Comparison of two varieties of *Plectranthus scutellarioides* based on extraction method, phytochemical compound, and cytotoxicity

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**Abstract.** *Plectranthus scutellarioides* have more than 173 varieties that can be distinguished from leaves foliage. Each variety produces certain second metabolite. Among all varieties, color blaze dark star was the one commonly used in Indonesia as traditional medicine. Optimization extraction process, qualitative phytochemical analysis, and preliminary assessment of toxicity of color blaze dark star and trailing queen variety of *Plectranthus scutellarioides* were investigated. Extraction process in color blaze dark star variety using Microwave assisted extraction show the highest %yield of extract but in trailing queen variety the highest %yield of extract was obtained from soxhletation method. Qualitative phytochemical analysis using thin layer chromatography from methanol, ethanol, and water extract with all method of extraction showed that both of varieties of *Plectranthus scutellarioides* contain alkaloid, flavonoid, tannin, and phenol. The highest toxicity with LC₅₀ 39 μg/ml was showed from ethanol extract using microwave assisted extraction of color blaze dark star variety.

**1. Introduction**

*Plectranthus scutellarioides* (syn. *Coleus scutellarioides*, *Coleus blumei*, *Plectranthus blumei*) have more than 173 varieties all over the world. Each variety has different leaves foliage. Color, smell, and flavour was affected by phytochemical constituent in the plant [1]. Phytochemical screening on Solenostemon genus show the most compound was diterpenoid compound with abietane or labdane skeleton. Monoterpenoid, sesquiterpenoid, dan phenolic also can be identified from five species of Plectranthus [2]. Study in some varieties of *Plectranthus scutellarioides* show difference in total phenolic [3], anthocyanin [4], chlorophyll and carotenoid [5], also isolated diterpenoide [6,7].

Color blaze dark star variety, known as “miana” or “iler”, usually be used in fresh, boil, or infuse form to treat some ailments like blain, abscess, ulcers, inflammation in ear and eye, the root was used for diarrhea and stomach pain [8], also can be used in diabetes mellitus, constipation, fever, and dysmenorrhea [9]. Ethnic Toraja used miana’s leaves to treat pulmonary tuberculosis [10], while trailing queen variety used as decorative plant. Difference in qualitative and quantitative of bioactive compound was affected by environment and genetic of the plant [11], also create new variety.
This study was presented to evaluate comparison of two varieties *Plectranthus scutellarioides* which are used traditionally to treat some ailment (color blaze dark star) and used as decorative plants based on %yield extract in some extraction method and solvent, qualitative compounds, and cytotoxicity.

2. Materials and Methods

2.1. Plant material
Leaves of color blaze dark star variety was collected from Gowa, Sungguminasa, South Sulawesi, while trailing queen was collected from Makassar, South Sulawesi at 10.00 am until 12.00 am to obtain optimum compound level. Determination and taxonomic identification was made from faculty of biology, UNM.

2.2. Extraction process
The leaves of both varieties *P.scutellarioides* were washed in water flow, dried in oven with temperature 400-500°C, sorting from other parts of plant, made into a coarse powder with mechanical grinder and the keep in the close tight container until used. The leaves was extracted using microwave assisted extraction, maceration, and soxhletation with methanol, ethanol, and water solvents. Extraction method with microwave was conducted during 30 minute with power at 50W, maceration was conducted by soaked the coarse powder three days, and soxhletation was conducted until the filtrat have clear level equal to all extraction method. The solvent was removed using rotary evaporator with reduced pressure until liquid extract become thick. All the 18 extracts were calculated for the percent yield of extract and showed in the table 1.

2.3. Phytochemical screening
Phytochemical screening was conducted by thin layer chromatography. For methanol and ethanol extract, solvent system that used was n-hexan : ethyl acetate (3:1), while for water extract, used ethyl acetate : methanol : water (5:1:0,5). This solvent system was selected due to their ability to separate constituent in both varieties of *Plectranthus scutellarioides* maximally. Specific reagent to identificate alkaloid (Dragendorff), flavonoid (sitroborat), terpenoid (vanillin-sulphate acid), and phenol (FeCl₃) was sprayed and observed under UV 366nm for flavonoid, while the other was observed visibly after heated five minutes at 105°C. Qualitative phytochemical analysis of all extract was showed in the table 2.

2.4. Brine shrimp lethality assay
The brine shrimp (Artemia salina) lethality assay was conducted according to McLaughlin 1982. Artificial seawater was prepared by dissolving 19.0 g in the 1 L distilled water. The brine shrimp eggs were sprinkled into artificial water in the separating funnel with lighting and aerator. After 24 hours, the hatched eggs were transferred to plastic chamber with several holes on the divider which was darkened. After the second 24 hours, nauplii (larvae) from the lighted side were used for the test. Concentrations of plant extracts was prepared with artificial seawater for dilution until desired concentration was obtained (10, 100, 1000 µg/ml), this step was done in triplicates. Ten nauplii were transferred by pipette into vial that contains diluted extract (all concentration) and added with artificial seawater until 5 ml and maintained at room temperature for 24 hours under the light. Mean of surviving nauplii were counted and recorded to calculate percentage of lethality along with mean of surviving nauplii from control vial. LC₅₀ of all extract was showed in table 3.
3. Results and Discussion

3.1. Extraction optimization

Three extraction methods along with three extraction solvents were used to obtain percent yield of extract which is highest in color blaze dark star and trailing queen variety. Microwave assisted extraction was produced the highest %yield of extract in color blaze dark star variety with water as the extraction solvent (35.8%) followed by soxhletation method with water solvent and soxhletation with methanol (23.2%; 20.8% respectively). While, the highest %yield of extract in trailing queen variety was obtained by soxhletation method with water solvent, followed by maceration with water and microwave with water (24.9%; 22%; 20.9% respectively).

| Varieties               | Extraction Process | Solvent | % yield of extract |
|-------------------------|--------------------|---------|-------------------|
| Color blaze dark star   | Maceration         | Water   | 17.6              |
|                         | Methanol           | 16.4    |
|                         | Ethanol            | 16.4    |
|                         | Water              | 23.2    |
|                        | Soxhletation       | Methanol| 20.8              |
|                         | Ethanol            | 11.8    |
|                         | Water              | 35.8    |
|                        | Microwave assisted | Methanol| 13                |
|                        | extraction         | Ethanol | 13.6              |
| Trailing queen          | Maceration         | Water   | 22                |
|                         | Methanol           | 18.6    |
|                         | Ethanol            | 19.7    |
|                         | Water              | 24.9    |
|                        | Soxhletation       | Methanol| 19.8              |
|                         | Ethanol            | 19.8    |
|                         | Water              | 20.9    |
|                        | Microwave assisted | Methanol| 10.2              |
|                        | Ethanol            | 8.3     |

Figure 1. Effect of method and solvent extraction on %yield of extract color blaze dark star variety
Figure 2. Effect of method and solvent extraction on %yield of extract trailing queen variety

Color blaze dark star and trailing queen variety contain fewer amounts of non polar substances in comparison to polar substances, as extractive value increases with the increase in the polarity of solvents. This result is parallel with study in evaluation of extraction yield on different extraction solvents of Orthosiphon stamineus leaves [12] and Phyllanthus niruri leaves [13].

3.2. Qualitative phytochemical analysis

Qualitative phytochemical analysis was performed in the all extracts and the results showed color blaze dark star and trailing queen have alkaloid, flavonoid, terpenoid, and phenol in all extract from different extraction method and solvent. Difference in method of extraction did not affect phytochemical compound of the extract with same extraction solvent qualitatively, but different number of spot was observed in the methanol and ethanol extract on both of P.scutellarioides varieties (Figure 3).

Table 2. Qualitative phytochemical analysis of extracts of Plectranthus scutellarioides

| Variety          | Extraction Method | Extraction Solvent | Alkaloid | Flavonoid | Terpenoid | Tannin/ Phenol |
|------------------|-------------------|--------------------|----------|-----------|-----------|----------------|
| Color blaze dark star | Maceration        | Water              | +        | +         | +         | +              |
|                   |                   | Methanol           | +        | +         | +         | +              |
|                   |                   | Ethanol            | +        | +         | +         | +              |
|                   | Soxhletation      | Methanol           | +        | +         | +         | +              |
|                   |                   | Ethanol            | +        | +         | +         | +              |
|                   | Microwave assisted| Water              | +        | +         | +         | +              |
|                   |                   | Ethanol            | +        | +         | +         | +              |
| Trailing queen    | Maceration        | Water              | +        | +         | +         | +              |
|                   |                   | Methanol           | +        | +         | +         | +              |
|                   |                   | Ethanol            | +        | +         | +         | +              |
|                   | Soxhletation      | Methanol           | +        | +         | +         | +              |
|                   |                   | Ethanol            | +        | +         | +         | +              |
|                   | Microwave assisted| Water              | +        | +         | +         | +              |
|                   |                   | Ethanol            | +        | +         | +         | +              |
Figure 3. TLC profile after sprayed with H$_2$SO$_4$ 10%, A) Methanol extract of color blaze dark star variety; B) Methanol extract of trailing queen variety; C) Ethanol extract of color blaze dark star variety; D) Ethanol extract of trailing queen variety

Trailing queen variety has 9 spots in ethanol extract, while color blaze dark star variety has 7 spots. In methanol extract, trailing queen variety has 8 spots and color blaze dark star variety has 7 spots. Amount of second metabolites can be different due to genetic factors or environmental conditions [14], as example, production of flavonoid in plant varies in order to fulfil their role as protection against UV-A and UV-B [15,16].

3.3. Preliminary assessment of toxicity

Brine shrimp lethality bioassay was used as preliminary assessment of toxicity all extract in both varieties of Plectranthus scutellarioides. LC$_{50}$ <1000 µg/ml was categorized as toxic, while LC$_{50}$ >1000 µg/ml categorized as non toxic [17]. Water extract showed non-toxic activity, except extract which was obtained by soxhletation. As traditional medicines, boiling the leaves of color blaze dark star variety in certain concentration may be poses some threat of acute toxicity. Extract ethanol has the highest toxicity than methanol and water extract by soxhletation and microwave method in both varieties of Plectranthus scutellarioides, while in maceration method, methanol extract was the highest. This results can be used to obtain anticancer isolated compound with optimization extraction method and solvent. Color blaze dark star and trailing queen variety showed the highest toxicity in ethanol extract with microwave assisted extraction (39 µg/ml; 41 µg/ml respectively). Soxhletation extracts in trailing queen are more toxic than color blaze dark star variety, it may be due to %yield of extract which was produce more in trailing queen. In crude extract form, the compound can have synergistic effect that mixed in fixed proportion to give toxic activity. Color blaze dark star variety more toxic in microwave assisted extraction and maceration method than trailing queen. But, in the future, trailing queen variety might be a potential plant to study bioactive isolated compound because it’s toxicity not so far from color blaze dark star variety and has more amounts of compound.
Table 3. Lethal concentration of extract of two varieties of *Plectranthus scutellarioides*

| Variety           | Extraction Method | Extraction Solvent | %Mortality depend on concentration (ppm) | LC\(_{50}\) (μg/ml) |
|-------------------|-------------------|--------------------|-----------------------------------------|---------------------|
|                   |                   |                    | 10          | 100           | 1000           |                   |
| Color blaze dark star | Maceration        | Water              | 3.33        | 23.33         | 16.67          | 47241             |
|                   |                   | Methanol           | 13.33       | 36.67         | 1000           | 55                |
|                   |                   | Ethanol            | 23.33       | 26.67         | 90.00          | 105               |
|                   | Soxhletation      | Water              | 10.00       | 10.00         | 63.33          | 820               |
|                   |                   | Methanol           | 13.33       | 23.33         | 93.33          | 122               |
|                   |                   | Ethanol            | 16.67       | 40.00         | 90.00          | 96                |
|                   | Microwave assisted | Water              | 10.00       | 16.67         | 43.33          | 2801              |
|                   | extraction        | Methanol           | 6.67        | 56.67         | 96.67          | 79                |
| Trailing queen    | Maceration        | Water              | 10.00       | 23.33         | 30.00          | 16566             |
|                   |                   | Methanol           | 3.33        | 16.67         | 93.33          | 182               |
|                   |                   | Ethanol            | 10.00       | 16.67         | 83.33          | 240               |
|                   | Soxhletation      | Water              | 0.00        | 13.33         | 50.00          | 653               |
|                   |                   | Methanol           | 3.33        | 56.67         | 96.67          | 93                |
|                   |                   | Ethanol            | 23.33       | 50.00         | 90.00          | 66                |
|                   | Microwave assisted | Water              | 10.00       | 16.67         | 33.33          | 12413             |
|                   | extraction        | Methanol           | 0.00        | 36.67         | 96.67          | 219               |
|                   |                   | Ethanol            | 36.67       | 50.00         | 90.00          | 41                |

4. Conclusion
Microwave assisted extraction is an extraction method that can produce the highest %yield of extract in color blaze dark star variety, and soxhletation method in trailing queen variety. Both of them using water as extraction solvent. The more polar solvents that are used, the more extract can be produce as well as bioactive compounds. All of extract from extraction method and solvent have alkaloid, flavonoid, phenol, tannin, and terpenoid, although there is difference in number of compound that observed in color blaze dark star and trailing queen variety. Trailing queen variety has more compound than color blaze dark star variety. Toxicity assay showed color blaze dark star variety was more toxic than trailing queen variety.

5. Acknowledgements
The authors are thankful to Trisakti Abubakar for the assistance in collecting leaves sample, all staff in the Pharmacognosy-Phytochemistry laboratory and Biofarmaka laboratory, Hasanuddin University, for facilities and support, also to Ministry of Research, Technology and Higher Education of the Republic of Indonesia for the sponsor so this study can be done.

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