# SURAT PERNYATAAN
# PENGALIHAN HAK PUBLIKASI

Menyatakan bahwa makalah berjudul *"POTENTIAL OF SALAM LEAVES (Syzigium polyanthum Walp.) AND HABATUSSAUDA SEEDS (Nigella sativa Linn.) AS ANTI-URIC ACID HERBAL MEDICINE MATERIALS; STUDY OF PRECLINICAL TESTING AND EXTRACT STANDARDIZATION"* Karya **Muhtadi, Andi Suhendi, Nurcahyanti W., dan EM. Sutrisna** *dari Fakultas Farmasi Universitas Muhammadiyah Surakarta telah dipresentasikan secara oral pada* **INTERNATIONAL SEMINAR ON CLIMATE CHANGE ENVIRONMENTAL INSIGHT FOR CLIMATE CHANGE MITIGATION**, *yang diselenggarakan oleh Departemen Teknik Sipil Fakultas Teknik bekerjasama dengan Departemen Ilmu Lingkungan Program Pascasarjarna Universitas Sebelas Maret pada tanggal 4-5 Maret 2011 di Hotel Sahid Kusuma Surakarta.*

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Surakarta, 5 Maret 2011

Panitia Pelaksana International Seminar on Climate Change 2011,

UNIVERSITAS MUHAMMADIYAH SURAKARTA
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No. 95/A.3-VIII/LPPM/III/2011

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Solo, 4-5 March 2011

(_____)

International seminar on climate change

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# Environmental Insight for Climate Change Mitigation

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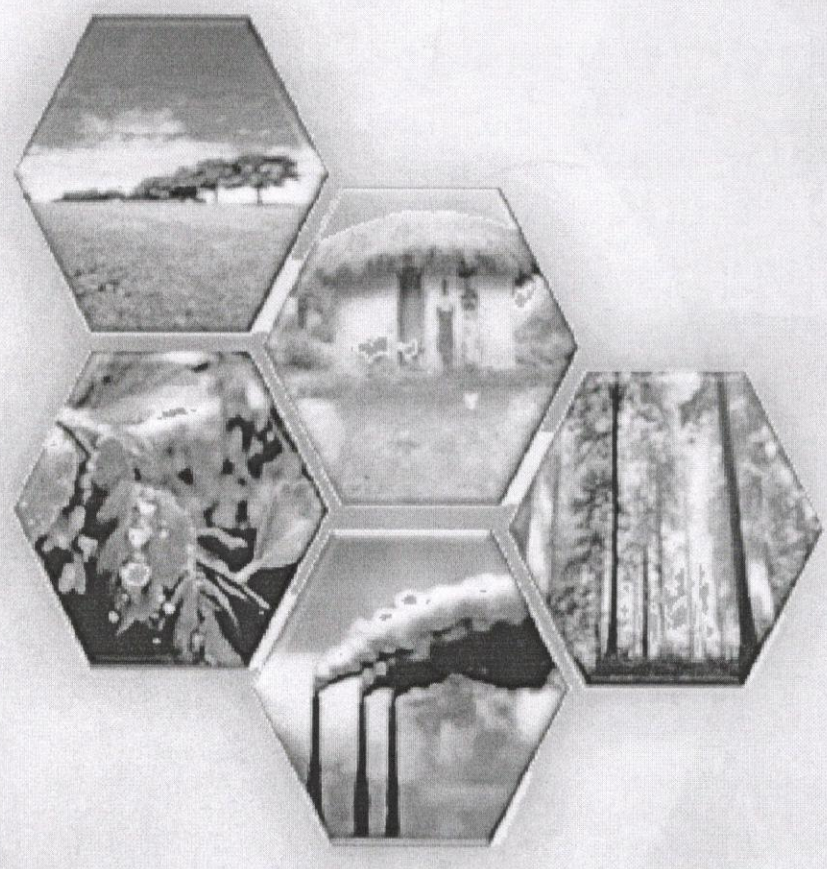

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# PREFACE

Many human activities induce climate change. The future change in our climate could be accelerating if an escalation of $CO_2$ concentration in atmosphere from industrial and daily human activities fails to be controlled. The impacts of climate change are widespread and complex, and are projected to various aspects according to the timing and magnitude of change, as well as according to adaptive capacity. It is clear, however, that climate change impacts have serious implications for the livelihoods of billions of people worldwide, and pose one of the greatest challenges to development in our time. Therefore, urgent actions are required to respond on a global scale to avoid irreversible damages imposed by climate change.

Assessments of climate change impact on a variety of area and facets are the first steps toward better planning in and developing the mitigation, preservation and adaptation strategies. This proceeding compiles articles categorized in six chapters which reflect the broad spectrum and perspectives of authors in dealing with impact of climate change and developing mitigation, preservation and adaptation strategies. The first chapter comprises investigations of climate change effect on climatology and ecosystem. The chapter also offers approaches to combat the effect and provide protocols which could help in recovering environment. Chapter two assesses interrelation between water resources and land management with environment and climate change including protocols to preserve the resources. Aspects of climate change impact on urban planning and housing are presented in chapter three. In chapter four, environmental awareness is implemented in construction activities and their product such as introduction of low energy building, utilization of waste materials and possible obtaining renewable energy from non fossil fuel. Politic, economic and social issues related to climate change are dealt in chapter five. And the last chapter suggests the use of vegetation for obtaining healthy food and medicine with less impact on environment.

The knowledge shared in this proceeding may contribute in our efforts to make the earth be a better place.

Solo, March2011

The Editors

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# POTENTIAL OF SALAM LEAVES (*Syzigium polynthum* WALP.) AND HABATUSSAUDA SEEDS (*Ambonicus coleus* LOUR) AS ANTI-URIC ACID HERBAL MEDICINE MATERIALS; STUDY OF PRECLINICAL TESTING AND EXTRACT STANDARDIZATION

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**Abstract**

Antihiyperuricemia activity testing has been carried out *in vivo* from a single and combination of extracts from salam leaves (*Syzigium polynthum* Walp) and habatussauda seeds (*Ambonicus Coleus* Lour.) Extraction of salam leaves and habatussauda seeds had been done by boiling its with water until the volume becomes a half, then filtered and pressed. The filtrate was dried by evaporation with the helping of waterbath and vacuum oven. Dried extracts of each ingredient, then proceed antihiyperuricemia preclinical testing in vivo on male white mice Balb-C strain that has been induced by potassium oxonic dose of 250 mg/kg. The results of antihyperuricemia preclinical testing showed that extracts of salam leaves and habatussauda seeds single dose of 200 mg/kg had lower activity levels of uric acid in blood serum of male white mice, respectively, 0.520 and 1.180 mg/dL. While antihyperuricemia activity of a combination of salam-habatussauda extracts is 0.900. Each of the extracts had been analyzed for standardization extract with the test procedure based on the parameters of Materia Medica Indonesia and the general standardize of medicinal plant extracts suggested by BPOM RI, which include non-specific and specific parameters. Overall, based on the results of standard tests common medicinal plant extracts, the two materials under study had met the recommended requirements.

Keywords: antihiyperuricemia activity in vivo, extract of salam leaves and habatussauda seeds, male white mice Balb-C, standardization extracts

## 1. INTRODUCTION

Indonesia is one of country that has a high diversity of living things so that by some ecological regions Indonesia is called the *"megabiodiversity"* or "a high diversity of living creatures." Based on the research report that 10% of plants, 12% of mammals, 16% of reptiles, 17% of birds, 25% of the fish in the world live in Indonesia, but Indonesia only 1.3% area of the size of Earth. The wealth of living creatures Indonesia was ranked third after Brazil and the Democratic Republic of Congo (Anonymous, 2001).

However, in the year 2008 had been reported that Indonesia as the country's most rapid rate of forest destruction in the world. Every year Indonesia is losing forest area of 1.8 million hectares. The damage that occurs in the upstream region (forest) also damage the downstream region (coastal) (Prasetyo, 2009). Damage to forest ecosystems is of course scientifically accompanied by the extinction of species that become the source of knowledge and benefits to human life, such as the availability of food, industrial materials and natural medicines.

Utilization of medicinal plants as traditional/herbal medicine in Indonesia is essentially a cultural part of Indonesia. The advantage of using the drug (herb) traditional in principle because of side

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effects is relatively small compared to modern medicine. Although empirically traditional medicine to cure various diseases, but efficacy and ability has not been scientifically proven, both preclinical and clinical. Also, not much is known what chemical compounds responsible for the efficacy of traditional medicine (Wijayakusuma, 2002). However, the utilization of Indonesian medicinal plants as a natural remedy that can provide economic benefits and improved health for the people so need to be developed.

This paper will present the research potential of *Salam* leaves extract *(Syzigium polynthum* Walp) and *Habatussauda (Ambonicus coleus* Lour) extract as an ingredient of herbal medicine to cure gout. Uric acid is a chemical compound final results from the metabolism of *nucleic acid* or purine metabolism in the body. Uric acid is carried to the kidneys through the bloodstream to be issued with urine. Healthy kidneys will regulate uric acid levels in the blood to keep it under normal circumstances. However, excessive uric acid will not be collected and metabolismed entirely by the body, there will be elevated levels of uric acid in the blood called hyperuricemia. Hyperuricemia which can further develop into *gout* (Klippel, 2000). Uric acid is one of the diseases associated with cardiovascular disease which many sufferers and lead to disability and death. Reportedly as many as 17 million people died of cardiovascular disease each year (Anonymous, 2008).

Results of previous studies of testing antihyperuricemia activity of *Salam* leaves extracts by the research team, indicated that *Salam* leaves decocta at a dose of 1.25 g / kg body weight can decrease lower blood uric acid levels in male white mice effectively (Handadari, 2007) and *Salam* leaves infusa at a dose of 2.5 g / kg body weight can decrease lower uric acid levels with allopurinol dose equivalent of 10 mg / kg (Ariyanti, 2007). While the *Habatussauda* has been reported as antibacterial activity (Hannan et al, 2008), treatment of diabetes (Khanam and Council, 2008), antitumor (Moses et al, 2004) and hepatoprotector (Yildiz et al, 2008).

Therefore, in order to explore the competitive advantage of natural ingredients native to Indonesia, by increasing the potential and capacity of the natural materials of herbal medicinal drugs become standardized (OHT), a quality or fitofarmaka, with mutual synergy potential of the Industry and Research Institutions (Universities), Therefore, research for the purpose of raising herbs into a better product (OHT or fitofarmaka) is very important to do.

## 2. RESEARCH METHOD

**2.1 Equipment:** injection volume of 3.0 ml syringe (Terumo), syringes for insulin injection 1.0 ml, oral syringe size of 15 *gauge,* flakon, mice weighing capacity of 2610 grams (Lark, China), analytical balance (A-Presica SCS), the capillary tube (Assistant), *mikrotube centrifuges* (Eppendorf), centrifuges (Mini-Spin), vortex, mikropipet size of 50-40 mL and 200-1000 mL, *blue tip, yellow tips,* tools glass (Pyrex), Stardust FC * 15 (DyaSys) a suite of tools distilled toluene (pyrex), *heating metel,* crush silica, electric furnace (Ney Vulcan), *Ovens* (Binder), analytical balance (Ohaus), *rotary vacuum evaporator* (Heildoph), *Vacuum Oven* (Vaciotem-T ), exicator, AA7000 Atomic Absorption Spectrophotometry (Shimadzu), UV-VIS Spectrophotometry Mini (Shimadzu), vortex, micropipet sizes, 1-10 mL, 5-40 mL, 50-200 mL and 200-1000 mL (Soccorex), *blue tip, yellow tips,* tools glass (Pyrex), and cuvet disposable.

**2.2 Ingredients:** water extract of *Salam* leaves and *Habatussauda* seed, potassium oxonic pa (Aldrich), allopurinol pa (Sigma), 0.9% NaCl, distilled water and uric acid reagent kit FS * TBHBA (DyaSys). n-hexane pa, ethyl acetate pa, ethanol pa, acetone pa, chloroform pa (Merck), concentrated $HNO_3$ (Merck), $HClO_4$ (Merck), HCl 2N, $H_2SO_4$ diluted Folin Ciocalteu (Merck), $Na_2CO_3$ 7%, $AlCl_3$ 10%, Potassium Acetate 1 M, destilata aqua, aqua bidestilata (Ikapharmindo), filter paper

(Sartorius), a solution of Pb and Cd standard of 1000 ppm (merck), raw aflatoxin mixture (B1, B2, G1 and G2) (Sigma Chemical Company), galic acid pa (Sigma), quercetrin pa (sigma), silica gel GF254 plate (merck),

**2.3 Animal experiments:** male white mice strain Balb-C with an average weight 30-40 g and aged 2-3 months.

**2.4 The Course of Study**

**2.4.1 Extraction**

Extraction was done by powder of *Habatussauda* dan *Salam* leaves boiled with water until the volume becomes half the initial volume, then filtered and the filtrate evaporated by *rotary evaporator* (RE) in order to obtain viscous extract.

**2.4.2 Making Hyperuricemia**

High uric acid levels (hyperuricemia) was made by injecting a potassium oxonic intraperitoneally 250 mg / kg or 5 mg/20 g body weight in mice.

**2.4.3 Introduction Preclinical Test**

Preclinical testing was conducted for the purpose of obtaining data on the dose of extract, time of blood sampling, and single extracts that are active in lowering uric acid levels.

**2.4.4 Treatment of Animals Test**

The test animals were divided into several treatment groups, which including the negative control group/hyperuricemia (potassium oxonic dose of 250 mg / kg), positive control (allopurinol dose of 10 mg / kg), water extracts of habatussauda and salam leaves with a single dose (200 mg / kg). Giving the test preparation performed one hour after induction hyperuricemia (potassium oxonic dose of 250 mg / kg).

**2.4.5 Blood Collection**

Blood sampling performed one hour after test preparation or two hours after induction hyperuricemia (potassium oxonic dose of 250 mg / kg), blood is drawn through the eyes of mice by piercing the veins *opthalmicus* branch located on *the median saccus orbitales* with capillary tube. The blood that flows through the capillary tube ependorf accommodated in the tube, after the blood clots were centrifuged to obtain serum.

**2.4.6 Determination of Uric Acid Levels**

Uric acid levels determined by enzymatic reaction using *uric acid* reagent FS * TBHBA. Blood serum was mixed homogeneous with *uric acid* reagent FS * TBHBA incubated for 10 minutes at 37 °C. Furthermore, the sample solution, standard and blank absorbance read using a spectrophotometer *StartDust* FC * 15 at a wavelength of 546 nm.

**2.4.7 Standardized Extract**

Standardization of extracts (materials) following standard procedures based on the Materia Medika Indonesia and the general standard parameters plant drug Extracts recommended by the BPOM RI, including specific namely water soluble compounds, total content of chemical levels (total flavonoid and phenolic content), and non-specific parameters namely shrinkage weights, water, total ash, acid insoluble ash content, aflatoxin contamination and contaminant levels of heavy metals (Pb and Cd)

**2.4.8 Chemical Content of Extracts**

1. Preliminary Test
   Test alkaloids with the reaction Meyer and Bouchardat; test phenolic and flavonoids test (Test Taubeck)
2. TLC Profile
   10% solution in acetone extracts on TLC using a stationary phase: silica gel GF $_{254}$ eluted with mobile phase: n-hexane: ethyl acetate (9: 1), detection with UV light 254 nm and 366 nm.

## 3. RESULTS AND DISCUSSION

**3.1 Preliminary Test**

Preliminary test conducted to determine how the model hyperuricemia on male white mice, namely by looking for an effective dose of potassium oxonic raise uric acid levels than normal conditions.

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**Table 1** – Data modeling hyperuricemia preliminary test

| Treatment | No. HU | Uric acid levels (mg / dL) | Average | SD |
|---|---|---|---|---|
| Normal control (no treatment) | 1 | 1.3 | 1.43 | 0.23 |
| | 2 | 1.7 | | |
| | 3 | 1.3 | | |
| Potassium oxonic Dose of 250 mg/kg | 1 | 3.1 | 3.07 | 0.95 |
| | 2 | 4 | | |
| | 3 | 2.1 | | |

From the preliminary test results can be seen that with potassium dose of 250 mg / kg have been able to raise uric acid levels of mice, from normal levels by an average of 1.433 to 3.067 mg / dL. This is consistent with a statement saying that the mice with hyperuricemia if their blood uric acid levels ranged from 1.7 to 3.0 mg / dL. And after the test statistics showed that between normal control and different groups of potassium oxonic very significant point with significance value of 0.205 ($p > 0.05$). Thus, the results of the method in the study can proceed further research is treatment with the test extract preparations.

### 3.2 Results and Discussion of Preclinical Testing

The test animals used in this study were white male mice *(Mus muculus)* having uricase enzymes that break down uric acid to form the final product allantoin which is easily soluble in water (Martin, 1987). To minimize biological variation, the researchers did control for several variables such as test animals by using more or less the same biological variation of some of them weighing about 30-40 g, age 2-3 months, Balb-C strain, the male sex and equal treatment that was placed in a cage with the same number of each cage and were given the same food as well as before the test animals were treated first fasted for ± 2 hours with a fixed given to drink *ad libitum.* This was done for the same conditions of test animals and to reduce the influence of food consumed on the test preparation is given in the study. And to reduce the stress level of the test animals were adapted to laboratory conditions for 7 days.

Selection of the male sex was based more on considerations that male mice did not have the hormone estrogen, although existed only in relatively small quantities and hormonal conditions on the male was more stable when compared with female mice because female mice experiencing hormonal changes at certain times such as on during the estrous cycle, pregnancy and breast-feeding in which these conditions could affect the psychological condition of the test animals. In addition, the level of stress on female mice were higher compared with male mice that may interfere at the time of testing.

Potassium oxonic was used as an inductor hyperuricemia because potassium oxonic was a competitive *inhibitor uricase* increase uric acid levels by preventing changes in uric acid into allantoin. Where is water-soluble allantoin and can be excreted through urine, so that the uricase enzyme inhibited by potassium oxonic then uric acid will accumulate and not eliminate in the form of urine.

Positive control used was allopurinol which was one or gout drugs are often used in treatment. Allopurinol was the only uricostaticum currently used in therapeutic, which works to reduce the formation of uric acid. While working to increase elimination of uric acid was called uricosurice (Mutschler, 1991). Allopurinol was a substrate xantin oxidase and eliminated through the kidneys mainly as oxipurinol (often also referred to as the one that is aloxantin) (Schunack and Mayer, 1990). Allopurinol or oxipurinol, inhibits xantin and uric acid, which in the low dose mechanism of inhibition taked place competitively and in high doses were not competitive work. Allopurinol which had half time in plasma about 40 minutes, hydrolysed by xantin oxidase into

metabolites (Mutschler, 1991). Metabolite of allopurinol-1-ribonucleotide, which could be expressed little in organ extracts, might be responsible for additional inhibition of *de novo* purine (Schunack and Mayer, 1990). Through the inhibition of oxidase xantin, then xantin and hipoxantin excreted in the urine, so more uric acid levels in blood and urine decreased (Mutschler, 1991).

The test preparation that was used to lower uric acid levels in this study was to extract water from *salam, habatussauda* and a combination of both. Dissolution method was used solvent extraction with water, where the method was similar to the use of vegetable material as a traditional medicine (herbal medicine) was to boil the ingredients and take the concentrates to be taken so that equality of treatment has traditionally and treatment in the study are identical. The difference in this study concentrates obtained after boiling evaporated in a *vacuum dryer* until produced dry extract. This was intended to maintain stability during storage because if stored in liquid form was very susceptible contaminated and overgrown by the fungus fast.

Determination of uric acid levels determined by enzymatic methods using reagent *Uric acid* FS * TBHBA *(2,4,6-tribromo-3hydroxybenzoic acid)* by using a spectrophotometer *Stardust FC 15.* The mechanism of uric acid that occurs was oxidized by the enzyme uricase with the help of $H_2O$ and $O_2$ into allantoin, carbon dioxide and hydrogen peroxide. Hydrogen peroxide formed reacts with 4-aminoantipirin and TBHBA be cuinonimine pink where the reaction was catalyzed by the enzyme peroxidase (POD). The amount of color intensity generated by the equivalent cuinonimine uric acid levels in the blood. Reaction mechanism can be seen in figure 1.

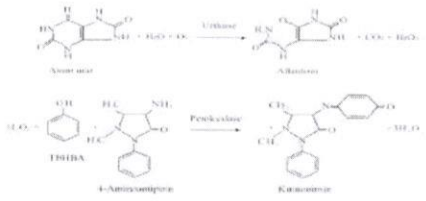

**Figure 1** - Reaction mechanism of formation of compounds cuinonimin (Schunack *et al.*, 1990)

Data of serum uric acid levels in mice after induced with potassium oxonic and tested extracts of a single dose of 200 mg / kg are shown in the following table:

**Table 2** - Data in serum uric acid levels after treatment with the extract

| No. HU | TREATMENT GROUP | BW (Gram) | URIC ACID LEVELS | | |
|---|---|---|---|---|---|
| | | | AFTER TREATMENT | AVERAGE | SD |
| 1 | Negative Control (Potasium oxonic 250 mg/kgBB) | 39.3 | 3.1 | 3.10 | 0.35 |
| 2 | | 30.5 | 3.7 | | |
| 3 | | 30 | 2.9 | | |
| 4 | | 36 | 2.9 | | |
| 5 | | 39 | 2.9 | | |
| 1 | Positive Control (Allopurinol 10 mg/kgBB) | 37.5 | 0.1 | 0.20 | 0.10 |
| 2 | | 36.5 | 0.2 | | |
| 3 | | 36 | 0.3 | | |
| 4 | | 39 | 0.3 | | |
| 5 | | 38.5 | 0.1 | | |
| 1 | Salam leaves extracts | 36 | 0.5 | 0.64 | 0.17 |

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| | | | | | |
|---|---|---|---|---|---|
| 2 | (200 mg/kgBB) | 36.5 | 0.5 | | |
| 3 | | 36 | 0.9 | | |
| 4 | | 38.5 | 0.6 | | |
| 5 | | 39 | 0.7 | | |
| 1 | | 31.5 | 1.8 | | |
| 2 | Habatussauda seeds | 31 | 1.5 | | |
| 3 | extracts | 35.7 | 1.2 | 1.20 | 0.56 |
| 4 | (200 mg/kgBB) | 35 | 1.2 | | |
| 5 | | 36 | 0.3 | | |
| 1 | | 38.5 | 0.7 | | |
| 2 | Salam – Habatussauda | 36 | 1.1 | | |
| 3 | extracts | 36 | 0.7 | 0.84 | 0.36 |
| 4 | (200 mg/kgBB) 1 : 1 | 38 | 0.4 | | |
| 5 | | 38.5 | 1.3 | | |

From the Table 2, the histogram can be made between treatment groups with average blood uric acid levels of male white mice as follows:

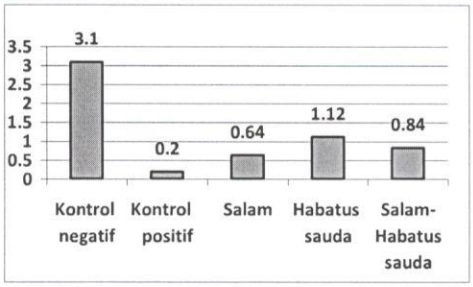


**Figure 2** - Histogram of the relationship between the treatment groups with average blood uric acid levels (mg / dL) in male white mice

From the ANOVA test results can be seen that the uric acid levels between the negative control with the positive control or with a group of extracts showed significantly different results with significance value of 0.000. This suggests that allopurinol and Salam leaves extracts, Habatussauda or a combination of both can lower blood uric acid levels in white male mice strain Balb-C when compared with negative control.

While compared with positive control, Salam and Habatussauda extracts differed significantly with consecutive values 0.058 (P> 0.05) and 0.000 (P <0.05). From the results obtained can be said that the two extracts with a single dose can lower uric acid levels of male white mice Balb-C strain but significant decrease given different from each other that is the significance value of 0.019 (P <0.05).

Meanwhile, after combined, the results were compared with positive controls also differed significantly with P value = 0.008 (P <0.05), and not significantly different when compared with a single Salam leaves extract (P = 0.373) and with Habatussauda (P = 0.116) . This means that the reduction produced by the combination of Salam-Habatussauda extracts can not be equivalent to the reduction produced by allopurinol. And the reduction produced by the combination of Salam-Habatussauda extracts almost identical to decline by a single Salam extract although still lower reduction produced by a single Salam leaves extract. Therefore it can be concluded that the Habatussauda extract less potent in lowering blood uric acid levels of male white mice than Salam leaves extract.

### 3.3 Results and Discussion of Standardized Extracts Testing

In the determination of standardized extracts, tests carried out include non-specific analysis is the analysis of drying shrinkage, specific gravity, moisture, ash, the residual solvents contents, pesticide residues, heavy metal, microbial contamination, and specific analysis that includes the identity of the extract , compounds dissolved in certain

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solvents, also test the chemical content of the extract. Results Salam leaves extract standardized test can be seen in the following table:

**Table 3** - Results of standardized testing extracts of non-specific parameters

| Extract | Non-specific parameters | | | | | |
|---|---|---|---|---|---|---|
| | Drying shrinkage | Water content | The ash content | Acid insoluble ash content | Contamination of heavy metals (µg / kg) | Microbial contaminants (aflatoxin) |
| Salam | 12,287 | 6,733 | 28,537 | 22,110 | 2,782 (Pb)<br>nd*) (Cd) | nd*) |
| Habatussauda | 14,969 | 7,100 | 7,147 | 5,374 | 6,405 (Pb)<br>0,0096 (Cd) | nd*) |

*Description: nd: not detected*

**Table 4** - Results of standardized testing extract specific parameters

| Extract | Specific Parameters | | |
|---|---|---|---|
| | Water-soluble extract | Total phenolic | Total flavonoids |
| Salam | 64,656 | 1,083 | 0,196 |
| Habatussauda | 28,651 | 0,664 | 0,400 |

### 3.4 Compound Identification

Each extract had been identified a chemical marker, and based on the results of TLC analysis proved the existence of fluoretin on Salam extract and luteolin on Habatussauda extracts.

Structural formulas :

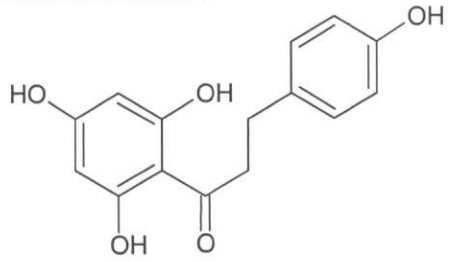


**Figure 3** - The structural formula of Fluoretin

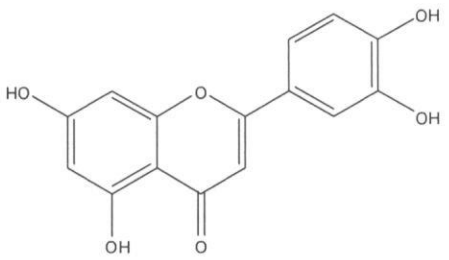


**Figure 4** - The structural formula of Luteolin

## 4. CONCLUSION

1. Based on data of antihyperuricemia preclinical testing, Salam leaaves extract more active than Habatussauda seeds extract on a single dose of 200 mg/kg BW, with a value activity of percentage decrease in uric acid levels provided a single dose of Salam and Habbtussauda extracts, repectively are 79.35% and 61.29%.
2. While the reduction produced by the combination of Salam and Habatussauda extract with the same ratio (1: 1) provide a different result is not significant with the positive control is by the percentage decrease of 72.90%.
3. Standardization of Salam leaves aqueous extract had also been done with the test procedure based on the material parameters Medika Indonesia and the general standard of medicinal plant extracts suggested by BPOM RI, which include non-specific and specific parameters obtained results in which the determination of total phenolic content in Salam leaves extracts using Folin Ciocalteu amounted to 1.083% and total flavonoid content, which is conducted by spectrophotometry visible colorimetry, obtained an average concentration of 0.196%. As for Habatussauda total phenolic content of 0.664% and total flavonoid content of 0.400%.

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