7 cells and cytokines IL-1β in osteoarthritis rat models of 70 % ethanol extract of *Arcangelisia flava* (L.) merr stems

Rizki Rahmadi Pratama [a], Riza Ambar Sari [b], Irawati Sholikhah [c], Hakiman Mansor [d], Hsin-I Chang [e], Sukardiman [f], Retno Widyowati [f,*]

[a] *Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Nanizar Zaman Joenoes Building, Campus C, Mulyorejo Street, Surabaya, 60115, East Java, Indonesia*
[b] *Department of Pharmacy, Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim State Islamic University, Malang, Indonesia*
[c] *Department of Chemistry, Faculty of Sains and Technology, Universitas Airlangga, Campus C, Mulyorejo Street, Surabaya, 60115, East Java, Indonesia*
[d] *Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, Malaysia*
[e] *Department of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan*
[f] *Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Nanizar Zaman Joenoes Building, Campus C, Mulyorejo, Surabaya, 60115, East Java, Indonesia*

A R T I C L E I N F O

Keywords:
Arcangelisia flava
Nitric oxide
IL-1β
Anti-inflammatory
Osteoarthritis

A B S T R A C T

Introduction: One of the most frequent types of arthritis is osteoarthritis, also referred to as a degenerative joint disease. Interleukin-1β (IL-1β) and nitric oxide (NO) are essential factors in the pain response; IL-1β and NO are responsible for increasing the production of matrix metalloproteinases (MMP) and a disintegrin-like and metalloproteinases with thrombospondin motifs (ADAMS) in chondrocytes. *Arcangelisia flava* (L.) Merr. Has been traditionally used to treat jaundice, liver disease, diarrhea, fever, and inflammation.
Methods: This study used *in vitro* and *in vivo* models to determine the effect of a 70 % ethanol extract of *Arcangelisia flava* (L.) Merr. stems on the inhibition of NO production in RAW 264.7 cells induced with lipopolysaccharide (LPS) and IL-1β in osteoarthritis rats induced with monosodium iodoacetate (MIA). The NO inhibition test was determined by the NO colorimetric assay using Griess reagent and measured by the ELISA plate reader. The measurement of joint diameter and hyperalgesia in osteoarthritis rats was carried out once a week for 7 weeks, and then the IL-1β levels were measured at weeks 3 and 7.
Result: The viability of cell line this extract was greater than 80 %, and the extract at 25, 50, and 100 μg/mL significantly inhibited NO production ($p < 0.0001$) in RAW 264.7 cells induced with LPS. Meanwhile, this extract at 10, 30, and 90 mg/200g BW increased latency time, reduced joint swelling, and reduced IL-1β levels in the serum in the osteoarthritis rat model.
Conclusion: 70 % ethanol extract of *Arcangelisia flava* (L.) Merr. Has the potential to be an anti-osteoarthritis drug.

* Corresponding author. Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Nanizar Zaman Joenoes Building, Campus C, Mulyorejo, Surabaya, 60115, East Java, Indonesia.
E-mail address: rr-retno-w@ff.unair.ac.id (R. Widyowati).

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1. Introduction

Osteoarthritis is one of the most common types of arthritis and is also called degenerative joint disease [1]. Osteoarthritis is distinguished by the occurrence of degradation in articular cartilage, inflammation inside the joint, and the replacement of subchondral bone. The underlying mechanisms and physiological processes responsible for this condition have yet to be fully understood [2]. Osteoarthritis is associated with a cartilage deficiency and the loss of meniscus tissue, which cushions the joints. Osteoarthritis is also caused by mechanisms that induce pathophysiological alterations in articular cartilage [3].

Chondrocytes are one of the cells that play a role in osteoarthritis and synthesize matrix to maintain a balance between synthesis and degradation. It facilitates the generation of proinflammatory cytokines. IL-1β and Tumour Necrosis Factor-α (TNFα) are the two most important proinflammatory cytokines associated with osteoarthritis [4]. IL-1β is an important proinflammatory cytokine in the progression of osteoarthritis, which impairs mitochondrial function and induces NO production in chondrocytes [5].

The presence of NO can stimulate the release of pro-inflammatory mediators and inhibit the synthesis of cartilage matrix components in chondrocytes, while simultaneously promoting the activity of matrix metalloproteinases (MMP) [6]. Therefore, suppression of NO and IL-1β is anticipated to reduce pro-inflammatory cytokine production and prevent the formation of various degraded proteins, such as matrix metalloproteinases (MMP) and A disintegrin-like and metalloproteinases with thrombospondin motifs (ADAMTS).

The most common symptom that prompts osteoarthritis patients to take medication is pain. The commonly recommended pharmaceutical drugs for this ailment encompasses oral nonsteroidal anti-inflammatory drugs (NSAID) [7]. Nonetheless, this treatment has adverse effects. Long-term use of NSAIDs is discouraged due to their gastrointestinal, cardiovascular, and nephrotoxic effects [8,9]. Traditional medicine can be an alternative for treatment that is effective and safe for long-term use.

Arcangelisia flava (L.) Merr. is a member of the Menispermaceae family. These species are prevalent in South-East Asia from China to New Guinea. This plant is indigenous to Borneo and has distinct, bright yellow wood. One of the local names in Indonesia is Kikoneng (West Java). The Dayak people in Kalimantan use boiled water to treat stomachaches, jaundice, malaria, fever, and sore eyes. People in Palupu District, West Sumatra Province, also use this plant empirically [10]. The leaf of this plant contains alkaloids, flavonoids, terpenoids, and saponins. The roots contain alkaloids from the isoquinoline group, including berberine, jatrorizin, and palmatine. The minor alkaloids columbamine, dehydrokoridalmin, homoaromolin, and talifendin are also found [11,12].

In previous investigation, an aqueous extract of yellow root at a 450 mg/kg BW decreased the expression of cyclooxygenase-2 in rats that were stimulated with Complete Freund's Adjuvant [13]. The main component of this plant, berberine, has anti-inflammatory activity via an inducible nitric oxide synthase (iNOS) inhibiting mechanism and inhibits differentiation of Th1/Th7 helper T cells [14]. Berberine also suppresses NF-kB signaling via the sirtuin1-dependent signaling pathway and is associated with inflammation [15]. Another compound, palmatine, has an anti-inflammatory effect on gastrointestinal tissue by increasing prostaglandin E2 (PGE2) levels and decreasing platelet-activating factor levels [16].

Current research focuses on IL-1β and NO production, two crucial components of pain response. An increase in IL-1β and NO production causes the release and activation of several proteins, including matrix metalloproteinases (MMP) and A disintegrin-like and metalloproteinases with thrombospondin motifs (ADAMTS). Therefore, this study was divided into two types: an *in vitro* test of NO inhibition using RAW 264.7 cells induced with LPS and an *in vivo* test of several parameters of anti-osteoarthritis activity using rats induced with MIA.

2. Material and methods

2.1. Material used

The combination of ethanol p.a. (Merck) and water was used for extraction. Dulbecco's modified eagle's medium (DMEM) (Thermo Fischer Scientific), dimethyl sulfoxide (DMSO) (Sigma-Aldrich), 10 % fetal bovine serum (FBS) (Thermo Fischer Scientific), 250 μg/mL amphotericin B, penicillin and streptomycin, lipopolysaccharide (LPS), 2,5-diphenyltetrazolium bromide (MTT), sodium nitrite, and Griess reagent used for *in vitro* assay, then carboxy methyl cellulose (CMC), 0.9 % physiological saline, monosodium iodoacetate (MIA) (Sigma-Aldrich, Darmstadt, Germany), 10 % ketamine (Agro-veterinarian, Nicaragua), IL-Iβ ELISA kit (Bioenzy, Germany), and meloxicam 7.5 mg (Sanbe Farma, Indonesia) used for *in vivo* assay. *Arcangelisia flava* (L.) Merr. stem powder obtained from Soren village, Central Kalimantan, Indonesia.

2.2. Extraction of Arcangelisia flava (L.) merr

Arcangelisia flava (L.) Merr. Was collected from Soren Village, Central Kalimantan, Indonesia, and determined at the Herbal Laboratory of Materia Medica, Batu, East Java, Indonesia (Designation Certificate No.074/620/102.20-A/2022). Fresh stems of this plant were cut into small parts, dried, and ground with a blend to obtain powder (1000 g). The powder was extracted by maceration method using 10 L of 70 % ethanol (1:10) at room temperature for 72 h. The filtrate was evaporated using a rotary evaporator at 40 °C with a speed of 50 rpm to form a viscous extract. The yield value of this extract was counted as % b/b and found to be 15.8 %.

2.3. Cell culture

The RAW 264.7 cell line was obtained from the Department of Biochemical Science and Technology at National Chiayi University in Taiwan. The cell line was cultured in DMEM Medium supplemented with 10 % FBS and 1 % penicillin-streptomycin solution. The

culture was maintained at 37 °C in a controlled environment incubator with a humidity level and CO_2 concentration of 5 % [17,18].

2.4. assay

RAW 264.7 cell lines were plated in a 96-well plate with a density of 2 x 10^4 cells per well, and the cells' viability was determined using an MTT colorimetric test (Sigma Aldrich, Germany) with a final volume of 100 μL. The cells were then allowed to incubate for 24 h and then exposed to various concentrations of the 70 % ethanol extract of *Arcangelisia flava* (L.) Merr. For the other 24 h. Next, the media were changed to 100 μL MTT and incubated for 5 h. After removing the supernatants, 100 μL of DMSO was added to dissolve the formazan. A microplate reader was used to measure absorbance at 570 nm after the plate had been gently shaken [17,18].

2.5. Nitric oxide production assay

A 24-well plate was utilized for culturing 2 x 10^5 RAW 264.7 cells per well. The plate was incubated at 37 °C for 24 h in a 5 % CO_2 environment. The cells were subjected to LPS treatment at 1 μg/mL for one night (24 h). After that, the cells were treated with various dilutions (25, 50, and 100 μg/mL). DMEM medium (without LPS), 1 μg/mL LPS, and 1 mM aspirin were used as a blank, negative, and positive control, respectively. The Griess Reagent was employed to ascertain the presence of nitrite in a 100 μL culture medium sample. Subsequently, the absorbance was measured using an ELISA reader at 550 nm [17,18].

2.6. Ethical considerations

Thirty male wistar rats (*Rattus norvegicus*) in healthy condition were procured from the Farma Veterinary Center in Surabaya, Indonesia. The rats with an age range of 2–3 months and weighing 200–300 g, underwent a seven-day acclimatization at the Animal Laboratory of the Faculty of Pharmacy, Airlangga University. The rats were kept in a controlled environment with a temperature of 22 ± 3 °C, relative humidity ranging from 30 to 70 %, and a light-dark cycle of 12 h each. Each enclosure measuring 45 cm in width, 30 cm in depth, and 20 cm in height accommodates a single rat. The enclosure exhibited waterproofness, durability, ease of cleaning, and quietness. The present study adheres to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, which underwent revision in 1985 and received approval from the ethics committee of the Faculty of Veterinary Medicine, Airlangga University (No.2.KEH.034.03.2023).

2.7. Osteoarthritis rat model

An osteoarthritis rats model was done by injecting MIA at 4 mg in 50 μL of saline solution into healthy animals intra-articularly [19]. The injection is given using a 27-G needle in the right joint knee. The procedure was conducted under the administration of an anesthetic, with a 10 % concentration of ketamine. Subsequently, the rat joint diameter and latency time were assessed using the utilization of the heated plate method [20,21].

Following three-week of MIA induction, blood samples were obtained to measure IL-1β levels before any intervention. The extract is given orally once daily for 28 days starting at weeks 4–7. In addition, the hyperalgesia and joint swelling in rats were assessed over seven weeks using a heated plate and a calibrated micrometer screw. The delay time in hyperalgesia was assessed using a stopwatch. The amounts of IL-1β in the serum were obtained during the seventh week. The injection led to an increase in the diameter of the joint rats on the same side physically. It resulted in a decreasing time for the rats to respond to heat stimulation (hyperalgesia) using heated plate functionally [20,21].

2.8. Animal grouping

Thirty rats were split into six groups of five each. They were the healthy group (fed and watered freely and not MIA-induced), the negative group (rats given 0.5 % CMC-Na and MIA-induced), the positive group (rats given 0.135 mg/200 gBW meloxicam and MIA-injected), and the treatment groups (given 70 % ethanol extract of *Arcangelisia flava* (L.) Merr. and MIA-injected). The three doses used for the treatment groups were 10, 30, and 90 mg/200g BW.

2.9. Hyperalgesia experiment

This study observed the hyperalgesia response of all groups on days 0, 7, 14, 21, 28, 35, 42, and 49 using a heated plate (Ugo Basile heated/Cold Plate 35,100, Gemonia, Italy). This method employs rats as subjects to express thermal pain through their observable responses. A rat is left unattended on a metal surface maintained at 55 ± 0.5°C. Response latency or time nocifensive response was recorded. Nocifensive behaviors include paw-pulling, paw-licking, swaying, stomping, and jumping. Mice are removed from the heated plate once a response is observed [20,21].

2.10. Joint swelling

Joint swelling was measured in the rats' right knee after intra-articular MIA injection. Measurements were taken from weeks 0 through 7. Using a screw micrometer calibrated in millimeters (mm), the joint diameter of the rats was measured to determine the

swelling during the development [20,21].

2.11. IL-iβ cytokine assay

At weeks 3 and 7, around 1–2 mL of rat blood was taken from the tail and centrifuged for 10 min at 3000 rpm to obtain serum. IL-Iβ levels were determined using a commercial rat IL-1 ELISA reagent (Bioenzy, Germany) according to the manufacturer's instructions. The results were then analyzed using an ELISA reader instrument (EZ-2000) [20,21].

2.12. Statistycal analysis

The results of the *in vivo* test were displayed as the mean ± standard deviation of five rats in each group using GraphPad Prism 9 (San Diego, USA) and SPSS 26. The data was used for a two-way analysis of variance (ANOVA) by comparing hyperalgesia and joint swelling data, followed by an LSD post hoc test. While IL-Iβ levels and NO production used one-way analysis of variance (ANOVA) to statistically compare them followed by LSD post hoc test, respectively. Statistical significance with a p-value less than 0.05 was employed.

3. Results

3.1. Viability test

The RAW 264.7 cell viability of 70 % ethanol extract from *Arcangelisia flava* (L.) Merr. stems at 6.25, 12.5, 25, 50, and 100 μg/mL indicated by the average value of % living cells in Fig. A.1. The increasing of concentration extract decreased the viability of RAW 264.7 cells. The extract with a percentage of live cells >80 %, indicated it was not toxic to RAW 264.7 cells, so it continued for further testing.

3.2. Nitric oxide

The NO production was measured based on the amount of nitrite in the media compared to the untreated control. RAW 264.7 cells induced by LPS released higher NO than the untreated control group (Fig. A.2) [22]. There were significant differences in NO production between the negative control group (LPS) and all groups ($p < 0.0001$). The extract at 25 μg/mL concentration inhibited NO production up to 37.9 %. When the concentration was increased to 50 and 100 μg/mL, the percent NO production decreased to 52.14 % and 55.44 %, respectively.

3.3. Rat models of osteoarthritis induced by monosodium iodoacetate (MIA)

The progression of the intra-articular MIA-induced osteoarthritis rat model was monitored for three weeks using a variety of parameters, such as the physical parameter of measuring rat joint diameter, the functional parameter of heated plate heat resistance, and the biochemical parameters of IL-1β cytokines levels. According to the data presented in Fig. A.3, it was observed that the negative group (MIA-induced) exhibited a significantly bigger diameter with an average measurement of 15.42 mm. In contrast, the healthy group (not exposed to MIA) had a comparatively smaller diameter with an average measurement of 11.32 mm. Based on the development parameters of knee diameter, it showed that MIA induction successfully produced a mouse model of osteoarthritis.

Behavioral parameters of osteoarthritis were evaluated by measuring pain levels in inflammatory conditions. It used the heated plate method by measuring heat resistance time (hyperalgesia). As shown in Fig. A.4, the negative group had a faster hyperalgesia time than the healthy group with an average latency of 3.39 s. Three weeks after receiving the MIA injection, the levels of IL-1β were measured using ELISA, the results showed four times higher than the healthy group with IL-1β levels of 1915.67 pg/mL in Fig. A.5.

3.4. Effect of Arcangelisia flava (L.) merr. Administration in osteoarthritis rats

The physical parameters of knee diameter and heat resistance time (hyperalgesia) were taken at weeks 4, 5, 6, and 7. Subsequently, quantification of IL-1β concentrations in the serum samples was performed during the seventh week. The results indicated variations in knee diameter measures between the negative control group and both the positive control group and the treatment group. Whereas the treatment group with a dose of 90 mg/200g BW did not show any difference in the average decrease in diameter (11.72 mm) compared to the meloxicam-positive control group (11.70 mm) in Fig. A.6. It concluded that this dose reduced the average knee diameter of rats in a manner comparable to the positive control (meloxicam).

Extracts with increasing doses showed an increase in latency time. The treatment group with three doses showed no significant difference from the positive control group, with an average heat retention time of 6 s. It indicates that the three doses of this extract can increase the heat retention time (hyperalgesia), as seen in Fig. A.7.

In the seventh week of the study, the levels of IL-1β in the blood serum of rats were measured. These measurements were then compared to the levels seen during the third week of the study (pre-test). The blood serum of rats administered extracts at doses of 10, 30, and 90 mg/200g BW exhibited a mean reduction in IL-1β levels of 608.0, 568.0, and 679.33 pg/mL, respectively, in comparison to the negative control group with a mean level of 1957.33 pg/mL (Fig. A.8). It shows that the three doses of this extract can reduce knee

swelling in rats by reducing IL-1β levels.

4. Discussion

In this research, the NO level in cells was a marker of inflammatory activity. Although NO is well-known for its physiological roles in the body, including immune protection against microbes, its excessive production has been related to several illnesses, including arthritis, diabetes, stroke, septic shock, autoimmune disease, chronic inflammatory disease, and atherosclerosis. iNOS is the primary regulator of NO production, but when excessive and prolonged NO production occurs, the potential for tissue damage associated with inflammation will occur [23]. LPS induces NO secretion in macrophage cells and creates chronic inflammation. The induction of inflammation in RAW 264.7 cells occurs with exposure to LPS, as evidenced by a substantial rise in the release of pro-inflammatory cytokines such as IL-6 and TNF-α, along with excessive NO production, which becomes highly elevated over a 24-h [24].

iNOS is a protein responsible for NO production and increases in this protein cause vasodilation during inflammation. Inhibition of iNOS could be a very useful option for treating of inflammation or disease associated with increased excessive NO production [25]. The present work investigates the impact of the extract on RAW 264.7 cells stimulated by LPS, with a particular focus on the concentration-dependent modulation of NO generation. The inhibition or reduction of NO production indicated the extract's anti-inflammatory properties. The extract at 25 μg/mL inhibited NO production at 37.9 %, and the increasing concentration to 50 and 100 μg/mL, NO production inhibited 52.14 % and 55.44 %, respectively (Fig. A.2). However, no statistically significant difference was seen in the inhibition of NO between concentration 50 and 100 μg/mL. Therefore, based on its ability to inhibit NO production, this extract is concluded to have anti-inflammatory activity.

In our previous study, 70 % ethanol extract of *Arcangelisia flava* (L.) Merr stems contain fifteen compounds in total with various groups of compounds such as alkaloids, flavonoids, furano-diterpenes, hydroxyquinolines, phenylpropanoids, phenols, and fatty acids. Compounds that were successfully analyzed include isopycnarrhine, pycnarrhine, sinapic acid, sinapaldehyde, pallidinine, 3-hydroxy-3',4',5'-trimethoxyflavone, demethyleneberberine, stepharanine, fibleucin, jatrorrhizine, berberine, docosanoic acid, n-methyltetrahydropalmatine, fissisaine, and palmatine. Based on these compounds, the 3-hydroxy-3',4',5'-trimethoxyflavone had free energy binding values of −7.72 kcal/mol, followed by fissialine −6.91 kcal/mol, and Demethyleneberberine −6.85 kcal/mol using an in-silico test on the target protein IL-1β (PDB ID: 1ITB [26].

One of the bioactive compounds in *Arcangelisia flava* (L.) Merr. is berberine which has anti-osteoarthritis activity by reducing LPS-induced iNOS protein [27,28]. Another compound contained in *Arcangelisia flava* (L.) Merr extract is jatrorrhizine. Cheng et al. reported that the jatrorrhizine contained in *P. arumense* inhibit the expression of NO by more than 60 % at 100 μg/mL [29]. It also inhibits iNOS and COX-2 by 45 and 29 %, respectively. The extract and jatrorrhizine from *P. amurense* were predicted to have an anti-inflammatory activity [30,31].

This study involved in-vitro tests on RAW 264.7 cells induced with LPS to measure the decrease in NO levels. Additionally, in-vivo tests were performed on osteoarthritis rat models induced MIA. This induction inhibits the function of glyceraldehyde-3-phosphate dehydrogenase in chondrocytes via a specific mechanism. It causes impaired glycolysis and chondrocyte cell death, resulting in histological and morphological alterations in the articular cartilage [32]. MIA induces an upregulation in the synthesis of IL-1β and TNF-α, subsequently leading to an elevation in the expression of COX-2 and matrix metalloproteinases (MMP) [33]. It has been demonstrated that elevated levels of IL-1β accelerate cartilage degradation in articular chondrocytes and inhibit cartilage matrix synthesis [34]. Osteoarthritis develops 2–8 weeks after MIA administration, and the average dose administered is 1–4 mg. This progressive condition is comparable to osteoarthritis in humans [19].

Following the administration of MIA through intra-articular injection, the diameter of the rat's right knee was assessed to ascertain the presence of edema and determine the tissue index of inflammation. The MIA-induced triggers an inflammatory response by inducing heightened vascular permeability, capillary extravasation, and cellular migration. The presence of knee swelling indicates synovial inflammation, which correlates with the development of osteoarthritis. The swelling persisted for days and reduced joint mobility in the rats [35]. Based on the results, 70 % ethanol extract of *Arcangelisia flava* (L.) Merr. stems showed promising effects in osteoarthritis rat models induced by MIA, seen from several parameters such as decreasing knee diameter (Fig. A.6), increasing heat retention time (Fig. A.7), and reducing IL-1β (Fig. A.8), which is the main inflammatory cytokine involved in osteoarthritis. Considering that IL-1β and NO are closely related to the expression of matrix metalloproteinases (MMP) and A disintegrin-like and metalloproteinases with thrombospondin motifs (ADAMTS). So *Arcangelisia flava* (L.) Merr. extract has anti-osteoarthritis potential.

Berberine in this extract improves the downregulation of type II collagen caused by IL-1β induction and significantly reduces MAPK expression which shows anti-catabolic and anti-inflammatory activity. In addition, MAPK activation is also associated with the development of osteoarthritis, so inhibiting MAPK activation is very important and inhibit osteoarthritis [36].

Another compound, demethylene berberine inhibits mitochondrial production and maintain mitochondrial homeostasis during inflammatory reactions. This compound also inhibit IL-1β maturation via a mitochondria-dependent mechanism. It effectively reduces the activity of myeloperoxidase (MPO), a factor closely related to inflammation and oxidative stress [37]. This compound also inhibits activation of the NFkB and interferon-signaling pathways, so the production of pro-inflammatory cytokines will not occur. Infusion of *Arcangelisia flava* (L.) Merr at 450 mg/kg decreases the expression of COX-2 in rats stimulated with Complete Freund's Adjuvant [13].

Based on previous studies in LC-MS/MS-QTOF and in-silico analysis of this plant [26] and also present studies in anti-osteoarthritis activity in-vitro and *in vivo*, further research needs to be carried out on the potential of this plant as an anti-osteoarthritis with different protein targets, it is also necessary to carry out standardization raw material and proceed to the clinical trial stage.

5. Conclusion

NO and IL-1β are two molecules that are involved in the development of osteoarthritis. Based on in-vitro research, 70 % ethanol extract of *Arcangelisia flava* (L.) Merr with different concentrations reduced NO production in RAW 264.7 macrophage cells activated by LPS. Besides that, as seen from in-vivo studies, this extract also increases heat retention time, reduces knee diameter, and reduces IL-1β levels in osteoarthritis rat models induced by MIA. Therefore, this extract has therapeutic potential for anti-osteoarthritis.

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CRediT authorship contribution statement

Rizki Rahmadi Pratama: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Riza Ambar Sari:** Writing – review & editing, Writing – original draft. **Irawati Sholikhah:** Project administration, Methodology, Formal analysis, Data curation. **Hakiman Mansor:** Writing – review & editing. **Hsin-I Chang:** Writing – review & editing. **Sukardiman:** Writing – review & editing, Conceptualization. **Retno Widyowati:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflicts of interest related to the publication of this study.

The authors whose names are listed immediately below certify that they have NO affiliations with or involvementin any organization or entity with any financial interest (Such as honoraria; educational grants; participations in speakers' bureaus; membership, employment consultancues, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (Such as personal or proffesional relationship, affiliation, knowledge or benefits) in the subject matter or materials discussed in this manuscript.

This statement is signed by all the authors to indicate agreement that the above information is true and correct.

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List of Abbreviations

OA	Osteoarthritis
IL-1β	Interleukin-1 Beta
MMP	Matrix metalloproteinases
ADAMTS	A disintegrin-like and metalloproteinases with thrombospondin motifs
NO	Nitric Oxide
LPS	Lipopolysaccharide
TNF-α	Tumour Necrosis Factor-Alpha
NSAID	Nonsteroidal anti-inflammatory drugs
COX-2	Cyclooxygenase-2
MIA	Monosodium Iodoacetate
PGE-2	Prostaglandin E2
IFN-γ	Interferon gamma
CMC-Na	Sodium Carboxy Methyl Celulose
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulfoxide
Th	T helper
FBS	Fetal Bovine Serum
ELISA	Enzyme-Linked Immunosorbent Assay

Appendices.

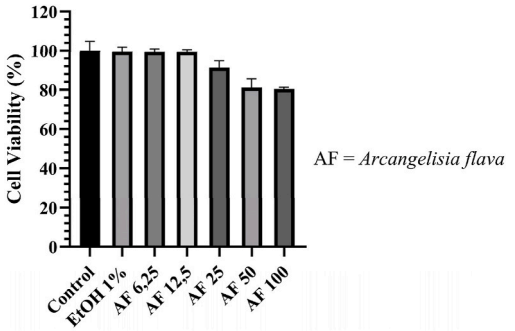

Fig. A.1. Cell viability of 70 % ethanol extract of *Arcangelisia flava* (L.) Merr. stems against RAW 264.7 based on **n = 8**, the data were presented as Mean ± SD.

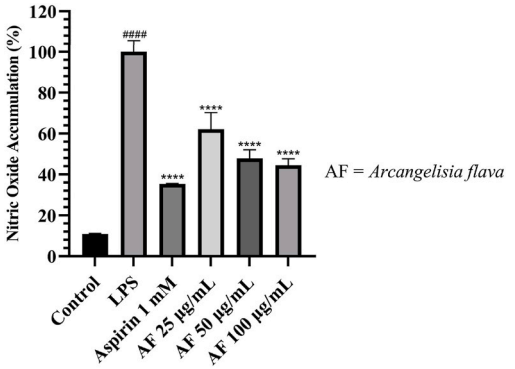

Fig. A.2. NO production results from 70 % ethanol extract of *Arcangelisia flava* (L.) Merr. stems (#### = $p < 0.0001$ compared to control, **** = $p < 0.0001$ compared to LPS) based on **n = 8**, the data were presented as Mean ± SD.

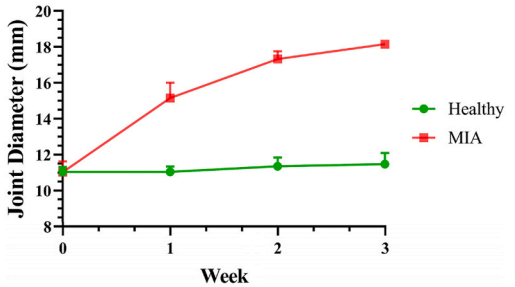

Fig. A.3. The pre-test joint diameter of rats during the development of an osteoarthritis rat model. Based on n = 5, the data were presented as Mean ± SD

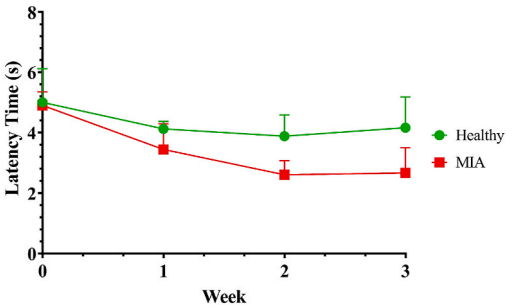

Fig. A.4. The pre-test latency time of rats during the development of an osteoarthritis rat model. Based on n = 5, the data were presented as Mean ± SD

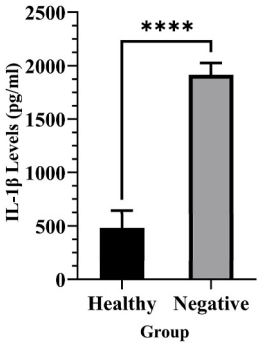

Fig. A.5. The pre-test levels of IL-1 in rat blood serum. IL-1 levels increased significantly after injection with MIA compared to the healthy group ($P < 0.0001$). Based on $n = 5$, the data were presented as Mean ± SD

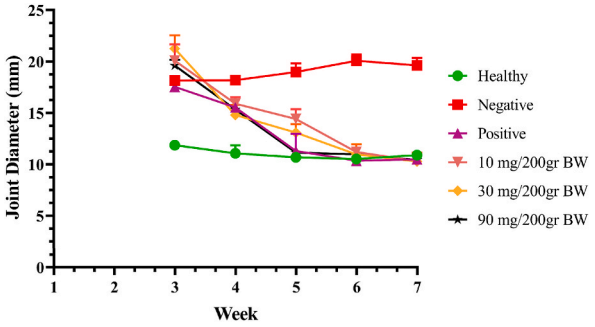

Fig. A.6. Rats joint diameter significantly decreased after treatment with *Arcangelisia flava* (L.) Merr. extract. Data are present as Mean ± SD ($n = 5$) for all groups.

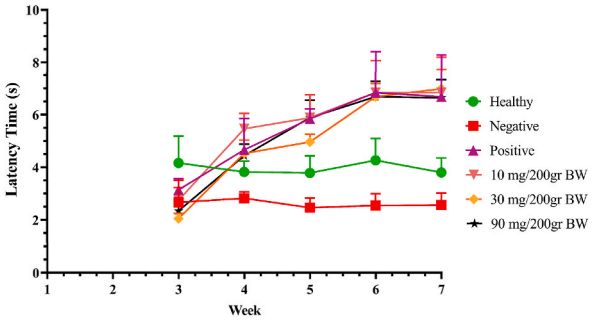

Fig. A.7. Rats latency time significantly increased after treatment with *Arcangelisia flava* (L.) Merr. extract. Data are present as Mean ± SD ($n = 5$) for all groups.

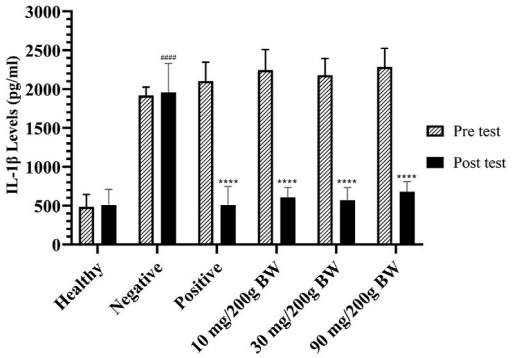

Fig. A.8. Results of measurements of IL-1 levels pre-test and post-test (#### = $p < 0.0001$ compared to healthy controls, **** = $p < 0{,}0001$ compared to negative controls). Data were present as Mean ± SD ($n = 5$) for all groups.

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