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Optimizing Combination of *Sambiloto* Herbal Water Fraction and *Salam* Leaf Water Fraction As Anti-Inflammation

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Abstract—*Sambiloto* herbs (*Andrographis paniculata* Nees) and *salam* leaves (*Syzygium polyanthum*), which are effective to reduce blood sugar level with different mechanism, have been suggested to produce a synergy as antioxidant and anti-inflammatory agent, hence the optimum combination formula is remained to be elaborated. By reducing blood sugar level and showing antioxidant, and anti-inflammatory activity, both plants were hypothesized of its ability ameliorating diabetes mellitus complexity. This study aimed to discover the optimal combination formula *sambiloto* herbs (*Andrographis paniculata* Nees) and *salam* leaves (*Syzygium polyanthum*) water fraction to produce anti-inflammation effect. Carrageenan was used to induced the inflammatory condition. Optimization was conducted by factorial design utilising 2 factors and 2 levels. Both factors, *sambiloto* herbs (*Andrographis paniculata* Nees) and *salam* leaves (*Syzygium polyanthum*) in the range of low level 1:10 and high level 10:1 with the used doses were 10 mg/kg BW at low level and 300 mg/kg BW for high level. The observed parameters were anti inflammation potential percent and edema rate. Conclusively, this study proposed that optimum combination formula of *sambiloto* herbal : *salam* leaves water fraction = 1.14 : 9.87 with 234 mg dose. The combination formula was theoretically resulted the optimum anti inflammation potential percent of 45.68%, and ER of 38.39% compared with other combination formulas.

Keywords—*salam* leaf; *sambiloto* herbal; water fraction; anti inflammation; factorial design

I. INTRODUCTION

There are many plants to treat diabetes including *sambiloto* herbal (*Andrographis paniculata* Nees) and *salam* leaf (*Syzygium*

polyanthum). Both plants work to decrease or reduce blood sugar by different ways, hence it is expected that both have a synergic effect as evidenced by Widjajakusuma's (2009) [1], tight blood sugar decrease effect is higher than the combination of two extracts than single extract. This combination is expected not only able to better decrease sugar level but it can prevent further complication from Diabetes mellitus through its ability in blocking free radical which is related to its antioxidant and anti inflammatory effect.

Based on Hadisoewignyo et al (2012) [2] research, it is found that in single use, *sambiloto* herbal water fraction has greater anti inflammation capability than *salam* leaf water fraction, but in their combination use with *salam* leaf water fraction to *sambiloto* herbal water fraction of 2 :1 provides greatest anti inflammation capability.

This research aimed to identify comparison between *sambiloto* herbal water extract fraction and *salam* leaf water fraction which possess optimum anti inflammatory effect. Optimize design used was factorial design with two factors and two levels using assisted by the design expert program.

II. MATERIALS AND METHODS

A. Instruments

Instruments used in this research were percolator, Rotavap, spectrophotometer UV-VIS, High Performance Chromatography (HPLC), UV lamp, and KLT plate.

B. Plant materials

Sambiloto herbal and *salam* leaf derived and

determined from Materia Medika Indonesia (MMI), Batu, Indonesia.

C. Chemical materials

Methanol, n-hexane, ethyl acetate, ethanol 96%, chloroform, quersetin, water for injection, NaCL 0,9%, ether, carrageenan, and tylose.

D. Animals

Animal used to evaluate anti inflammation and ulcerogenic tests were Wistar male white rats (*Rattus norvegicus*) mean age of 2 to 3 months, 150-200 g body weight. The project was previously approved by the Animal's Ethics Committee of the Laboratory Research and Testing of Integrated of Gadjah Mada University, Indonesia.

E. Standardization of Simplicia

In simplicia standardization of herbal sambiloto and salam leaf, ash level determination, ethanol solution extract content determination and water soluble extract content determination tests were performed according to Materia Medika requirements [3].

F. Sambiloto Herbal Etanol Extract Water Fraction and Salam Leaf Ethanol Extract Water Fraction Production

Sambiloto extract and Salam leaf extract production were performed in percolation manner. Stages to be performed were following: simplicia powder of sambiloto/salam leaf is weighted and entered to percolator; the powder is macerated with ethanol 96% for 24 hours and sometimes is stirred. After 24 hours, ethanol extract is filtered with sieve paper (macerate I) and the residue is re-macerated with ethanol 96%; after 24 hours, ethanol extract is filtered (macerate II). Then Macerate I and II are combined and thickened using evaporator following with heating in a porcelain dish on stem bath until thick extract is obtained. A thick extract obtained is weighted and dissolved with 90% methanol-water. Staged fractionation is performed in separate funnel with n-hexane by shaking for 5 minutes and n-hexane fraction is separated. Fractionation is performed several times until n-hexane fraction is colorless. All n-hexane fractionation obtained is combined and thickened in the evaporator. Methanol-water fraction is vaporized in a porcelain dish above steam bath until the methanol vaporizes and water fraction is obtained. Water fraction is fractioned with ethyl acetate in separate funnel like in n-hexane fraction. All ethyl acetate fractions obtained are combined and thickened in the evaporator. Water fraction obtained from fractionation residue wit ethyl acetate is thickened

in a porcelain dish on the steam bath. At the end of extraction total extract, hexane fraction, ethyl acetate fraction and water fraction from sambiloto and salam leaf are obtained.

G. Standardization of Extract

Tests performed on extract standardization are organoleptic tested, pH determination, water level test, water diluted essence level test, ethanol diluted essence level test, and chromatogram profile from the merit material.

H. Optimization Design

Factorial design in this research used 2 factors and 2 levels for respective factors. Factors used are salam leaf water fraction concentration: sambiloto herbal water fraction comparison and the water fraction dose used. For salam leaf water fraction: sambiloto herbal water fraction concentration comparison, low level used was 1 : 10 and high level 10 : 1, while for water fraction dose factor used, low level 100 mg/kg BW and high level of 300 mg/kgBW. Design framework can be seen in table 1.

Table 1. Optimization with factorial design

| Groups | Salam leaf: sambiloto herbal water fraction concentrate ratio | Water fraction dose | Factor Interaction |
|--------|---|---------------------|--------------------|
| I | - (1: 10) | - (100 mg) | + + |
| II | - (1: 10) | + (300 mg) | - |
| III | + (10: 1) | - (100 mg) | |
| IV | + (10: 1) | + (300 mg) | + |

I. Anti inflammation test procedure (Anonym, 1993)

The rat is fasted \pm 18 hours before testing, drink is still given. In testing day, the rat is weighted and grouped randomly, divided into 5 groups, one group as inflammation control and the four groups remaining are given with testing material by: sambiloto herbal water extract suspension, Salam leaf water extract suspension, combined sambiloto herbal and salam leaf extracts suspension, and ibuprofen suspension. Each group consist of 8 rats. In group control every rat is given with 1 mL/100 g body weight of 0.9% NaCl solution by oral. In the test group, every rat is given testing medicine preparation by oral of 1 mL/100 g body weight. One hour after testing and control solution administration, left paw of all rats are injected by intra planar with 0,1 mL of carrageenan suspension. Left paw volume is measured by immersing them into plethysometer tools for 30 minutes, 1, 2, 3, and 4 hours after carrageenan suspension injection. Edema rate percent (%ER) mean is calculated by the formula as in equation

(1).

$$\%ER = \frac{V_t - V_0}{V_0} \times 100 \quad (1)$$

V_0 is feet volume before injected with carrageenan, V_t is feet volume after injected with carrageenan at time t . While EC is %ER of the control group and Et is %ER on treatment group (Ghamdi, 2001). The Antiinflammation capability percent is determined as in equation (2).

$$\text{Persen daya antiinflamasi} = 100\% - \left(\frac{AUC_p}{AUC_k} \times 100\% \right) \quad (2)$$

AUC_p is treatment group AUC and AUC_k is control group AUC.

III. RESULT AND DISCUSSION

A. Standardization of Simplicia

Simplicia quality test performed include: dried shrinking, total ash content, level of water soluble, and level of ethanol soluble. Observation result can be seen in table 2.

Table 2. Result Of *Salam* Leaf And *Sambiloto* Herbal Simplicity Standardization

| No | Test Parameter | Salam Leaf | | Sambiloto Herbal | |
|----|--------------------------|--------------|------------------|------------------|------------------|
| | | Require-ment | Test Result | Require-ments | Test result |
| 1 | Ash Content | $\leq 5\%$ | $6,28 \pm 0,04$ | $<12\%$ | $11,92 \pm 0,14$ |
| 2 | Level of water soluble | $\geq 12\%$ | $5,45 \pm 0,24$ | $\geq 18\%$ | $17,03 \pm 0,38$ |
| 3 | Level of ethanol soluble | $\geq 8\%$ | $11,55 \pm 0,03$ | $\geq 9,7\%$ | $13,53 \pm 0,10$ |

Total ash content determination is aimed to provide internal and external mineral content figure derived from initial process to the forming of dried simplicity. Total ash content determination result of *sambiloto* herbal simplicity has fulfilled the standard requirement that is not more than 12% [4] and *salam* leaf has not fulfilled the standard requirement that is not more than 5% [5]. Level of water soluble and level of ethanol soluble determination test showed that *sambiloto* herbal has fulfilled the requirement of not less than 18% and 9,7 % [4] while level of water soluble of *salam* leaf has not fulfilled standard

Table 5. Standardization Of Water Fraction

| Water Fraction | Organoleptic | pH | Ash Content |
|--|---|-----|-----------------|
| <i>Sambiloto</i> Herbal ethanol extract water fraction | Color: Green blackish Taste : Bitter Smell : Characteristic | 6,0 | $3,69 \pm 0,18$ |
| <i>Salam</i> Leaf Etanol Extract Water Fraction | Color: Dark brown Taste : tasteless Smell : characteristic | 5,0 | $1,85 \pm 0,07$ |

requirements and level of ethanol soluble has fulfilled the requirement of not less than 8%.

B. Preparation of *Sambiloto* Herbal and *Salam* Leaf Extracts

Preparation of *sambiloto* extract and *salam* leaf extract are both using percolation method. The final result of extract preparation produced *sambiloto* extract with rendement in 19.7%; while for *salam* leaf extract with 20.3% rendement.

C. Standardization of Extract

In extract standardization, tests performed were organoleptic (color, taste, and smell), pH, and ash content and the result is listed in Table III.

Table 3. Standardization of Extract

| Extract | Organoleptic | pH | Ash Content |
|---------------------------|--|-----|-----------------|
| <i>Sambiloto</i> Extract | Color : green blackish Taste : bitter Smell: characteristic Color: dark brown | 6,4 | $5,85 \pm 0,14$ |
| <i>Salam</i> Leaf Extract | Taste: tasteless Smell: Characteristic | 5,6 | $2,25 \pm 0,29$ |

D. Extract Fractionation

Preparation of fractioned extracts from *sambiloto* and *salam* leaf is performed by fluid-fluid method. The result of fractionation is listed in table 4.

Table 4. Result Of *Sambiloto* Extract And *Salam* Leaf Extract Fractionation

| Fraction | <i>Sambiloto</i> | | <i>Salam</i> Leaf | |
|----------|------------------|-----------------|-------------------|-----------------|
| | Weight (g) | Sucrose content | Weight (g) | Sucrose Content |
| n-Hexane | 9,30 | 9,24% | 16,7 | 16,17% |
| Ethyl | 33,9 | 33,67% | 19,4 | 18,78% |
| Acetate | | | | |
| Water | 56,2 | 55,81% | 66,9 | 64,95% |

E. Standardization of Fraction

In water fraction standardization tests performed were following organoleptic (color, taste, and smell), pH, and ash content, the result is listed in table 5.

F. Anti inflammation test

In this research, rat paw volume is observed using plethysmometer and edema volume is measured which occurred about 0 hours to 4th hour. Then a graph depicting relationship between mean edema volume and time (figure 1) is made. Edema peak is obtained due to carrageenan induction in inflammation control group, occurring in 2.5 to 3 hours. Smallest edema volume occurred with use of *Salam* leaf water fraction: *sambiloto* herbal water fraction combination in 10:1 ratio, both at 10 mg/kgBW dose and 300 mg/kgBW dose. While the combination with greatest edema volume is a *salam* leaf water fraction: *sambiloto* herbal water fraction at 1 : 10 ratio at 100mg/kgBW dose.

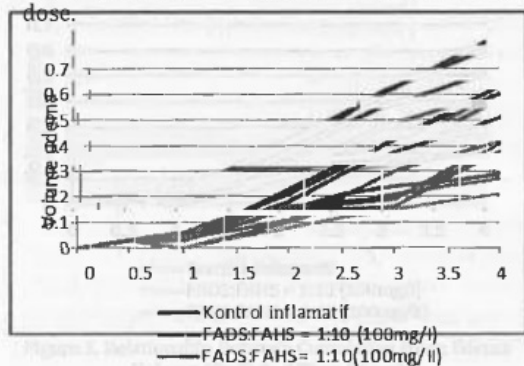


Figure 1. Relationship Between Cumulative Mean Edema Volume (Cm³) And Time (Hour)

G. Optimization with Factorial Design

Optimization of *salam* leaf water fraction and *sambiloto* herbal water fraction comparison and optimum dose searching is performed by using a factorial design method associated with the design expert program. Based on anti inflammation capability percent data mathematical equation as the following is obtained:

$$Y = 35,16 + 7,81 X_A + 8,59 X_B + 1,25 \times 10^{-3} X_A X_B \quad (3)$$

Y is anti inflammation capability percent (%) response. X_A is the value of the *salam* leaf water fraction and *sambiloto* herbal water fraction comparison. X_B is dose value and $X_A X_B$ is value of interaction of *salam* leaf-*sambiloto* herbal water fraction and dose. Of the equation (3) contour plot as in figure 2 is obtained.

Based on equation (3) and contour plot in Figure 2, it is seen that dose factor has greater effect on anti inflammation capability resulted compared with the *salam* leaf water fraction and *sambiloto* herbal water fraction comparison factor. The interaction of both factors has a small effect on response resulted.

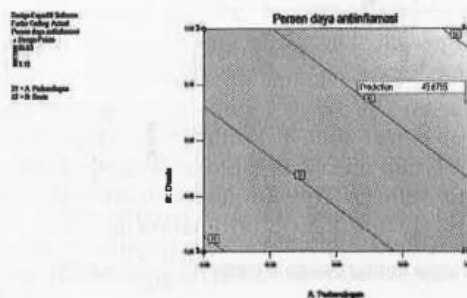


Figure 2. Contour plot of anti inflammation capability percent.

Based on the edema rate (ER) date, a mathematical equation is obtained as follows:

$$Y = 39,70 - 3,3 X_A + 3,52 X_B + 2,18 X_A X_B \quad (4)$$

Y is ER response(%). X_A is the value from *salam* leaf water fraction and *sambiloto* herbal water fraction comparison, X_B is dose value, and $X_A X_B$ is value of interaction of *sambiloto* herbal-*salam* water fractions and dose comparison. Of the equation (12) contour plot as in figure 3 is obtained.

Based on equation (4) and contour plot in Figure 3, it is seen that *salam* leaf water fraction and *sambiloto* herbal water fraction comparison factor can reduce ER value, hence in the formulation of the two materials combined the comparison to be used should be considered, since it will influence their ability in blocking edema.

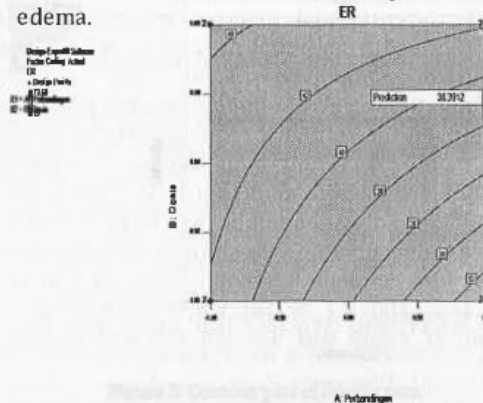


Figure 3. Contour plot of edema rate.

Contour plot from each response is then superimposed hence the optimum area with the desired anti inflammation capability would be obtained. Yellow area (figure 4) depicts the optimum area prediction with the desired response.

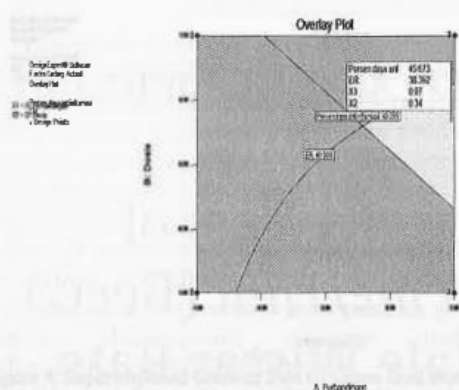


Figure 4. Superimposed Contour Plot Of Salam Leaf Water Fraction And Sambilotto Herbal Water Fraction Combination

Based on the yellow area in the superimposed contour plot optimum formula can be selected that is: formula that use salam leaf water fraction: sambilotto herbal water fraction comparison of 9,865 : 1,135 to 234 mg doses, providing theoretical results for anti inflammation capability percent of 45,68% and ER of 38,39%.

IV. CONCLUSION

Smallest edema volume occurred on use combination of *salam* leaf water fraction: *sambilotto* herbal water fraction by 10:1 ratio at 100 mg/kgBW or 300 mg/kgBW doses. Optimum formula can be made by using *salam*

leaf water fraction to *sambilotto* herbal water fraction ratio of 9,865:1,135 at 234 mg dose which give theoretical results in an inflammation capability percent of 45,68% and ER of 38.39%.

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