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Anti-Inflammatory Activity of Stem Barks Ethanol Extracts of *Garcinia xanthochymus* On Male White Rats

Hanafis Sastra Winata [1], Muhammad Andry[2], Muhammad Amin Nasution[3*], Firman Rezaldi[4] , Ade Shindy F Br Sembiring[5]

[1]*Departement of Pharmaceutical Biology, Faculty of Pharmacy, Institut Kesehatan Helvetia, Helvetia, Medan, Sumatera Utara, 20124, Indonesia.*
[2]*Departement of Pharmaceutical Chemistry, Faculty of Pharmacy, Institut Kesehatan Helvetia, Helvetia, Medan, Sumatera Utara, 20124, Indonesia.*
[3*] *Faculty of Pharmacy, Universitas Muslim Nusantara Al Washliyah, Medan, Sumatera Utara, 20147, Indonesia.*
[4]*Pharmacy study program at the Mulia High School of Health Sciences, Madiun, East Java, Indonesia*
[5]*Faculty of Pharmacy, Institut Kesehatan Helvetia, Helvetia, Medan, Sumatera Utara, 20124, Indonesia.*

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[*)] Corresponding author:
E-mail: mhdaminnst@umnaw.ac.id

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Abstract

Inflammation is a complicated set of tissue alterations resulting from tissue damage caused by microorganisms, trauma, toxins, heat, and pain. The *Garcinia xanthochymus* is a fruit-bearing tree. Plants of the genus garcinia are abundant in secondary metabolites with potential pharmacological action, including as flavonoids, steroids, and triterpenes. The content of secondary metabolites that are useful as anti-inflammatories are flavonoids. This study aims to investigate the anti-inflammatory efficacy of the stem bark of *Garcinia xanthochymus* based on the reduction in edema volume. The inquiry was experimental. Through maceration, an ethanol 96% extract of the stem bark was created. The animals were separated into five groups, each with five white male rats. The test given was a suspension of stem bark ethanol extract at 200, 400, and 600 mg/kg BW, a negative control of 1% Na CMC suspension, and a positive control of 2.25 mg/kg sodium diclofenac. Observations were made for 6 hours, and then data were analyzed using a two-way analysis of variance (ANOVA) test. The results showed stem bark ethanol extract was proven to have an anti-inflammatory effect in white male rats induced by carrageenan beginning dosages are 200, 400, and 600 mg/kg, marked by a statistically significant change from the negative control ($p<0.05$). The 600mg/kg dose group showed no significantly different results from the positive control group ($p>.05$). These results concluded that the dose group 600mg/kg was the best dose of stem bark ethanol extract had an anti-inflammatory activity based on reducing the volume of leg edema of white male rats.

Keywords: Stem Bark of *Garcinia xanthochymus*; Anti-Inflammation; Male White Rats

Introduction

Inflammation is the body's attempt to deactivate or eliminate invading organisms, relieve irritation, and prepare for the repair of damaged tissue (Andayani et al., 2018; Amin et al., 2020). In general, there are two categories of anti-inflammatory drugs: steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs. Most people generally use synthetic drugs, which have various harmful side effects on the body, such as stomach ulcers, dyspepsia, cardiovascular disease, osteoporosis, and moon face on the face (Rahayu et al., 2016; Husna et al., 2021).

Indonesia is one of the countries with the most incredible biodiversity. The real potential is to develop herbal medicines produced from medicinal plants. Several studies have shown that plants produce secondary metabolites with various molecular structures and biological functions, whereas herbal plants have fewer side effects than synthetic drugs and are safer to use (Pratama et al., 2020; Wahyuni et al., 2017).

Plants of the genus *Garcinia* are rich in secondary metabolites. One of the species included in the Garcinia genus is *Garcinia xanthochymus*. The *Garcinia xanthochymu* is a fruit-producing tree native to Southeast Asia (Chen et al., 2017). The content of the *Garcinia xanthochymus* plant includes flavonoids. COX

inhibition by flavonoid compounds proves that the compounds in *Garcinia xanthochymus* reduce symptoms of inflammation and pain (Winarsih, 2014). Phytochemicals show that the ethanol extract of *Garcinia xanthochymus* stems bark positively contains polar and nonpolar compounds (Andry & Winata, 2022).

In a previous study by Winata et al., 2021 regarding the content of the *Garcinia xanthochymus* plant using nonpolar solvents where the inflammatory inhibition of the *Garcinia xanthochymus* plant was obtained, researchers were interested in using a different type of solvent, namely ethanol which is a polar compound, where ethanol attracts more compounds containing secondary metabolites in the extract plant.

Methods

This study uses a laboratory experimental approach. This research was conducted at the phytochemical and pharmacology laboratories at the University of North Sumatra with the ratification of the FMIPA ethics committee certificate number 0406/KEPH-FMIPA/2021. Statistical tests carried out in this study used a two-way analysis of variance (ANOVA) test.

Preparation of *Garcinia xanthochymus* Stem Bark extract

Extraction was done by maceration using 5 liters of 96% ethanol as a solvent. Put 500 g of Garcinia xanthochymus powder into a container, pour 75% 96% ethanol extractor liquid or the equivalent of 3.75 L, then cover and leave for five days while stirring frequently. Obtained macerate and dregs from the maceration process (maserat 1). The resulting dregs were returned to the filter vessel with the remaining 25% 96% ethanol solvent equivalent to 1.25 L, then kept away from light for two days while frequently stirring (macerate 2). The results of maceration 1 and 2 are combined, stirred, and evaporated using a rotary evaporator at a temperature below 40ºC.

Simplisia Characterization Examination

Determination of the amount of water

The water content was determined using the Azeotropic technique (toluene distillation). In a round-bottom flask, 200 mL of toluene and 2 mL of distilled water were combined and distilled for two hours. After cooling the toluene for 30 minutes, the amount of water in the receiving tube was measured to be within 0.05 mL. After adding 5 g of simplicia powder to the flask, the flask was heated for 15 minutes. After the toluene has reached a boil, the drip rate is changed to around two drops per second until the majority of the water has been distilled, at which point it is raised to four drops per second. Continue distillation for five minutes, then let the receiving tube to cool to ambient temperature. After all of the water has been distilled, toluene is used to rinse the interior of the cooler. After thoroughly separating the water and toluene, the amount of water was measured with an accuracy of 0.05 mL. The difference between the two volumes of water read is based on the water content of the being studied material (Kementerian Kesehatan RI, 2013; Mayasari & Laoli, 2018).

Determination of the content of water-soluble essence

A total of 5 grams dry powder was macerated for 24 hours in 100 mL of water-chloroform (2.5 mL of chloroform in 1 liter of distilled water) in a corked flask with intermittent shaking for the first 6 hours, then left for 18 hours, and finally filtered. 20 mL of the initial filtrate was evaporated to dryness in a flat-bottomed evaporation plate that had been weighed, and the remaining was heated at 105ºC until the weight remained constant. On the basis of the dried substance, the percentage of water-soluble juice is computed. (Kementerian Kesehatan RI, 2013; Mayasari & Laoli, 2018).

Determination of the concentration of soluble essence in ethanol

In a corked flask, 5 grams of the powder was macerated for 24 hours in 100 mL of 96% ethanol while being shaken intermittently for the first 6 hours, then left for 18 hours. It was then rapidly filtered to prevent ethanol evaporation. Twenty milliliters of the filtrate were evaporated to dryness in a heated and tara-coated flat-bottomed vaporizer. The remaining is heated to 105 degrees Celsius until its weight is consistent. The ethanol-soluble extract percentage determined on the dried material is 96% (Kementerian Kesehatan RI, 2013; Mayasari & Laoli, 2018).
2018).

Determination of total ash content

A total of 2 grams of powder that has been meticulously crushed and weighed is placed in an incandescent porcelain crucible and then leveled. The crucible was gently incandescent till the charcoal ran out; the incandescence was conducted at 600°C for three hours, then cooled and weighed until a steady weight was reached. The ash content was computed based on the dried substance (Kementerian Kesehatan RI, 2013; Mayasari & Laoli, 2018).

Determination of acid insoluble ash content

The ash obtained during the measurement of the ash content was heated for 5 minutes in 25 mL of diluted hydrochloric acid; the portion that was insoluble in acid was collected, filtered, ignited, and then brought to a constant weight. On the basis of the dried sample, the acid-insoluble ash content was computed (Kementerian Kesehatan RI, 2013; Mayasari & Laoli, 2018).

Phytochemical Screening

Alkaloids Test

As much as 0.5 g of *Garcinia xanthochymus* extract was weighed before 1 mL of 2 N hydrochloric acid and 9 mL of distilled water were added, boiled in a water bath for 2 minutes, cooled, and filtered. The filtrate was utilized in the subsequent experiments:

1) Add two drops of Mayer reagent to three drops of the filtrate to form a white/yellow precipitate.
2) Add two drops of Bouchardat reagent to three drops of filtrate to generate a brown-black precipitate.
3) Add two drops of Dragendrof reagent to three drops of filtrate to form a brick-red precipitate.

If at least two or three of the aforementioned tests indicate the

presence of alkaloids, then the simplicia is deemed to have alkaloids. (Shaikh & Patil, 2020; Alqethami & Aldhebiani, 2021; Kabede *et al.,* 2021).

Flavonoids Test

One gram of *Garcinia xanthochymus* extract was mixed with 10 cc of boiling water. The mixture is then boiled for approximately five minutes and filtered while still hot. 5 mL of the filtrate were collected, 0.1 g of Mg powder, 1 mL of concentrated HCl, and 2 mL of amyl alcohol were added, and the mixture was agitated and allowed to separate. Flavonoids are positive if the amyl alcohol layer is colored red, yellow, or orange (Shaikh & Patil, 2020; Alqethami & Aldhebiani, 2021; Kabede *et al.,* 2021).

Tannins Test

One gram of *Garcinia xanthochymus* extract was weighed, cooked for three minutes in distilled water, chilled, and then filtered. The filtrate is next treated with 1-2 drops of 1% iron (III) chloride reagent; if it becomes blue-black, the presence of tannins is confirmed (Shaikh & Patil, 2020; Alqethami & Aldhebiani, 2021; Kabede *et al.,* 2021).

Glycoside Test

The extract of *Garcinia xanthochymus* was weighed, then filtered with 30 mL of a combination of 96% ethanol and water (7:3), 10 mL of 2 N hydrochloric acid was added, and the liquid was refluxed for 2 hours, cooled, and filtered. Take 30 mL of the filtrate, combine it with 25 mL of distilled water and 25 mL of 0.4 M lead (II) acetate, then mix, let stand for 5 minutes, and filter. The filtrate was filtered three times with 20 mL of a 3:2 chloroform-isopropanol combination. Take a layer of water, add 2 mL of water and five drops of Molisch reagent, and then gently add 2 mL of pure sulfuric acid to make a purple ring showing the presence of sugar (Shaikh & Patil, 2020; Alqethami & Aldhebiani, 2021; Kabede *et al.,* 2021).

Saponins Test

Garcinia xanthochymus extract was weighed at 0.5 g, applied to a test tube along with 10 mL of hot distilled water, chilled, and then rapidly shaken for 10 seconds; foam or foam developed for at least 10 minutes and measured between 1 and 10 cm in height. If the foam remains after adding one drop of a 2 N hydrochloric acid solution, the presence of saponins is indicated (Farag *et al.,*2018; Roghini & Vijayalaksmi, 2018).

Steroids/Triterpenoids Test

One gram of *Garcinia xanthochymus* extract was macerated with 20 mL of n-hexane for two hours before being filtered. The filtrate is evaporated in a plate for evaporation. Two drops of anhydrous acetic acid and one drop of concentrated sulfuric acid are added to the remaining. A purple or red hue that transforms into a blue-green hue indicates the presence of triterpenoid steroids. (Farag *et al.,*2018; Roghini & Vijayalaksmi, 2018).

Anti-Inflammatory Activity Test

Preparation of suspension of Garcinia xanthochymus stem bark ethanol extract

The ethanol extract of *Garcinia xanthochymus* stem bark was weighed 10 grams In addition, 1% Na CMC suspension was added to the mortar was little by little while grinding; the suspension preparation of Injecting the *Garcinia xanthochymus* stem bark extract into a 100 mL volumetric flask was adequate to achieve a 100 mg/mL concentration (Rahman & Jahan, 2021).

Paw Edema Test

As many as 25 white male rats as test animals were randomLy divided into five groups of five, as follows:

a. Negative control, given 1% Na CMC suspension (oral)
b. Positive control, given 2,25 mg/kgBW Na-Diclofenac suspension (oral)
c. Test group dose 1, given a suspension of ethanol extract of *Garcinia xanthochymus* stem bark 200 mg/kgBW (oral)
d. Test group dose 2, given a suspension of ethanol extract of *Garcinia xanthochymus* stem bark 400 mg/kgBW (oral)
e. Test group dose 3, given a suspension of ethanol extract of *Garcinia xanthochymus* stem bark 600 mg/kgBW (oral)

First, the rats fasted for approximately 18 hours before the experiment started (drinking water was still given). The hind legs of the rats to be induced were marked using a marker so that the measurements were the same each time. Then, the normal volume of the rat's feet was measured (V_0) by dipping the rat's paw in the plethysmometer. Then each group was given 0.1 mL of 1% carrageenan solution on the sole intra-plantar. After 40 minutes, the rats were given a suspension of the test material, and then the volume of the rat's feet was measured at 1, 2, 3, 4, 5, and 6 hours after being induced by carrageenan (Fitri *et al*., 2021).

The formula for measuring the value of edema and the percentage of edema inhibition is as follows (Sharma *et al.,* 2020) :

$$\% \text{ Inflammation} = \frac{Vt - Vo}{Vo}\ x\ 100\%$$

Information:

Vo = Normal foot Volume

Vt = Volume of inflammation after the time (t)

$$\% \text{ Inflammation Inhibition} = \frac{a - b}{a} x\ 100\%$$

Information:

a = Percentage of negative control inflammation

b = Percent inflammation of the test sample

Results

Simplicia Characterization

The simplicia characterization includes determining water content, total ash content, acid insoluble ash content, water-soluble extract content, ethanol-soluble extract content, and drying losses to ensure uniform quality of simplicia so that it meets the simplicia and extract standard requirements. Water content is less than 10% which is equal to 8.62%. Concentration

was determined using two solvents, namely water, and ethanol. The determination of water-soluble extract yielded a value of 17.74%. Determination of the concentration of the ethanol-soluble extract reveals the compound removed from the ethanol solvent (polar or non-polar). Insoluble in ethanol are glycosides, steroids, alkaloids, flavonoids, substances with low solubility, lipids, and saponins. The concentration of the extract which dissolves in ethanol is determined to be 29.54 percent (Utami, 2020).

Phytochemical Screening

Phytochemical screening is one way to identify the content of secondary metabolites of a natural product. *Phytochemical screening* is an initial stage that can provide an overview of the content of certain compounds in the natural product to be studied. Research conducted on the ethanol extract of Garcinia xanthochymus stem bark obtained positive results related to its phytochemical screening in which the extract contained secondary metabolites, namely saponins, glycosides, steroids, alkaloids, flavonoids, and tannins, where the secondary metabolite content, which is helpful as an anti-inflammatory agent is a flavonoid.

Anti-Inflammatory Activity Testing

Changes in the volume of the rat's paws can indicate the amount of inflammation that occurs in the rat's paws. The percentage of inflammation inhibition or inflammatory inhibition indicates how effectively the induction of carrageenan lambda produces each test sample or comparison drug used in suppressing rat foot edema. Induction of carrageenan results in The formation of inflammation consisting of two phases (Andry, M., & Faisal, 2022).

The first phase (early phase) is 1-2 hours after the injection of carrageenan, the release of serotonin and histamine to the site of inflammation and an increase in prostaglandin synthesis in the damaged tissue. In the second phase (late phase), prostaglandin release occurs 3 hours after the induction of carrageenan then edema develops rapidly and lasts at a maximum of about 5 hours after induction (Adyasari *et al*., 2018). The measurement results of percent inflammation and percent inflammation inhibition can be seen in Figure 1 and Figure 2.

Table 1. Result of simplicia Characterization of stem bark of *Garcinia xanthochymus*

No	Parameter	Result (%)
1	Water Content	8.62%
2	Content of water soluble extract	17.74%
3	Content of ethanol soluble extract	29.54%
4	Total ash content	3.70%
5	Content of acid insoluble ash	0.69%

Table 2. Result pytochemical screening of stem bark of *Garcinia xanthochymus*

No	Secondary Metabolites	Result
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Tanins	+
5	Glycosides	+
6	Steroids/triterpenoids	+

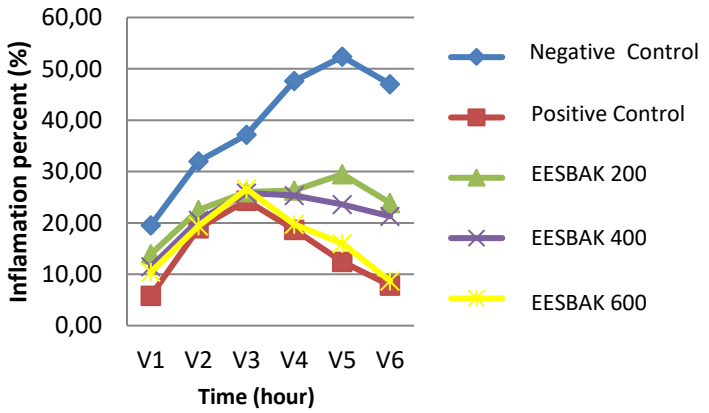

Figure 1. Graph of percent inflammation of the soles of mice,
*EESBAK(Extract Ethanol Stem Bark of *Garcinia xanthochymus*
*EESBAK 200 is a significant inflamation

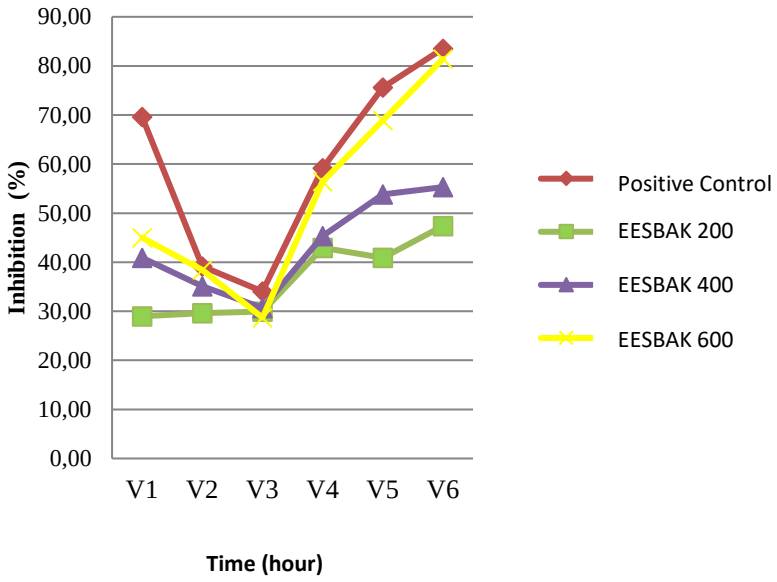

Figure 2. Graph of percent inhibition of rat paws
*EESBAK(Extract Ethanol Stem Bark of *Garcinia xanthochymus*),
*EESBAK 200 is a significant antiinflamation

Discussion

Research on the ethanol extract of Garcinia xanthochymus stem bark obtained positive results related to its phytochemical screening. The extract contained secondary metabolites, namely glycosides, steroids, alkaloids, and flavonoids, where the secondary metabolite content is helpful as an inflammatory inhibitor. Flavonoids can protect lipid membranes against destructive absorption and suppress inflammatory mediators such as histamine and prostaglandins. The bioactive content in the ethanol extract of Garcinia xanthochymus stem bark is helpful for treatment. The peculiarity is that plant extracts contain bioactive compounds. The results of the phytochemical screening showed that the ethanol extract of the stem bark of Asam Kandis positively contained polar and non-polar compounds (Utami, 2020; Andry & Winata, 2022). Based on Figures 1 and 2 on the leg edema test, male rats were given ethanol extract of stem bark at doses of 200, 400, and 600 mg/kg, 1% CMC Na negative suspension, and 2.25 mg/kg diclofenac sodium positive control. The best dose for reducing inflammation is 600 mg/kg.

The anti-inflammatory test included two parameters: measuring the volume of inflammation and inflammation inhibition in the left paw of experimental animals induced by carrageenan. The volume of edema in the rats' soles was measured every 1 hour for 6 hours after induction of carrageenan. Before treatment, each rat fasted for approximately 18 hours before the experiment started (drinking water was still given); this aims to avoid the possibility of the influence of food on the content of nutritious ingredients in the ethanol extract of *Garcinia xanthochymus* stem bark which can affect the anti-inflammatory effect it causes. Rats were acclimatized in the study room for ± one week. The purpose of acclimatization is so that the rats are not stressed and get used to their new residence.

In the graphic observation, the percentage of inflammation in the negative control group (Na CMC) showed an increase in the percentage of inflammation until the 5th hour and a decrease in the percentage of inflammation starting at the 6th hour, with a percentage of inflammation of 47%. The average percentage of inflammation in the *Garcinia xanthochymus* stem bark extraction suspension test sample group was 600mg /kgBW at the 6th hour, which was 8.70%, was close to the percentage of inflammation, the average positive control of sodium diclofenac was 2.25mg/kgBW, which was 7.84%. The greater the percentage of inflammation on average, the higher the edema in the left foot of the rats formed. In graphical observations of the percentage of inflammation inhibition, it can be seen that administration of EEKBAK suspension at a dose of 600 mg/kg BW was effective in suppressing edema that occurred at the sixth hour, reaching 81%, almost the same as the positive control group (Sodium diclofenac) was able to obtain a percentage of 83.52% inhibition. The higher average inflammatory inhibition indicated the ability of each test sample or comparison drug to suppress rat foot edema by induction of carrageenan lambda. It can be seen that the higher the dose given, the higher the percentage of anti-inflammatory inhibition. But another possibility is that doses that exceed the effective and higher doses will produce a lower or decreased effect because there are several types of drugs in high doses that release histamine directly from mast cells, causing blood vessels to become permeable to plasma fluid and cause inflammation (Adnyasari *et al*., 2017)

Based on the homogeneous set test using the Tukey HSD Post Hoc Test, the sodium diclofenac group (positive control) and the *Garcinia xanthochymus* 600 mg/kg group showed no significant or the same results (p>0.05). *Garcinia xanthochymus* suspension 600 mg/kg is the best dose of *Garcinia xanthochymus* stem bark extract in testing anti-inflammatory activity and obtains almost the same value as diclofenac sodium.

Conclusion

Based on research conducted, the ethanol extract of Garcinia xanthochymus stem bark contains secondary metabolites of flavonoids which are helpful as inflammatory inhibitors. The best dose of this study was 600 mg/kg, which can be seen based on the decrease in the volume of paw edema in white male rats.

Conflict of Interest

The authors declare no conflict of interest.

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Author contribution

Hanafis Sastra Winata: research conceptualization, data collection, Muhammad Andry: research conceptualization and research data compilation Muhammad Amin Nasution: Manuscript Writing, Script Revision, Firman Rezaldi: Script Writing and research data collection, Ade Shindy F Br Sembiring: sampling and data compilation study

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