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Original Article

Activity of Pegagan Embun (*Hydrocotyle sibthorpioides* Lam.) Extracts in Capsule Dosage Form to IgM and IgG Levels in Humans

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ABSTRACT

Objective: Traditionally, Pegagan embun (*Hydrocotyle sibthorpioides* Lam.) has been used to enhance the immune system. Previous studies confirmed that *Hydrocotyle sibthorpioides* Lam. enhanced antibody titers and was practically non-toxic by animal tests. This study aimed to determine the effect of *Hydrocotyle sibthorpioides* Lam. extract on IgM and IgG levels in humans. **Methods:** The participants involved 20 healthy volunteers aged 20-25 years were divided into two groups with a placebo as a comparison. The capsules containing *Hydrocotyle sibthorpioides* Lam extract were in the treatment group at a dose of 67 mg and the placebo group was given capsules containing lactose orally for three days. IgM and IgG levels were measured before and after consuming the capsules. The ELISA method tested IgM and IgG levels and detected them using specific antibodies that form complexes with enzymes and substrates. **Results:** The results showed the average baseline IgM level of 1.480 mg/mL and after consumption of 1.405 mg/mL for placebo. For *Hydrocotyle sibthorpioides* Lam. extract capsules, the baseline was 1.492 mg/mL and 1.854 mg/mL after consumption. For IgG, before given 8.941 mg/mL and after given 8.710 mg/mL for placebo, while before given *Hydrocotyle sibthorpioides* Lam. extract capsules 8.946 mg/mL and after given *Hydrocotyle sibthorpioides* Lam. extract capsules was 10.234 mg/mL. Statistical results showed a significant increase in IgM and IgG levels after consuming *Hydrocotyle sibthorpioides* Lam. extract capsules for three days ($p<0.05$). **Conclusion:** It can be concluded that 67 mg of *Hydrocotyle sibthorpioides* Lam. extract capsules can increase IgM and IgG levels in humans.

Key words: Extract, *Hydrocotyle sibthorpioides* Lam, IgM, IgG, ELISA, Volunteers.

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INTRODUCTION

Medicinal plants have medicinal properties and are used to heal and prevent disease.[1] Indonesia has many medical plants; about 30,000 species of flora are found in Indonesia's tropical forests, and around 9,600 species have been recognised as medicinal. Since ancient times, medicinal plants processed into medicine have been widely used by the Indonesian people, both in the form of single fresh, mixed, and herbaceous, better known as traditional medicine.[2]

The immune system can protect humans from foreign substances and pathogens such as viruses, bacteria, parasites, and fungi.[3] There are two types of immune responses: specific and non-specific.[4] Non-specific immune responses work quickly to protect humans from microorganisms, while specific immune responses show specific responses to certain microbes.[5] Immunostimulants can boost the body's defence mechanisms. Immunostimulant compounds can enhance the body's specific and non-specific defence mechanisms through cellular or humoral responses.[6] Certain plants contain compounds that exhibit immunostimulatory activity.[7] One such plant is the herb *Hydrocotyle sibthorpioides* Lam. which is widely used in traditional Chinese medicine to boost human immunity.[8]

The ethanol extract of *Hydrocotyle sibthorpioides* Lam. has immunostimulant activity. The previous study showed that ethanol extract of *Hydrocotyle sibthorpioides* Lam. can increase antibody titers at doses 10, 50, and 200 mg/kg BW against male white mice.[9] Previous study related to the extract of *Hydrocotyle sibthorpioides* Lam. possessed pharmacological activity to improve the immune system, with doses of 10, 50, and 200 mg/kg BW showing immunostimulatory effects through increased activity and capacity of macrophages, the number of leukocytes, and the number of macrophages, macrophage capacity, total leukocyte count, lymphocyte percentage, and significantly reduced TNF-α and total macrophage levels tested in male white mice.[10] The broad utilisation of *Hydrocotyle sibthorpioides* Lam. in the field of pharmacology, significantly as an immunostimulant, can also increase the activity of NK cells, CD8 cells[11] increase the number of erythrocytes, the number of reticulocytes, hematocrit values, and haemoglobin levels.[12] It concluded that *Hydrocotyle sibthorpioides* Lam. extract has immunomodulatory activity.

In previous preclinical studies, acute and sub-acute toxicity tests of *Hydrocotyle sibthorpioides* Lam. extract was also reported, where the results obtained from the doses given, 7 mg/kg BW, 35 mg/kg BW, and 150 mg/kg BW to the test animals, showed no damage to the histology of the liver and kidneys of male white mice.[13] As well as carbon clearance,[14] creatinine clearance,[15] and SGOT and SGPT levels after administration of *Hydrocotyle sibthorpioides* Lam. extract to test animals also had no toxic effects.[16] The lethal doses (LD50) value for the ethanol extract of *Hydrocotyle sibthorpioides* Lam. amounted to 128.83 g/kg BW, from the LD50 category, then the ethanol extract of *Hydrocotyle sibthorpioides* Lam. is classified into the range of practically non-toxic (LD50 >15,000 mg/kg BW).[13]

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IgG, which makes up 80% of the body's antibodies, is the most abundant immunoglobulin.[17] Antibodies secreted by the salivary glands include IgA, which is crucial in mucosal surface defence.[18] IgM (6%) is an antibody that activates the complement system.[19] IgD (1%) is involved in immune tolerance.[20] IgE (1%) is involved in immediate hypersensitivity reactions; this antibody causes mast cells to release large amounts of histamine, causing severe vasodilation.[19]

IgM is the first antibody produced and expressed on the surface of B cells. IgM plays a role in the primary immune response to microbial antigens, as it is the first antibody produced during infection.[21] IgM circulates in the blood, lymph, and B cell surfaces (in monomeric form).[21] The percentage of this antibody in serum is 5-10%, which acts as an antigen receptor for these cells and is also a molecular solute in the blood.[21] Due to its large size, IgM is found mainly in the intravascular space (in the bloodstream).[21] Since IgM is produced in the immune response, its efficiency in binding to antigens is critical until sufficient amounts of IgG have been synthesised.[22] Although IgM usually has low affinity binding sites for antigens, it has ten combining sites per molecule that can synergise with each other on the same molecule when binding to microbes.[22] Thus, the overall tightness of binding of IgM molecules (avidity) to microbes is relatively high, making this class of antibodies very effective in eliminating microbes.[22] IgG levels also rise higher and last longer due to the presence of B cells and memory T cells due to the first antigen exposure.[22]

Based on the explanation, the study aimed to examine the activity of *Hydrocotyle sibthorpiodes* Lam. extract on IgM and IgG levels in human serum. It was conducted in the form of capsule preparation because it is easy to prepare and easy to use.

MATERIALS AND METHODS

Tools

The tools used were analytical balance (Ohaus), dropper, spatel, beaker glass (Pyrex), serum rack and tube, mortar and pestle, rotary evaporator (Buchi®), grinder, funnel, volumetric flask (Pyrex), Erlenmeyer (Pyrex), centrifuge, UV-Vis spectrophotometer (Thermo Scientific Genesys 10S UV-Vis), UV-lamp (Camag), measuring cup (Pyrex), thermo-shaker (BioSan), vortex (Ika Vortex Genius 3), volumetric flask (Pyrex), volume pipette (Pyrex), brown bottle, KLT vessel, oven, porcelain crucible (Pyrex), desiccator, test tube (Pyrex), tab density tester (Electrolab), disintegration tester (Lorderan), flowtester, microplate absorbance spectrophotometer (BIO-RAD).

Materials

Hydrocotyle sibthorpiodes Lam., distilled water, 70% ethanol, ethanol P, rutin, silica gel 60 F254 (Sentana), phytochemical reagents (Mayer reagent, Dragendorff reagent, HCl, metal Mg, FeCl3, HgCl2, AlCl3, Sodium acetate), mobile phase (n-butanol and acetic acid), Whatman filter paper, aluminium foil, capsule shell, magnesium stearate, aerosol, Saccharum lactis, Manihot amylum, and IgM and IgG ELISA kits (BT LAB).

Preparation of ethanol extract from *Hydrocotyle sibthorpiodes* Lam.

Hydrocotyle sibthorpioides Lam. extract was collected from 5000g of fresh *Hydrocotyle sibthorpioides* Lam. plants, then cleaned from dirt by washing using running water, then aerated to dry the simplisia. The dried simplisia was crushed using a blender and sieved using sieve no. 48. The sample that has been finely ground, then macerated by inserting 1 part of the powder and adding 10 parts of the solvent into a dark bottle as a maceration container, soaked with 70% ethanol for the first 6 hours while occasionally stirring, then let stand for 18 hours. All macerates were collected, and the solvent was evaporated using a rotary evaporator until a thick extract was obtained. After obtaining the thick extract, the yield calculation was carried out. The yield must reach at least the number specified in each extract monograph.[23]

Capsule formulation

All ingredients were weighed 335mg for each capsule. Each ingredient for the composition of the test capsule consisted of *Hydrocotyle sibthorpiodes* Lam. extract, aerosil, amylum Manihot, Mg stearate, and lactose. Put the extract of *Hydrocotyle sibthorpiodes* Lam. into a mortar, then add some lactose little by little, grind until homogeneous, continue with the addition of amylum Manihot, ground again until homogeneous, then aerosol and Mg stearate was added, grind again until homogeneous then remaining lactose was added little by little. Furthermore, ground it again until the powder was homogeneous and placed into an empty capsule shell with a size of 0, which had a 300-500 mg capacity. The capsule formulation is presented in Table 1.

Standardisation of condensed extract *Hydrocotyle sibthorpioides* Lam.

The ethanol extract of Hydrocotyle sibthorpioides Lam. that has been produced underwent extract characterisation, including testing non-specific and specific parameters and analysing the extract's chemical content. It was done to ensure that the extract obtained meets safety and quality standards. Characterisation of non-specific parameters were drying shrinkage of 5.72%, total ash content of 2.25%, acid insoluble ash content of 0.07% and water content of 5.56%. On the other hand, the specific characterisation consists of organoleptic tests, identity parameters, chemical content tests, thin layer chromatography, and determination of total flavonoid content.

Evaluation of powder contents of *Hydrocotyle sibthorpiodes* ;""

The formulated powder was subjected to evaluation tests before being put into capsules to ensure the effectiveness of the powder. The evaluation tests conducted were flow rate, compressibility, and angle of repose tests. The evaluation of the capsule showed in Table 2.

Capsule evaluation of *Hydrocotyle sibthorpiodes* Lam. extract

The organoleptic test (weight uniformity and dissolution time) was used to evaluate the effectiveness of the capsules. The characterisation of the capsule is shown in Table 3.

Table 1: Capsule formula.

Composition	F1	F1
Hydrocotyle sibthorpioides Lam. Extract	-	67 mg
Aerosil	2%	2%
Amylum manihot	2%	2%
Magnesium stearate	1%	1%
Lactose	qs	qs

Table 2: Evaluation of powder-containing capsule extract.

Evaluation Type	Results	Condition
Flow rate	8.21 gram/s	4-10 gram/s
% compressibility	14.3%	11-15%
Angle of repose	32.31^0	21-35^0

Table 3: Capsule evaluation results.

Evaluation Type	Results	Observations
Organoleptic	Qualified	Dry, fine, dark green powder, slightly bitter, no particles attached to the capsule shell
weight diversity	Qualified	<10%
Disintegration time	2 min 41 second	<15 minutes

Clinical trial protocol

Of the 20 research subjects, 10 healthy volunteers were given the test preparation of *Hydrocotyle sibthorpiodes* Lam. extract capsules and 10 healthy volunteers were given a placebo capsule preparation containing only lactose, which was consumed orally at a dose of 1 capsule per day after meals in the morning for 3 days.

Blood specimen collection and testing

Blood specimens were collected by health professionals under the supervision of the doctor in charge of the study after healthy volunteers had fasted for 6-12 hours. A volume of 3 mL of blood was taken through the mediana cubital vein at the elbow fold of the hand, which was first cleaned above the stab site with 70% alcohol and allowed to dry. Next, a damming tie (tourniquet) was attached to the upper arm, and the mediana cubital vein was pierced at a 45-degree angle. The aspirated blood will flow into the syringe, then remove the tourniquet and pull the needle while still pressing the puncture hole with alcohol cotton and the former puncture is covered with plaster. The blood obtained was tested for lymphocyte levels and then centrifuged for 30 minutes at 3000 rpm. Then, the serum was used to test IgM and IgG levels using an ELISA kit.

Data analysis

The data was analysed statistically using the paired T-test analysis method between pre and post-data, then continued with the Independent T-Test.

Ethical approval

Ethical approval was obtained from the Faculty of Medicine Ethics Committee, Universitas Andalas, with the contract number of the ethics letter: 1072/UN.16.2/KEP-FK/2022.

RESULTS

The measurement results of IgM levels of healthy volunteers are shown in Table 4, and the correlation of IgM levels with the administration of *Hydrocotyle sibthorpioides* Lam. extract capsules and placebo is shown in the bar chart in Figure 1.

The measurement results of IgG levels of healthy volunteers are shown in Table 5, and the correlation of IgM levels with the administration of *Hydrocotyle sibthorpioides* Lam. extract capsules and placebo is shown in the bar chart in Figure 2.

This study's results indicate a significant difference between the average IgM and IgG levels before and after the provision of *Hydrocotyle sibthorpiodes* Lam. extract capsule preparation. Based on the results obtained in this study, higher IgM levels were shown in the healthy volunteer group after consuming *Hydrocotyle sibthorpiodes* Lam. extract capsules. The average IgM levels before and after in the group of volunteers who consumed *Hydrocotyle sibthorpiodes* Lam. extract capsule preparation were 1.492 ± 0.388 mg/mL and 1.854 ± 0.198 mg/mL. For the placebo group, they were 1.480 ± 0.294 mg/mL and 1.405 ± 0.222 mg/mL, respectively. In comparison, the average IgG levels before and after in the group of volunteers who consumed *Hydrocotyle sibthorpiodes* Lam. extract capsule preparation were 8.946 ± 0.466 mg/mL and 10.234 ± 0.915 mg/mL. For the placebo group, they were 8.941 ± 0.843 mg/mL and 8.710 ± 0.528 mg/mL, respectively.

Based on the results of the paired T-test that has been carried out, it can be seen that there is a significant difference between the mean levels of IgM with a value of $p<0.05$ (Table 6) and IgG with a value of $p<0.05$ (Table 7) before and after consumed *Hydrocotyle sibthorpiodes* Lam. extract capsules.

Table 4: IgM levels in healthy volunteers before and after consuming *Hydrocotyle sibthorpiodes* Lam. extract capsules and placebo for 3 days.

Placebo samples	IgM Levels (mg/mL)		Hydrocotyle sibthorpiodes Lam. samples	IgM Levels (mg/mL)	
	Pre	Post		Pre	post
A1	1.515	1.466	A11	1.301	2.056
A2	1.384	1.356	A12	2.051	1.923
A3	1.180	1.359	A13	1.322	1.864
A4	1.553	1.468	A14	1.867	2.135
A5	1.568	1.451	A15	1.362	1.888
A6	1.212	1.322	A16	1.722	2.016
A7	1.192	1.034	A17	1.898	1.668
A8	1.690	1.450	A18	0.931	1.524
A9	2.150	1.908	A19	1.509	1.852
A10	1.360	1.233	A20	0.955	1.616
Average	1.480	1.405	Average	1.492	1.854
St. Deviation	0.294	0.222	St. Deviation	0,388	0.198

Table 5: IgG levels in healthy volunteers before and after *Hydrocotyle sibthorpiodes* Lam. extract capsules and placebo for 3 days consumed.

Placebo samples	IgG Levels (mg/mL)		Hydrocotyle sibthorpiodes Lam. samples	IgG Levels (mg/mL)	
	Pre	Post		Pre	post
A1	8,820	8,476	A11	9.490	11.848
A2	8,058	9,848	A12	9.021	10.856
A3	9,067	8,211	A13	8.436	10.234
A4	9,003	8,747	A14	8.765	9.246
A5	8,136	8,058	A15	8.774	9.977
A6	7,483	8,820	A16	9.657	10.907
A7	9,682	8,469	A17	9.329	8.872
A8	9,211	9,006	A18	8.136	9.756
A9	10,106	9,136	A19	9.021	10.991
A10	9,848	8,329	A20	8.829	10.329
Average	8,941	8,710	Average	8.946	10.234
St. Deviation	0,843	0,528	St. Deviation	0.466	0.915

Table 6: Paired T-test results of IgM levels of healthy volunteers in placebo and *Hydrocotyle sibthorpiodes* Lam. groups.

Group		t	Df	Sig. (2-tailed)
IgM	Pre-Post Hydrocotyle sibthorpiodes Lam Group	-3.507	9	.007
IgM	Pre-Post placebo	1.986	9	.078

Table 7: Paired T-test results of IgG levels of healthy volunteers in placebo and *Hydrocotyle sibthorpiodes* Lam. groups.

Group		t	df	Sig. (2-tailed)
IgG	Pre-Post Hydrocotyle sibthorpiodes Lam	-5.258	9	.001
IgG	Pre-Post placebo	.689	9	.508

Table 8: Independent T-test results of IgM levels of healthy volunteers in placebo and *Hydrocotyle sibthorpiodes* Lam. groups.

Group		df	Sig. (2-tailed)
IgM	Equal variances assumed	18	.000
	Equal variances not assumed	17.766	.000

Table 9: Independent T-test results of IgG levels of healthy volunteers in placebo and *Hydrocotyle sibthorpiodes* Lam. groups.

Group		df	Sig. (2-tailed)
IgG	Equal variances assumed	18	.000
	Equal variances not assumed	14.652	.000

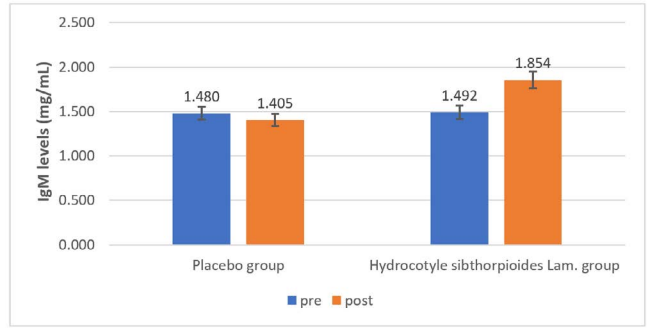

Figure 1: Chart of IgM level measurement results in healthy volunteers before and after consuming *Hydrocotyle sibthorpiodes* Lam. extract capsules and placebo for 3 days.

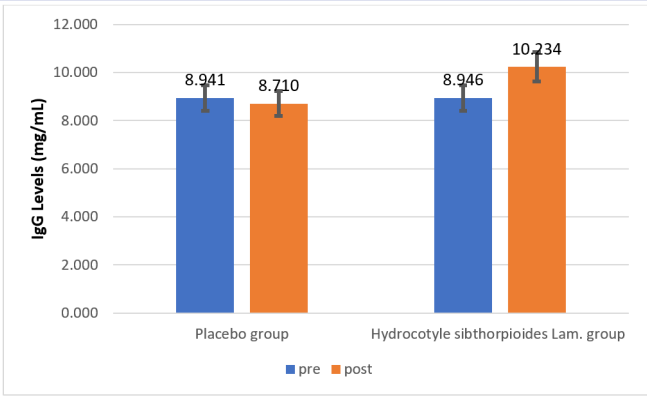

Figure 2: Bar chart of IgG level measurement results in healthy volunteers before and after *Hydrocotyle sibthorpiodes* Lam. extract capsules and placebo for 3 days consumed.

There was not a significant difference between the mean levels of IgM (Table 6) and IgG (Table 7) before and after administration of placebo capsules ($p>0.05$). Then, the results obtained from the two test groups were compared using an independent T-test. There was a significant difference between the mean levels of IgM (Table 8) and IgG (Table 9). after healthy volunteers consumed *Hydrocotyle sibthorpiodes* Lam. extract capsule preparation compared to placebo ($p>0.05$).

DISCUSSION

Pegagan embun (*Hydrocotyle sibthorpioides* Lam.) has many pharmacological activities, traditionally to relieve swelling, diuretic, expectorant, inflammatory, and neutralise toxins (detoxification).[14] The immunostimulant activity of *Hydrocotyle sibthorpiodes* Lam. is evident from the flavonoid chemical content in its ethanol extract.[24] Previous preclinical studies in animal tests have proven that *Hydrocotyle sibthorpiodes* Lam. extract has immunomodulatory effects, and toxicity tests that have been conducted state that *Hydrocotyle sibthorpiodes* Lam. extract is practically non-toxic.[9,25] Therefore, this study was conducted to see the activity of *Hydrocotyle sibthorpiodes* Lam. extract capsules on IgM levels through an immunomodulatory approach in humans. The implementation of clinical trials of herbal medicines must be based on benefit claims describing the usefulness or benefits that promise a positive change for its users, which must be under empirical history supported by sufficient scientific evidence through relevant preclinical trials. The required scientific support evidence is safety data, at least the LD50 value of herb simplistic, and to reduce bias, it is recommended to use subject randomisation.[26]

The Immunomodulatory activity test of *Hydrocotyle sibthorpiodes* Lam. extract was conducted on humans. The average IgG and IgM levels in the placebo group decreased because the placebo capsule only contained excipients that did not increase IgG and IgM levels in the body. As for the treatment group given *Hydrocotyle sibthorpioides* Lam. capsules, it increased because *Hydrocotyle sibthorpioides* Lam. was proven preclinically to be efficacious as an immunomodulator that can increase the body's immune system, one of which is antibodies that play a role in the body's defence.[14] Immunomodulators are materials that can modulate the body's immune system, both immunostimulants and immunosuppressants. In addition, this founding is also comparable to other studies conducted on bengkoang tuber extract, which has an immunostimulant effect which can also increase antibody levels.[27]

In Tables 6 and 7, the paired T-test showed significant results in the test group given *Hydrocotyle sibthorpioides* Lam. This result showed a significant difference in IgG and IgM levels before and after treatment.[28] This result can be used as a reference to assess how the effect of giving *Hydrocotyle sibthorpioides* Lam extract, while in the placebo group, the results given did not have significant differences in results, so it can be concluded that by giving a placebo, there is no effect on changes in IgG and IgM levels observed.[29]

After observing the IgG and IgM levels before and after treatment, the IgG and IgM levels were compared with the placebo group. It can be observed in Tables 8 and 9 that there is a significant difference in IgG and IgM levels after being compared with a placebo. The result indicates that the test capsules significantly changed IgG and IgM levels compared to placebo capsules.[28,29]

CONCLUSION

Based on the results of the study to determine the immunomodulatory effect of *Hydrocotyle sibthorpiodes* Lam. extract in capsule dosage form consumed by healthy humans at a dose of 67 mg, the effect was a significant increase in IgM and IgG levels.

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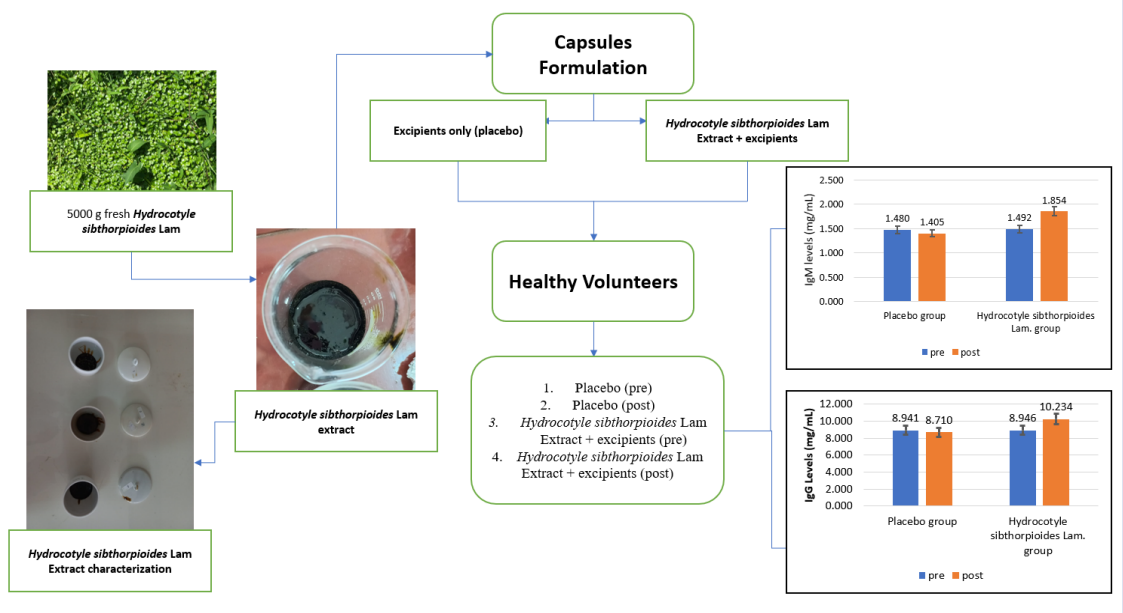
GRAPHICAL ABSTRACT



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