Application of Ultrasonic-Assisted Fractionation with Full-Factorial Design for the Production of Alkaloid-Rich Fraction from *Ficus septica* Leaves

Muhammad Hafizh Ridho, Andayana Puspitasari Gani, Subagus Wahyuono, Nanang Fakhrudin*

1 Faculty of Pharmacy, Universitas Gadjah Mada, 55281 Yogyakarta, Indonesia

* Corresponding author: Nanang Fakhrudin | Email: nanangf@ugm.ac.id

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Abstract: *Ficus septica* Burm. F (Awar-awar) in Indonesian herbal medicine traditionally used for the treatment of various disease. Previous studies indicated that the *F. septica* leaves contained phenantroindolizidine alkaloids with anticancer activity. Fractionation of the ethanol extract of *F. septica* leaves using *n*-hexane was able to separate chlorophylls and other inert non-polar constituents from the extract. This fractionation process can be optimized using ultrasonic-assisted fractionation to yield *n*-hexane insoluble fraction (HIF) that is rich in alkaloids. This study aimed to produce HIF from the ethanol extract of *F. septica* leaves by using optimized ultrasonic-assisted fractionation with *n*-hexane as a solvent and full factorial as experimental design. *Ficus septica* leaves were macerated in ethanol (60%) and the extract was used for the ultrasonic-assisted fractionation process. The duration of fractionation (5-30 minutes), the extract : *n*-hexane ratios (1:0.1 - 1:1.66 ml/ml), and the ultrasonic power (21-106 Wrms) were optimized to determine the optimal condition for each variable. These optimal variables were used for the production of HIF from the extract by using full factorial design in the ultrasonic-assisted fractionation process. The total alkaloid content was measured using spectrophotometry and was used as parameter for the optimization process. We found that the optimal condition for the fractionation process based on each single variable optimization were 5 minutes of the fractionation duration, 64-70Wrms of the ultrasonic power, and 1:0.7 - 1:0.8 of the extract : *n*-hexane ratio. By using these optimized variables, the ultrasonic-assisted fractionation using full factorial design yielded up to 0.035% total alkaloid content which is almost double from those in the extract (0.019%). This study provided a basic experimental model for the production of alkaloid-enriched HIF from *F. septica* leaves in the industrial or pilot scale, and might contribute to the development of Indonesian herbal medicine products from natural resources.

Keywords: Awar-awar; Alkaloid; Anticancer, Herbal medicine, Full Factorial Design

1. INTRODUCTION

Currently, cancer is still one of the deadliest diseases in the world because its difficulty to cure and its rapid invasion in the human body. Cancer also represents the disease that most consumes health care budget after heart diseases and kidney failure [1]. Although there are many chemotherapy drugs available in the market for cancer treatment, many studies indicated that the resistance and side toxic effects remained the main problem and accompanied chemotherapy. Resistance and undesired side effects lead to therapeutic ineffectiveness and might reduce the patient quality of life. This encourages the need for alternative chemotherapy agent or co-chemotherapy approach that
work complementary with chemotherapy to improve therapeutic efficacy and the patient quality of life. Several studies have shown that administering complementary products along with the chemotherapy agents might work synergically or additionally increasing therapeutic efficacy and improving patient quality of life [2-4]. Additionally, the meta-analysis study showed that the use of traditional herbal medicine increased the efficacy of cancer therapy as well as improved the quality of life of cancer patients [5, 6].

Indonesia is mega biodiversity country with abundant medicinal plants that have the potential to be developed as traditional medicinal products to treat cancer. One of these potential plants is *Ficus septica* Burm. F. (Awar-awar). The leaves of *F. septica* are empirically used for various health problems, such as the treatment of skin diseases, appendicitis, ulcers, rattlesnake bites, warts and shortness of breath [7]. Previous studies demonstrated that the ethanol extracts of *F. septica* leaves and its fractions showed cytotoxic activity on cancer cells *in vivo* and *in vitro* [8, 9], and increased cancer cells sensitivity upon the treatment with the chemotherapy agent, doxorubicin [10-12]. The ethanol extract of *F. septica* leaves has been shown to have a cytotoxic effect on T47D and MCF-7 breast cancer cells, with IC₅₀ of 13 µg/ml and 6 µg/ml respectively [3,14], and it also has antiproliferative effect on breast cancer cells [15]. The low IC₅₀ against cancer cells in several experiments indicated that *F. septica* is a potential source of anticancer agents. The compounds responsible for the anticancer activity of *F. septica* leaves extract are phenanthroindolizidine alkaloids such as tylophorine, 14α-hydroxyisocrebrine N-oxide, ficuseptine A, 14-hydroxy-3,4,6,7-tetramethoxyphenanthroindolizidine, 14-hydroxy-2,3,4,6,7-pentamethoxyphenanthroindolizidine, tylocrebrine, isotylocrebrine, antofine, and 2 seco-phenanthroindolizidine alkaloids: septicine, and secoantofine [16-18]. Previous research showed that fractionation of *F. septica* ethanol extract using n-hexane resulted in the n-hexane insoluble fraction (HIF) that has a potent cytotoxic activity [9, 12]. The HIF contained alkaloids and demonstrated cytotoxic activity on cancer cells. It also enhanced the sensitivity of cancer cells against anticancer drug, doxorubicin [11]. However, the aforementioned study did not determine the amount of total alkaloid and the fractionation method has not been optimized. In this study, we optimized the production of the HIF from the ethanol extract of *F. septica* leaves by using ultrasonic-assisted fractionation method with a full factorial design. N-hexane was used as the solvent of choice for the fractionation process.

Liquid-liquid fractionation using n-hexane was able to remove invaluable substances including chlorophylls, lipids and waxes to yield the HIF [11]. However, conventional fractionation is lack of efficiency and effectiveness because it consumes high energy, takes long time duration, and uses excessive solvent [5] and therefore, it is not suitable for industrial application. Ultrasonic-assisted fractionation is the commercially available and advance method capable of increasing fractionation yield with less energy and solvent, as well as shorter time duration. In the HIF production, the ultrasonic wave might enhance total alkaloid content by generating mass transfer rapidly and powerfully [6] that can dramatically increase the fractionation effectiveness and efficiency. In the ultrasonic-assisted fractionation, the main mechanism is a mass transfer. This fractionation improves effectiveness and efficacy by increasing the mass transfer via the intensifying of intermolecule interactions among the solvent and the sample [19]. Additionally, the utilization of ultrasonic power breaks the particles size into smaller parts and increases sample dissolution [20]. The effectiveness and efficiency of an ultrasonic-assisted fractionation is influenced by several factors such as solvent ratio,
fractionation duration, and mechanical strength (energy) [7][8]. Here, we optimized these factors by using a full factorial design approach to obtain an optimal condition for the ultrasonic-fractionation process. Since alkaloids are the active compounds responsible for the anticancer activity in the HIF, thus, the total alkaloid content of the HIF was used as an indicator in the optimization of fractionation process. First, the effects of each single variable (solvent ratio, fractionation duration, and mechanical strength) were investigated, and then the full factorial design of fractionation was employed to investigate the variables yielding the highest alkaloid content.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Material

_Ficus septica_ leaves were harvested from Tawangmangu district, Jawa Tengah, Indonesia. The leaves were washed with running water and then let dried at room temperature. The leaves were dried in the oven at temperature of 60 ºC for 48 h, and then cut into a smaller size and powdered to obtain dried powder for the extraction process.

2.1.2. Chemicals

The chemicals used in this experiment were Sodium hydroxide (Merck), phosphate buffer saline consist of Sodium phosphate (Merck), and citric acid (Merck), Bromocresol green (Merck), common pro-analytical grade solvents including chloroform and _n_-hexane (Merck), water, and ethanol (technical grade).

2.1.3. Instrument

The Ultrasonic-assisted fractionation was done using Lab Ultrasonic for radial probe instrument equipped with Nextgen 750 as power generator (SinapTec). A peristaltic pump BT 100S equipped with a pippet was used to separate the _n_-hexane insoluble fraction (lower fraction) from the _n_-hexane soluble fraction (upper fraction). A spectrophotometer (Shimadzu UV1800) was used to determine the total alkaloid content. The glasswares consisted of separating funnel (Duran) volumetric flask (Duran), cyclindrical glassware (Iwaki), centrifuge, and sonicator bath, were also used in this research.

2.2 Methods

2.2.1. Plant Extraction

The dried _F. septica_ leaves powder were extracted in ethanol (60%) for 12 h. Our preleminary study indicated that the extraction of _F. septica_ leaves using 60% of ethanol produced the highest amount of alkaloid compared to that using the lower and the higher ethanol percentage. The filtrate was separated from the residue using filter filtration on a Buchner funnel. The filtrate was evaporated on a rotary evaporator (IKA), and the evaporation process (60 ºC) was terminated when the total solid of the extract reached 35-40%, measured in a moisture analyzer (Metler Toledo). The extract was collected for the fractionation process.

2.2.2. Ultrasonic-Assisted Fractination

The liquid extract of _F. septica_ leaves (700 mL) with total solid of 35-40% was put into a cylindrical glassware. _N_-hexane was used as the solvent to produce the soluble and insoluble fraction. The ultrasonic-assisted fractionation was done by applying ultrasonic power (0-106 Wrms) through the ultrasound radial probe at 20 KHz. After the fractionation process was completed, the fractions were let in room temperature for 20 h until 2 fractions (the lower and upper layer) formed. The upper
fraction was the n-hexane soluble fraction; whereas the lower was the n-hexane insoluble fraction (HIF). The HIF was used for this study. There were 2 part of this study, first part (One factor one level) and second part (full-factorial design). The purpose of first part of fractionation was to observe effect of each parameters (duration of fractionation, ratio of extract : hexane, ultrasound power) and full factorial design fractionation used to investigate which parameters have effect to rise total alkaloid content.

2.2.3. Fractionation duration optimization

The liquid extract (350 mL) was stirred until homogenous. The extract was then poured into 700 ml cylindrical glassware, and added with 350 ml of n-hexane. The ultrasonic-assisted fractionation was done at the strength of 90 Wrms and ratio of extract : solvent was 1:1. Duration in these fractionation were 5 minutes, 10 minutes, 20 minutes and 30 minutes. The fraction was left in the room temperature for 20 hours before the n-hexane soluble fraction was discarded and the n-hexane-insoluble fraction (HIF) was collected. The total alkaloid content was measured using a spectrophotometer at the wavelength of 420 nm.

2.2.4. Extract : solvent ratio optimization

The extract added with n-hexane with the extract : solvent ratio of 1:0.1 ; 1:0.3 ; 1:0.6 ; 1:1 ; 1:1.3 and 1:1,6 in a 700 cylindrical glassware. The ultrasonic-assisted fractionation was done at the strength of 90 Wrms with the duration obtained from 2.2.3 (5 minutes). The next step was similar to that was described previously in the section 2.2.3.

2.2.5. Ultrasonic strength optimization

The extract was added with n-hexane with the volume according to the optimized extract : n-hexane ratio obtained from section 2.2.4 (1:0.3) in a 700 ml cylindrical glassware. The ultrasonic-assisted fractionation was done at the strength of 21, 42, 64, 85, and 106 Wrms. The duration of the fractionation referred to the optimized fractionation duration obtained from section 2.2.3 (5 minutes).

2.2.6. Application of factorial design in the fractionation

To investigate whether each parameter has a significant influence on the fractionation process and to observe interactions among the parameters, factorial design was employed in the study design. Factorial design is the form of design of experimental that consist of planning, analysis, setting and interpretation of data in experiment. Factorial design used factor and level. Factor (parameter) is a observed variable that may impact the result of experiment. Level is value of factor that observed in experiment. If a experiment consist of 2 level, it used low point (-1) and high point (1) [21]. In this experiment, 2 levels and 2 factors were used in the factorial design. The ultrasonic power and the ratio of the extract to the solvent (n-hexane) represented the factors. The ultrasonic power at 42 Wrms (lower point) and at 70 Wrms (higher point), and the extract : solvent ratio of 1:0.1 (lower point), and 1:0.8 (higher point) represented the levels in the factorial design.

2.3. Spectrophotometry Analysis

The HIF (10 ml) was centrifuged at 3000 rpm for 10 minutes. This fraction was taken 0.2 mL into separating funnels and added with BCG (5 mL) and phosphate buffer (5mL). This mixture was partitioned using 5x2 ml of chloroform. The lower fraction was separated and then centrifugated at
3000 rpm for 5 minutes. The absorbance was measured using a spectrophotometer at the wavelength of 420 nm.

2.3.1. Preparation of Bromocresol Green (BCG)

To measure the total alkaloid content in the HIF using spectrofotometry method, bromcresol green was used as a chemical reagent to form alkaloid-bromcresol-green complex for generating a standard curve. This method referred to Shamsa et al and Qarah et al [22][24]. Shortly, bromcresol green (69,80 mg) was dissolved in 3 ml of NaOH 2 N and added with aquadest in a 1000 volumetric flask.

2.3.2. Preparation of phosphate buffer saline with pH 4.7

Phosphate buffer saline(pH 4.7) was prepared by mixing 2 M sodium phosphate (71.60 g in 1000 mL aquadest) with 0.2 M citrit acid (42.02 g of citrit acid in 1000 mL aquadest). This mixture was adjusted to pH of 4.7.

2.3.3. Calibration curve for visible spectrophotometer

Chloroquine phosphate (1 mg) was dissolved in 25 ml aquadest and sonicated in the water bath for 10 minutes. Phosphate buffer (pH 4.7) and BCG solution each at the volume of 5 mL was mixed with the chloroquine phosphate solution (at volume of 0.2; 0.4; 0.6; 0.8; 1 and 1.2 ml) each in different separating funnels. These mixtures were added with 5x2 ml chloroform in the separating funnels. The lower phase (chloroform fraction) was separated and then centrifugated for 5 minutes at room temperature. The absorbance of the BCG-Chloroquine complex was measured in a spectrophotometer at the wavelength of 420 nm.

2.3.4. Preparation of phosphate buffer saline with pH 4.7

Phosphate buffer saline(pH 4.7) was prepared by mixing 2 M sodium phosphate (71.60 g in 1000 mL aquadest) with 0.2 M citrit acid (42.02 g of citrit acid in 1000 mL aquadest). This mixture was adjusted to pH of 4.7.

2.4. Statistical Analysis

The total alkaloid contents among group of treatments were compared using a statistical analysis. First, the data distribution was analyzed using Saphiro-Wilk to determine whether the data distribution is a normal (P > 0.05). To investigate the data homogeneity, Levene test was used (P > 0.05). Data with normal distribution and homogenous were analyzed by using one-way Anova to determine their significant level (P < 0.05).

3. RESULT AND DISCUSSION

Currently the utilization of natural products for the treatment and prevention various diseases, including cancer is gaining many interests. One of the medicinal plants from Indonesian origin is F. septic a (awar-awar). The leaves of F. septic a contain phenantroindolizidine alkaloids that have anticancer activity. There is no established method of alkaloid separation or fractionation from the leaves of F. septic a. The common method of separating the compounds of interest from the extract is by fractionation. Ultrasonic-assisted fractionation is an upgrade from conventional or manual
fractionation aiming to increase the fractionation effectivity and efficiency. The ultrasonic-assisted fractionation has been used by several pharmaceutical industries. This study aimed to produce the alkaloid-enriched fraction (HIF) from the ethanol extract of *F. septica* leaves using an ultrasonic-assisted fractionation with a full factorial design. Several previous studies have shown that ultrasonic-assisted fractionation was able to increase the fractionation effectivity and efficiency due to the increase in temperature, mass transfer, and generation of bubble cavitation mechanism [26][19].

3.1. Measurement of total alkaloid content using visible spectrophotometer

As a measure in the fractionation process, the total alkaloid content in HIF was used. The total alkaloid content was determined using spectrophotometer. The interaction between alkaloids in HIF and bromocresol green produces alkaloid-bromocresol green complex that can be quantified in a spectrophotometer at the wavelength of 420 nm [22]. Because the alkaloid components in the *F. septica* leaves are mainly dominated by phenatroindolizidine alkaloid, chloroquine was used as a compound standard due to structural similarity. The reaction between chloroquine and bromocresol green was used to generate a calibration curve (Figure 1) for determination of total alkaloid content in the HIF.

![Figure 1](image-url). Calibration curves and linear regression equations of chloroquine as a standard for the determination of total alkaloid content using spectrophotometer.

3.2. The effect of fractionation duration to the total alkaloid content

Duration of fractionation affects the amount of mass transfer of the compound of interest toward the solvent. On the other hand, longer duration of ultrasonic-assisted fractionation increases temperature which is not good for compound stability. Ultrasonic also lead degradation of substances in HIF. In this study (Figure 2), duration of fractionation had no effect to increases total alkaloid content. Total alkaloid content in HIF were 0.110, 0.093, 0.111, and 0.096 % using 5, 10, 20, and 30 minutes of fractionation duration, respectively. The fractionation duration that was used for the full factorial design was 5 minutes because 5 minutes of ultrasonic-assisted fractionation produced the highest amount of alkaloid content in the HIF. Thus, fractionation duration of 5 minutes was used for the multivariate design of this study using minimal fractionation duration (5 minutes).
Figure 2. Effect of duration of fractionation to total alkaloid content. Duration of fractionation had no effect to increases total alkaloid content. Total alkaloid content was obtained: 0.110% (5 min); 0.093% (10 min); 0.1114 % (15 min); and 0.0959% (30 min).

3.3. The effect of extract : solvent ratio to the total alkaloid content

The ethanolic extract of *F. septica* leaves was fractionated with n-hexane in different volumes. The extract : n-hexane ratios were designed at 1:0.1, 1:0.3, 1:0.6, 1:1, 1:1.3, and 1:1.16. In this fractionation process, 3 different solvents comprising ethanol, water, and n-hexane were involved in the fractionation process. Theoretically, n-hexane was an apolar solvent that separated the chlorophylls, lipids, and other inert non polar subsituents from the extract and resulted in the n-hexane soluble fraction (HIF) with a higher alkaloid content. However, the amount of n-hexane in the fractionation was not necessarily linear with the total alkaloid content in the HIF. Additionally, the excessive n-hexane increases cost of production. Therefore, the extract : n-hexane ratio required to be optimized as it affects total alkaloid content. We found that the extract : n-hexane ratio of 1:0.1, 1:0.3, 1:0.6, 1:1, 1:1.3, and 1:1.6 yielded the total alkaloid content of 0.020, 0.0270, 0.023, 0.020, 0.022, and 0.018 %, respectively (Figure 3). The extract : hexane ratio of 1:0.1 and 1:0.3 significantly increased the total alkaloid content of the HIF. Interestingly, the ratio higher that 1:0.3 until 1:1.6 did not alter total alkaloid content. Therefore, the extract : n-hexane ratio of 1:0.1 (lower point) and 1:0.33 (higher point) were used in the full factorial design to obtain the optimal condition for fractionation that involves several variables.
Figure 3. The effect of the extract : solvent ratio to the total alkaloid content. N-hexane was used as a solvent, and the extract : n-hexane ratios were set at 1:0.1 until 1:1.6. The extract : n-hexane ratio of 1:0.1 and 1:0.3 significantly increased the total alkaloid content (*P < 0.05) of the HIF compared to the extract.

3.4. The effect of ultrasonic power to the total alkaloid content

Figure 4. The effect of ultrasonic power to the total alkaloid content in the HIF. The ultrasonic power applied for the fractionation process was ranging 21 to 106 Wrms. The total alkaloid content was at peak and showing statistically significant compared to the extract at 64 Wrms (*P < 0.05).

The ultrasonic power applied in the fractionation process affected the yield of the targeted compound by increasing the mass transfer and cavitation to create dynamic stress toward the solvent [23]. In the other hand, the ultrasonic power could raise the temperature leading to the compound destabilization. In this study, for the fractionation process we applied the ultrasonic power of 21, 42,
64, 85, and 106 Watt root mean square (Wrms) and yielded the total alkaloid content of 0.027, 0.028, 0.032, 0.021, and 0.016 %, respectively. Figure 4 demonstrated that the ultrasonic power of 21-106 Wrms, but not higher than 64 Wrms increased the total alkaloid content in the HIF. Nevertheless, the peak of total alkaloid content was reached at 64 Wrms. By using 64 Wrms the total alkaloid content of the HIF showed a statistical significant compared to the 0 Wrms (the extract/before fractionation). Consequently, the ultrasonic power of 42 Wrms (lower point) and 70 Wrms (higher point) was applied in the full factorial design.

3.5. Optimization of total alkaloid content in the insoluble hexane fraction using full-factorial design method

After the lower (-1) and higher (1) values from each parameters were obtained from the previous single variable optimization, these values were used in the full-factorial design to optimize the condition for the ultrasonic-assisted fractionation of F. septica leaves extract. In this study, we used 2 variables (extract : solvent ratio, and ultrasonic power) with 2 levels (the lower point (-1) and the high point (1)), and 1 centre point. From the single variable optimization, we found that the duration of fractionation was 5 minute, the ratio of the extract to n-hexane was 1:0.1 (low point) and 1:0.8 (high point), the ultrasonic power was 42 Wrms (the lower point), and 70 Wrms (the higher point). Table 1 provided the result of the total alkaloid content obtained using the full-factorial design.

Table 1. Total alkaloid content obtained by the full-factorial design. The full-factorial design used 2 levels (the lower point (-1) and the higher point (1)), with 2 variables (extract : n-hexane ratio, and ultrasonic power). In the extract : n-hexane ration, the ratio of 1:0.1 and 1:0.8 were set as the lower and higher point, respectively; whereas in the ultrasonic power, 42Wrms and 70Wrms were set as the lower and higher point, respectively. The center point defined the mean between the lower and higher point.

| Level | Ultrasonic power (Wrms) | Extract : n-hexane ratio | Total alkaloid content (%) |
|-------|-------------------------|--------------------------|-----------------------------|
| 1     | 1                       | 70                       | 1:0.8                       | 0.031±0.0042 |
| 1     | 1                       | 70                       | 1:0.8                       | 0.035±0.0008 |
| 1     | 1                       | 70                       | 1:0.8                       | 0.033±0.0025 |
| -1    | 1                       | 42                       | 1:0.8                       | 0.030±0.0003 |
| -1    | 1                       | 42                       | 1:0.8                       | 0.026±0.0017 |
| -1    | 1                       | 42                       | 1:0.8                       | 0.025±0.0008 |
| 1     | -1                      | 70                       | 1:0.1                       | 0.030±0.0011 |
| 1     | -1                      | 70                       | 1:0.1                       | 0.026±0.0010 |
| 1     | -1                      | 70                       | 1:0.1                       | 0.023±0.0013 |
| -1    | -1                      | 42                       | 1:0.1                       | 0.023±0.0011 |
| -1    | -1                      | 42                       | 1:0.1                       | 0.023±0.0004 |
| -1    | -1                      | 42                       | 1:0.1                       | 0.025±0.0011 |
| 0     | 0                       | 56.2                     | 1:0.4                       | 0.028±0.0006 |

| Extract | 0.019±0.0010 |

Based on the results presented in Table 1, a mathematical equation representing the corelation between the ultrasonic power and extract : n-hexane ratio variables with the total alkaloid content can be described as 0.01883 + 0.0002 X1 - 0.00113 X2 + 0.000311 X1X2 + 0.00002 Ct Pt (X1: ultrasonic power; X2: extract : n-hexane ratio; and Ct Pt = 0.0273825). From this equation, we were able to obtain
the optimum condition for the production of HIF from the *F. septica* leaves by using ultrasonic-assisted fractionation. The condition were 5 minutes of fractionation duration, (64-70W) of ultrasonic power, and 1:0.7 - 1:0.8 extract : n-hexane ratio. This method yielded up to 0.035% alkaloid content which is almost double from the total alkaloid content in the extract (0.019%). The ultrasonic power, the extract : n-hexane ratio, and the interaction of both variables affected the total alkaloid content in the ultrasonic-assisted fractionation process. These interactions were illustrated as a contour plot in the Figure 5. Each color in Figure 5 represented the total alkaloid content of the HIF produced using different combination of ultrasonic power and extract: n-hexane ratios. The dark green area at the upper right and the dark blue area at the lower left are the highest and lowest alkaloid content. Figure 5 showed that the extract : n-hexane ratio of 1:0.7-1:0.8, and the ultrasonic power of 64-70 W were the optimum fractionation condition to produce HIF with the highest alkaloid content. The total alkaloid content in the HIF obtained using this optimized method is 0.035% or almost double from the extract. In this study, we found that ultrasonic-assisted fractionation together with the full-factorial design could be used to produce alkaloid-enriched fraction (HIF) from the extract of *F. septica* leaves. These findings provided an important basis for the production of the alkaloid-enriched HIF in the pilot or industrial scales.

**Figure 5.** Contour plot showing the effects of the extract : solvent ratio and the ultrasonic power on the total alkaloid content in the HIF production from the *F. septica* leaves fraction. The optimal condition was obtained at the dark green area (the extract : n-hexane ratio of 1:0.7-1:0.8, and the ultrasonic power of 64-70 W).

### 4. CONCLUSION

This study demonstrated that the ultrasonic-assisted fractionation along with the full factorial design optimization can be used to produce HIF with enriched alkaloid from the extract of *F. septica* leaves. This method enriched the alkaloid content almost double than the extract and provided a scientific basis for the further production in larger scale.

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