Simplex Lattice Design Analysis for Optimizing the Sangketan Extract (Achyranthes aspera) production

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Abstract. The need for medicinal raw materials is increasing, in line with the increasing of traditional medicine utilization. Sangketan (Achyranthes aspera) is one of the potential medicinal plants, considering this plant contains active compounds that are beneficial for health. In an effort to increase productivity, quality, and continuity of medicinal plants, improvement in cultivation techniques is needed. The right use of growing media, will provide optimal environmental conditions for plant growth. Animal manure and rice husk charcoal are organic material, that can be used as a mixture of growing media. The purpose of this study was to determine the optimal combination of growing media, that could support the extract production from Sangketan. In Sangketan cultivation, given treatment at growing media composition of soil + rice husk charcoal, with comparison 1:1; 1:2 and 2:1. Whereas the fertigation uses goat manure fertilizer, with a concentration of 1 kg per 5 liters of water, dose of 60 ml per plant every two weeks. Sangketan is harvested after 4-5 months, then quantitative analysis is performed of resulting extracts. The research data was applied using simplex lattice design (SLD), to obtain the optimal combination of growing media. Sangketan resulting extracts was applied using SLD, obtained the equation $Y = 8.94 \text{ (Charcoal)} + 11.585 \text{ (Soil)} + 14.26 \text{ (Charcoal.Soil)}$. Results of this study can identify and evaluate, technologies that support the sangketan development as a raw material for traditional medicine.

1. Introduction
The need for medicinal raw materials is increasing, in line with the increasing of traditional medicine utilization. In the present era of drug development and discovery of newer drug molecules, many plant products are evaluated on the basis of their bioactive constituents. The curative properties of medicinal plants are mainly due to presence of various complex chemical substances of different compositions which occur as secondary metabolites [1]. In an effort to increase productivity, quality, and continuity of medicinal plants, improvement in cultivation techniques is needed. One such effort is to use organic materials for growing media. Proper growing media is one of the requirements for successful cultivation of medicinal plants. The right use of growing media, will provide optimal environmental conditions for plant growth. A good growing media has the ability to provide optimum water and air [2].

Animal manure and rice husk charcoal are organic material, that can be used as a mixture of growing media. Organic matter contains a number of nutrients that can improve the physical, chemical and biological properties of the soil [3]. In the cultivation of medicinal plants it is recommended to use organic materials, because if use chemical fertilizers it is feared it can have a negative effect in the form of chemical residues.
Sangketan (*Achyranthes aspera*) is one of the potential medicinal plants, considering this plant contains active compounds that are beneficial for health. *Achyranthes aspera* holds a reputed position as a medicinal herbs in different systems of medicine in India. According to Ayurveda, it is bitter, pungent, heating, laxatives, stomachic, carminative and useful for the treatment of vomiting, bronchitis, heart diseases, piles, abdominal pains, ascites, dyspepsia, dysentery, blood diseases etc. In Ayurveda, two varieties, red and white are mentioned. It is described in *Nighantus* as purgative, pungent, digestive, a remedy for the inflammation of internal organs, piles, itch, abdominal enlargements and enlarged cervical glands. The diuretic properties of the plant are well known to the natives of Indian and European physicians. Different parts of the plant form ingredients in many native prescriptions in combination with more active remedies [4].

2. Materials and Methods

2.1 Collection plant material

*Sangketan* plants that are used as raw material are harvested after 4-5 months, as for the composition of the growing media as follows:

\[ P_1 = \text{growing media composition } 1 : 1 \ (1/2 \text{ piece of land : 1/2 piece of rice husk charcoal}) \]
\[ P_2 = \text{growing media composition } 1 : 2 \ (1/3 \text{ piece of land : 2/3 piece of rice husk charcoal}) \]
\[ P_3 = \text{growing media composition } 2 : 1 \ (2/3 \text{ piece of land : 1/3 piece of rice husk charcoal}) \]

The addition of Rice Husk Charcoal (RHC) into composting of organic matters was able to accelerated the composting process through higher decomposition rates. It is expected that RHC functions as a bulking agent, increases the microbial activity and retains the moisture and nutrient N during the composting process [5].

The seedlings used are from seedbed seedlings that have reached 5-10 cm in height, fresh, not attacked by pests and diseases, normal growth forms and not deformed. Whereas the fertigation uses goat manure fertilizer, with a concentration of 1 kg per 5 liters of water, dose of 60 ml per plant every two weeks.

2.2 Simplex lattice design studies

Quantitative analysis, 500 grams of *Sangketan* dry powder in the test tube was extracted with petroleum ether for 2 hours at 50°C then filtered. The extract is then heated to dry and placed on a saucer. Then extracted again with methanol for 2 hours at 50°C and filtered. From the experiment shows the presence of alkaloids, saponins, tannins and cardiac glycosides, flavonoids, carbohydrates and steroids are present in methanolic extract [6]. The extract is then heated to dry and placed on a saucer. The data obtained in the weight of methanol dry extract, used as a reference level of secondary metabolite production in *Sangketan*. In general, the study revealed that mixture of rice hull charcoal and pyrolygenic acid (MRPA) was effective in improving agronomic characters, panicle number, ripening ratio and yield of rice. The treatments affected the total lipid content more than the starch content. Furthermore, application of MRPA is more appropriate in improving both the antioxidant and nutritional quality of rice [7].

3. Results and Discussion

The research method used was a Completely Randomized Design, three replications were carried out in each composition of the growing media. *Sangketan* plants that are used as raw material are harvested after 5 months, crop weight as follows (Table I).

| Harvest/Treatment | Growing Media |
|-------------------|--------------|
| Wet               | P1 | P2 | P3 |
| 24.86             | 22.90 | 27.16 |
| Dry               | 3.73 | 3.44 | 4.08 |
The research data was applied using simplex lattice design (SLD), to obtain the optimal combination of growing media. *Sangketan* resulting extracts was applied using SLD, obtained the equation (1).

\[ Y = 8.94 \text{ (Charcoal)} + 11.585 \text{ (Soil)} + 14.26 \text{ (Charcoal.Soil)}. \]

The equation coefficient is obtained from the average calculation of *Sangketan* yield extract (Table II). The optimization steps are determining the mixture composition using the simplex lattice design method, done with a certain total mixture. Each ingredient must be determined minimum and maximum limits, so we can know the effect response of each ingredients mixture [8].

| Table 2. Average sangketan yield extract (%) |                 |                 |
|---------------------------------------------|-----------------|-----------------|
| Replication  | Charcoal | Soil | Charcoal Soil |
| I             | 8.94     | 11.59 | 14.26          |
| II            | 9.39     | 11.68 | 14.36          |
| III           | 8.49     | 11.49 | 14.16          |
| average       | 8.94     | 11.585| 14.26          |

Enhancement of nutrient availability for plants positively affected plant secondary metabolite synthesis. Furthermore mycorrhization may influence the metabolic pathway of anthocyanin in plants and activates the key-enzymes, which are responsible for biosynthesis of secondary metabolite. Rice husk compost application in combination with mycorrhiza application improved plant nutrient uptake rate and enhanced growth/yield and plant secondary metabolites status [9]. The analysis for the marker compounds of *Sangketan* in which TLC profile was obtained based on the Rf value. The gray band under UV light 254 nm at Rf value 0.47 was associated to triterpenoids for *A. aspera*. Observation of these bands can be associated to oleanolic acid compound [10]. Results of this study can identify and evaluate, technologies that support the *sangketan* development as a raw material for traditional medicine.

4. Conclusion
The quantitative data of *Sangketan* yield extract was applied using simplex lattice design, obtained the equation \( Y = 8.94 \text{ (Charcoal)} + 11.585 \text{ (Soil)} + 14.26 \text{ (Charcoal.Soil)}. \) Application with simplex lattice design, can show the right formula related to the growing media composition of soil + rice husk charcoal. So that it supports optimal production in *Sangketan* organic cultivation.

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