

Anti-Inflammatory Activity of *Ormosia Calavensis Azola* (Bahai) Leaf Extract

Jellian B. Pedong¹, Melinda C. Getalado^{1,2}

¹Department of Physical Sciences, College of Science,

²University Research and Development Services,

^{1,2}University of Eastern, Northern Samar, Philippines

How to cite this paper: Jellian B. Pedong | Melinda C. Getalado "Anti-Inflammatory Activity of *Ormosia Calavensis Azola* (Bahai) Leaf Extract" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-3 | Issue-4, June 2019, pp.1687-1690, URL: <https://www.ijtsrd.com/papers/ijtsrd25223.pdf>



IJTSRD25223

Copyright © 2019 by author(s) and International Journal of Trend in Scientific Research and Development Journal. This is an Open Access article distributed under the terms of the Creative Commons



Attribution License (CC BY 4.0) (<http://creativecommons.org/licenses/by/4.0>)

Inflammation is the human body's response to injuries arising from a part which is under attack by viruses or bacteria, such as inflamed wounds. Other inflammations are caused by internal disorders in our body, such as arthritis and joint rheumatism. This is characterized by enlargement of the part where the injury is present.

Many plants constitute to the development of medicines and other health remedies for today. But, in many areas where technological medicine is scarce, people used old traditional ways to relieve themselves from injuries and inflammations. These people often use plant parts to give them relief from common illness, and one of these plants is *Ormosia calavensis azola* in locally known as Bahai.

The Bahai plant can be found in Cagayan; in the Ilocos province of Luzon; Surigao and Zamboanga in Mindanao, particularly in forest at low and medium altitudes. This tree found scattered in dipterocarp forests. The bark of a Bahai tree is gray or dark and rough. The under-bark is a pale yellow and less than 25 millimeters in thickness. The bark has no sap and the terminal buds are not enclosed by leaves.

Based on research, *Ormosia calavensis azola* wood extract is potent against microorganisms. The extract had a great in

ABSTRACT

This study determined the anti-inflammatory activity of *Ormosia calavensis azola* (Bahai) leaf extract. The physical properties test shows that the plant extract is acidic, less dense in water and polar. The alkaloids, flavonoids, leucoanthocyanin, saponin, tannin and terpenoids were positive in Bahai leaf extract. Application of the three treatments shows the following results; the negative control rapidly increases the thickness of paw with reddish color of inflammation after treated with carrageenan. Both the positive control and the plant extract had significant reduction effect on the inflammation. These results implied that Bahai leaf extract is an effective anti-inflammatory substitute. The researcher recommends the following perform further study of the compounds structure present in the Bahai plant; perform further study of anti-inflammatory using the positive control indomethacin; perform further study using other Bahai plant parts like roots and bark; perform further study of plant extract in other uses such as high blood pressure, dysentery and etc.

Keywords: anti-inflammatory activity, *Ormosia calavensis azola* (Bahai)

1. INTRODUCTION

Plants have been on this world for so many years that they evolve to adapt in a certain environment. Many plants produce toxins to defend themselves from herbivores and other plant eating insects. Others are simply used as foods to humans and animals, and also, others are used as herbs. Herbs are plants that are used to treat certain kinds of diseases and illness in humans and animals; they are also used to counteract venom and other irritating pain in our body such as inflammations.

vitro potential for antibacterial activity against three tested bacteria. The inhibitory effect of the extract of *Ormosia calavensis azola* against this bacterial strain can introduce the plant as potential candidates for the treatment of ailment caused by these pathogens. The presence of these compounds usually further supports and justified the traditional use of the plant for treatment of skin infections and abdominal disorders. It also confirmed the toxicity of *Ormosia calavensis azola* wood extract.



Figure1. *Ormosia calavensis azola* (Bahai) sample

METHODOLOGY

A total of two hundred (200) grams of Bahai leaf samples was collected and used in the entire study. The leaf of the Bahai plant were washed and pruned from its shoots using a scissor and collected into a beaker.

The leaf of the plant was weighed and finely cut. For the extraction procedure, about 200 g of finely cut leaf as soaked in pure methanol. The flask was covered and the leaf was soaked overnight. After soaking, the mixture was filtered through clean cheesecloth to separate the leaf followed by filtration using a Buchner funnel. The filtrate was distilled by heating to the boiling point of methanol. The solvent-free leaf extract of Bahai was stored in clean labelled airtight bottle inside the refrigerator ready for use. The crude leaf extract was measured and transferred into clean bottle. The extract was ready for testing of secondary metabolites and anti-inflammatory agent.

Test for Physical Properties

Color determination was tested on the extract of Bahai leaf using the sense of sight of a total of five respondents. About two (2) mL of Bahai leaf extract was measured and put in a clear test tube. Then, using the sense of sight, the respondents described the color of the extract contained in the test tube. The obtained perceptions of the color from the respondents were gathered for proper color determination.

Odor determination was tested on the Bahai leaf extract using the olfactory sense of a total of five respondents. About two (2) mL of Bahai leaf extract was measured in a clear test tube. Then, using the sense of smell, the respondents described the odor of the leaf extract contained in the Petri dish. The obtained perceptions for the odor of the extract from the respondents were gathered for proper odor determination.

pH determination was tested on the Bahai leaf extract using a pH paper. About 5 mL of the extract were poured in a 10 mL beaker. Then, a pH paper was dipped into the extracts. After a period of one (1) minute, the pH value was compared and recorded. The procedure was repeated thrice. Average pH was also computed.

About Ten (10) mL of the Bahai leaf extract was measured on an analytical balance, the weight of the leaf extract used (in mL). The procedure was repeated thrice. Average density was also computed. The equation used is shown below:

$$\text{Density} = \frac{\text{mass}}{\text{volume}}$$

For testing the miscibility of the Bahai leaf extract, three (3) solvents were used, namely; Hexane, water and acetone. About 2 mL of the Bahai extract were put into nine (9) clear test tubes. Then, two (2) mL each of hexane was poured into three test tubes, same goes to another three test tubes, but it was poured with two (2) mL each of water, and lastly, the remaining three test tubes with the leaf extracts of Bahai extract was poured with two (2) mL each of acetone solvent. For one (1) full hour, the nine test tubes were observed to determine the miscibility of the extract in three (3) different solvents. After the given hour, the researcher recorded the result whether it is (1) miscible or (2) slightly miscible and (3) not miscible.

Secondary Metabolites Screening

Test for the secondary metabolites of the Bahai alcoholic extracts was done. The following procedures were used in the tests with three replications:

Test for Alkaloid

About 5 g of the Bahai leaf was extracted with 25 mL diluted hydrochloric acid. The acidic filtrate was rendered alkaline with ammonium hydroxide and extracted with three successive portions (each mL of chloroform). The chloroform extracts was evaporated until incipient dryness and the residues have been dissolved in 2 mL of dilute hydrochloric acid and tested with Mayer's and modified Dragendorff's reagent.

Mayer's reagent: when added to the residue solution, turbid or white precipitate will be formed; this indicates the presence of alkaloids.

Dragendorff's reagent: when added to the residue solution an orange precipitate was confirmed; this indicates the presence of alkaloids.

Test for Anthraquinones

Leaf extract of about 5 mL was prepared and evaporated to incipient dryness over a steam bath. 10 mL of distilled water was added to the residue and was filtered separately with 5 mL ammonia solution and shaken, and then it was compared to the control tube.

The Borntrager's test detects presence of anthraquinones. A red colorization in the lower ammoniacal layer indicates the presence of anthraquinones.

Test for Flavonoids

About 0.5 g of Bahai leaf extract was dissolved in ethanol, warmed and then filtered. Three pieces of Magnesium chips was added to the filtrate followed by a few drops of concentrated hydrochloric acid. A pink, orange or red to purple is the evidence for the presence of flavonoids.

Test leucoanthocyanin

About 5 mL of Bahai leaf extract was defatted by taking up the residue with 3 mL hexane and 2 mL water or with petroleum ether. The hexane was discarded or the petroleum ether extract. Aqueous extract was diluted with 10 mL 80% ethanol and were filtered. The filtrate was divided into two test tubes, one portion as a control, and then one portion of the alcohol filtrate was treated with 0.5 mL HCL. A positive result is indicated by the formation of a strong violet color.

Test for Saponin

The capillary test was used to determine the presence of saponins. If the level of the plant extract in the capillary tube is half or less than in the other tube containing water. The presence of the saponins may be inferred. Load the capillary tube with plant extract by immersing the tube to a height of 10 mm in the plant extract.

Test for Tannin

About 5 mL of Bahai leaf extract as added with 3 mL gelatine salt reagent. The formation of the jelly-precipitate indicates the presence of tannin.

Test for Terpenoids

About 2 mL of Bahai leaf extract was added 2 mL of acetic anhydride and concentrated sulphuric acid. The formation of blue or orange rings indicates the presence of terpenoids.

Test for triterpenes

The Lieberman-Burchard test was used to test for the presence of triterpene. A range of colors from blue to green,

red, pink, purple or violet indicates the presence of the triterpene.

Equivalent of 3 g of plant extract was evaporated to incipient dryness over a steam bath. Then, cooled to room temperature. The material was defatted by taking up the residue 6 mL of hexane and 3 mL of water. Partition by gently shaking the mixture in the test tube. The upper hexane layer was pipetted. The treatment was repeated with hexane until most of the color pigments has been removed and discard all the hexane properly. The aqueous layers was treated with 5 mL chloroform and gently shake. The mixture was allowed to stand and chloroform extract was dried by filtering through about 100 mg anhydrous sodium sulphate held over the dry filter paper. The filtrate was divided in two portions, use one for control. Other portion was treated with 3 drops of acetic anhydride then 1 drop of concentrated sulphuric acid. Observation was done for any immediate color change.

Anti-inflammatory test

A total of twenty (20) albino mice were used to determine the anti-inflammatory activity of the Bahai leaf extract using carrageenan induced paw edema. Mefenamic acid was used as positive control, while water a used as the negative control.

Preparation of the Test Animals using Carrageenan Induced Paw Edema

Each albino mice were weighed according to the body and the mass of mice preferably 25-30g. To the right paw of each mice 0.05 mL stock solution of carrageenan/ gelatin was injected extradermally. Dosage was 2 mL/ gbw of mice, time treatment was recorded for each mice. The researcher used gelatine powder as alternative for carrageenan, since it is also a product of sewage same as the carrageenan. A 3.25g of mefenamic acid (Positive control) was dissolved in saline solution drop by drop until the mefenamic acid was dissolved. Carrageenan of about 0.25g was dissolved in saline solution. Prepared 50 mL of negative control and 30 ml of pure extract. Nine (9) mice per trial, 27 mice in all and all identified will be put a tape.

Testing of the Extract, Positive and Negative control

The mice was grouped into three and treated them as follows: treat 1 (negative control) - inject intradermally to the right hind paw 0.05 mL of distilled water per 25g mouse; treatment 2 (Positive control)- inject intradermally to the right hind paw 0.05 mL of the stock solution of mefenamic acid per 25g mouse; treatment 3 (plant extract)- administer orally using dropper 0.25 mL of extract per 25g of mice, time treatment was recorded for each mice, the volume of the right paw was measured for every mice using vernier caliper. One hour after the above treatment, to the right paw of each mice 0.05 mL stock solution of carrageenan / gelatine was injected extradermally. The researcher used gelatin powder as alternative for carrageenan, since it is also a product of sewage same as the carrageenan.

RESULTS AND DISCUSSION

The Bahai leaf extract was collected from Scout city, University of Eastern Philippines, Catarman N. Samar. Results of the study are herein presented. The physical characterization in terms of color and odor, pH, density and solubility. The secondary metabolites are in terms of alkaloid, Anthraquinone, flavonoids, leucoanthocyanin,

saponins, tannin, terpenoids, triterpene and the anti-inflammatory activity.

The **physical properties** of Bahai leaf extract were determined in terms of color, pH, density and solubility. The color of the Bahai leaf extract was observed and recorded by the five respondents. The odor was obtained by wafting the air above the extract. The color of Bahai leaf extract was brownish while the odor of the Bahai leaf extract was unpleasant. The pH results of Bahai leaf extract was determined using the pH paper indicator. The average pH of the Bahai leaf extract is 4.7 which mean that the extract was acidic. The density was obtained by dividing the mass of the extract by volume of the extract. It was done three trials.

The solubility of Bahai leaf extract was determined using three solvents (water, hexane and acetone). The solubility of Bahai leaf extracts on three different solvents. leaf extracts were soluble to both water and acetone but insoluble to hexane. This implies that the extract is polar. All were insoluble in hexane, hexane being a non polar solvent.

Table1. Summary results of the physical properties of Bahai leaf extract.

PHYSICAL PROPERTIES	RESULTS
Miscibility	Polar
Color	Brownish
Density	0.96 g/mL
Odor	Unpleasant
pH	4.7

Secondary metabolites. The test for alkaloid was done using Dragendorff's reagent and Mayer's reagent. In the test of the leaf extract starting from trials 1 to 3 same results were obtained. There was orange precipitate formed when it was treated with Dragendorff's and Mayer's reagent. This means that alkaloid is present in the Bahai leaf extract.

The modified Bontrager's test was used to determine the presence of Anthraquinone. A red color indicates a positive result of Anthraquinone. The results starting from trials 1 to 3 show the same results. The leaf extract gives negative results when it was treated with ammonia. It reveals that the leaf of Bahai do not contain Anthraquinone.

In the test for flavonoids, three pieces of magnesium chips was added to filtrate followed by a few drops of concentrated hydrochloric acid. A pink, orange or red to purple coloration is the evidence for the presence of flavonoids. There was a red colored formed when it was added of Mg chips and drops of conc. HCL. This means that flavonoids are present in the Bahai leaf extract.

Bate-Smith and Metcalf method was used to determine the presence of leucoanthocynin. A strong red or violet color indicates a positive result. Bahai leaf extract treated with 0.5 mL conc. HCL, within minutes there is a change in color happened, which indicates a positive result.

Capillary tubes were used to determine the presence of saponins. If the height of the extract is half or less than in the other tube containing distilled water, then the presence of saponin may be inferred. The results for the saponins test of the Bahai leaf extract revealed that it is positive.

The 10% gelatine salt reagent was used to determine the presence of tannin. The leaf extracts has a positive result

when it was treated with 10% gelatine salt reagent which indicate a positive result in tannin.

Terpenoid was determined using acetic anhydride and conc. H_2SO_4 . In all the results starting from trial 1 to 3 there is a formation of blue or orange rings which indicates the presence of terpenoid.

The liberman-Burchard test was used to test for the presence of triterpene. A range of colors from blue to green, red, purple or violet indicates the presence of the triterpene. The results of the triterpene test in Bahai leaf extract from trails 1 to 3 has no color appearance, which indicate a negative result. Bahai leaf extract treated with 0.5 ml conc. HCl, within minutes there is no change in color happened, which indicates a negative result.

Table2. Summary results of the secondary metabolite of Bahai leaf extract.

SECONDARY METABOLITES	RESULTS	INTERPRETATION
Alkaloid Mayer's reagent Dragendorff's reagent	White precipitate Orange precipitate	Positive Positive
Anthraquinone	Clear solution	Negative
Flavonoids	Red coloration	Positive
Leucoanthocyanin	Strong violet colored solution	Positive
Saponins	Higher than the extract	Positive
Tannin	White jelly precipitate	Positive
Terpenoids	Orange ring precipitate	Positive
Triterpene	Clear solution	Negative

In **Anti-inflammatory activity** the negative control rapidly increases the thickness of paw with reddish color of inflammation after treated with carrageenan. Both the positive control and the plant extract had significant reduction effect on the inflammation. These results implied that Bahai leaf extract is an effective anti-inflammatory substitute.

Table3. Average reading of inflammations thickness over a period of 5 hours

Treatment	Average thickness (mm)		
	Trial 1	Trial 2	Trial 3
(-)control	0.62	0.66	0.66
(+)control	0.60	0.60	0.53
Plant sample	0.51	0.46	0.45

CONCLUSIONS

The Bahai leaf extract has a pH of 4.7, density of 0.96 g/mL. Extract is polar. Its color was brownish with unpleasant odor. The alkaloid, flavonoids, leucoanthocyanin, saponins, tannin and terpenoids they are the secondary metabolites have present in Bahai extract. Bahai leaf extract is an effective anti-inflammatory substitute. Bahai leaf extract was comparable with the commercial drug.

References

[1] Bhatt SV, Verma Y. 1993. Antimicrobial of *Ormosia calavensis* extract. An experimental and clinical evaluation. *Phytother Res; The World Book Encyclopedia*, Vol 1: 353.

[2] Consolacion Y. Ragasa, Angel Lyn Kristin C. CoJohn A. Rideout. 2005. Antifungal metabolites from *Ormosia calavensis*. *Natural Product Research*, 19(3): 231-237.

[3] Guevara Q. 2005. A guide book to plant phytochemical screening. UST Publishing House, Espana, Manila.

[4] Mahesh PG, Velasco RN, Demigillo JM, Rivera WL. 2010. Antimicrobial Activity of some medicinal plant against plant and human pathogen. *World Journal of Agricultural Science*, 4(5): 839-843.

[5] Saadabi and Abuzaid. 2011. Antimicrobial Effect of *Ormosia calavensis* extract in preadipocytes and adipocytes. National Center for Biotechnology Information, US National Library of Medicine 8600 Rockville Pike, Bethesda MD.