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# Phytochemical Screening of Essential Oil Vetiver Roots *(Vetiveria zizanioides (L.) Nash)* From Garut District

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## Abstract

Indonesia is uniquely positioned among the countries in the world where most of the land consists of tropical forests. Besides Haiti and the Bourbon Pacific, Indonesia is among the three largest producers of vetiver oil in the world. Garut Regency is the center of vetiver oil production in Indonesia. Vetiver root (Vetiveria zizanoides L. Nash), is known to contain terpenoid compounds that have antioxidant bioactivity, especially β-vetivena, β-vetivenon, and α-vetivenon compounds. Phytochemical screening is a way to determine the content of secondary metabolites in a natural material. This study aims to determine the content of secondary metabolites in vetiver essential oil from Garut Regency. This research is qualitative in nature using a descriptive design of laboratory experiment method. The vetiver essential oil produced by the steam distillation method was tested for secondary metabolite content using thin-layer chromatography by looking at the stains that appeared on the chromatogram and the Rf value of the tested compounds. Secondary metabolite compounds tested include alkaloids, flavonoids, tannins, steroids, terpenoids, and saponins. Vetiver essential oil contains steroid compounds characterized by the onset of blue stains with an Rf of 0.9 and terpenoid compounds characterized by the onset of bright blue stains with an Rf of 0.5.

**Keywords:** Essential Oil, Vetiver, Thin Layer Chromatography

## INTRODUCTION

Indonesia is a global region that is largely made up of tropical forests. Indonesia has the region's third-largest tropical forest area after Brazil and the Democratic Republic of Congo, and these forests contain unique biological resources. Indonesia is also a tropical region with high humid temperatures that create conditions for the growth of various plant species (Mainawati et al., 2019).

Vetiver is originally from India, where it is a tall, tufted, and flavorful grass. The stems are straight, the leaves are long and narrow, and the roots are substantial. Throughout history, vetiver has been used as a perfume and traditional medicine ingredient because the volatile oil contained

in vetiver has biological functions (Wibowo et al., 2019). Besides Haiti and Bourbon Pacific, Indonesia is considered among the three largest vetiver oil producers in the world. Garut Regency is the production center of vetiver oil in Indonesia. Field studies in Randu Kurung Village, Samarang Subdistrict showed that Kamojang area of Samarang Subdistrict and Darajat area of Pasirwangi Subdistrict are the main sources of vetiver essential oil in Garut Regency (Nuramalina, et al., 2019).

According to the world of health, essential oils are used as a treatment and body care which is the current trend of "back to nature" such as vetiver oil. In some studies on compounds contained in vetiver (*Vetiveria zizanoides* L. Nash), it is known to contain terpenoid compounds that have antioxidant bioactivity, especially β-vetivena, β-vetivenon, and α-vetivenon. Recent studies have shown that vetiver has various biological benefits, including anti-inflammatory, antioxidant, antifungal, antibacterial, antidepressant, and antihyperglycemic (Zahoor et al., 2019).

Secondary metabolites are compounds found in plants whose existence is not often known, but these compounds are very useful to be used as a source of the plant's medication. Secondary metabolite compounds in plants that have efficacy as drugs include flavonoids, alkaloids, terpenoids, tannins, saponins, and steroids (Kafied et al., 2023). Phytochemical screening is an important first step that provides an overview of the content of certain compounds in natural materials to be researched.

## RESEARCH METHOD

Research is qualitative in design using descriptive laboratory experiment method. The sample used in this study is vetiver essential oil produced from PT Van Aroma.

### Determination of Plants

Determinations of plants were carried out at the Padjajaran University Laboratory which aims to determine the character of vetiver plants (*Vetiveria zizanioides* (L.) Nash) used in research.

### Research Procedure

### Identification of Alkaloid Compounds

Phase motion consists of ethyl acetate, methanol, and water in the ratio (100:13.5:10) saturated in the chamber. In the chromatogram, vetiver essential oil was bottled and then inserted into the chamber containing eluent. After finishing the elution process, the chromatogram was dried and observed under UV 254 nm and UV 366 nm. Then measured and measured the propagation distance of each spot that arises and the phase of motion from the bottling point so that the Rf value is obtained. The reagent spray for alkaloid compounds is Dragendorff reagent the formation of a brown or orange color

indicates the presence of alkaloid compounds (Hanani, 2015).

**Identification of Flavonoid Compounds**

The phase consisted of n-butanol, acetic acid, and water in the ratio (4:1:5) which was saturated in the chamber. A chromatogram was photographed with vetiver essential oil and then inserted into the chamber containing the eluent. However, after the elution process was completed, the chromatogram was dried and observed under UV 254 nm and UV 366 nm. Then measured and quantified the propagation distance of each spot that arises and the mobile phase from the point of bottling so that the Rf value is obtained. The spray reagent for flavonoid compounds is ammonia, the onset of incentive yellow stains indicates the presence of flavonoid compounds. The yellow stain caused by ammonia vapor will disappear gradually when the ammonia evaporates (Hanani, 2015).

**Identification of Tannin Compounds**

Phase motion consists of methanol and water in the ratio (6:4) saturated in the chamber. The chromatogram was photographed with vetiver essential oil and then inserted into the chamber containing the eluent. After the elution process was completed, the chromatogram was dried and observed under UV 254 nm and UV 366 nm. Then measured and noted the propagation distance of each spot that arises and the mobile phase from the point of bottling so that the Rf value is obtained. Reagent spray for tannin compounds is with FeCl3 5% the emergence of a black stain indicates the compound tannin (Banu & Nagarajan, 2014).

**Identification of Steroid Compounds**

Phase motion consists of chloroform and methanol in the ratio (9:1) saturated in the chamber. On the chromatogram, vetiver essential oil was bottled and then inserted into the chamber containing the eluent. After the elution process was complete, the chromatogram was dried and observed under UV 254 nm and UV 366 nm. Then measure and record the propagation distance of each spot that arises and the mobile phase from the point of bottling so that the Rf value is obtained. The reagent spray for steroid compounds is Lieberman Burchard reagent heated at $105^0$C for 5 minutes, the formation of blue-green stains indicates the presence of steroid compounds (La Jawa et al., 2020).

**Identification of Terpenoid Compounds**

Phase motion consists of n-hexane and chloroform in the ratio (2:7) saturated in the chamber. A chromatogram with vetiver essential oil was inserted into the chamber containing eluent. After the elution process was completed, the chromatogram was dried and observed under UV 254 nm and UV 366 nm. Then measured and recorded the propagation distance of each

spot that arises and the mobile phase from the point of bottling so that the Rf value is obtained. The spray reagent for terpenoid compounds is Lieberman Burchard reagent heated at $105^0$C for 5 minutes the formation of blue-violet or red-violet color indicates the presence of terpenoid compounds (Ramadhan et al, 2023).

**Identification of Saponin Compounds**

Phase motion consists of chloroform, methanol, and water in the ratio (13:7:2) saturated in the chamber. The chromatogram was photographed with vetiver essential oil and then inserted into the chamber containing eluent. After the elution process was complete, the chromatogram was dried and observed under UV 254 nm and UV 366 nm. Then measured and recorded the retention distance of each spot that arises and the mobile phase from the bottling point so that the Rf value is obtained. The spray reagent for saponin compounds is Lieberman Burchard reagent heated at 110 degrees Celsius for 10 minutes the formation of green, yellow, or red color indicates the presence of saponin compounds (Sahabur, 2019). The test was carried out triplo (three repetitions).

## RESEARCH RESULT AND DISCUSSION

## RESULT

**Plant Determination Results**

According to the results of the determination carried out at the Jatinangor Herbarium, Biosystematics and Molecular Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjajaran University, the plants used in this study are true vetiver with the scientific name *Chrysopogon zizanioides* (L.) Roberty synonym *Vetiveria zizanioides* (L.) Nash which comes from the *Poaceae* family.

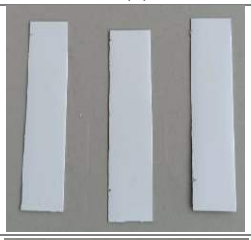
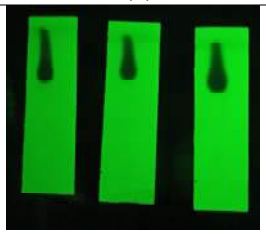
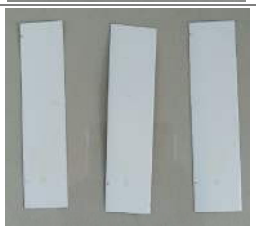
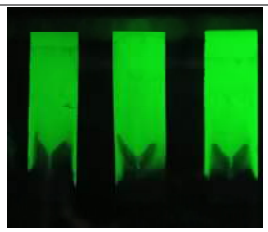
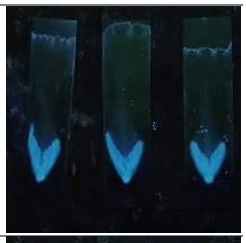
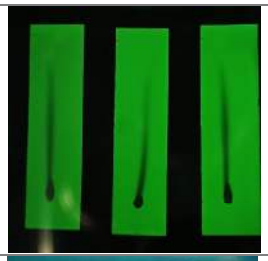
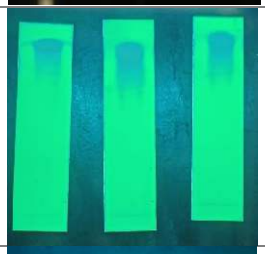
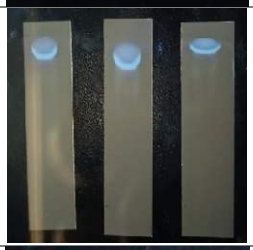
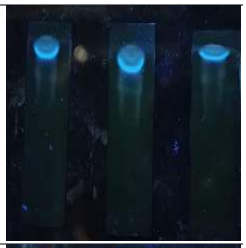
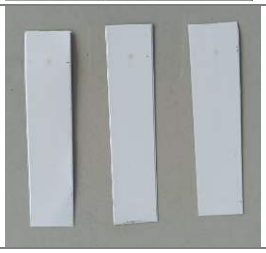
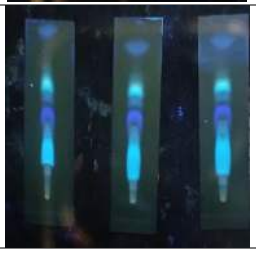
**Table 1.** Secondary metabolite compound content of vetiver essential oil

| Compound | Eluent | Reagent | Positive Reaction | Result |
|---|---|---|---|---|
| Alkaloid | Atil Aceta: Metall:Air (100:13,5:10) | Dragendroff | The formation of a brown or orange color | Negative |
| Flavonoid | N-butanol: acetic acid:water (4:1:5) | Ammonia | The onset of yellow stains on incentives | Negative |
| Tannins | Methanol: water (6:4) | FeCl 5% | The appearance of black stains | Negative |
| Steroids | Chloroform: methanol (9:1) | Lieberman Burchard | The appearance of green-blue stains | Positive |
| Terpenoid | n-hexane: chloroform (2:7) | Lieberman Burchard | The formation of a blue-violet or red-violet color | Positive |

| | | | | |
|---|---|---|---|---|
| Saponins | Chloroform: methanol: water (13:7:2) | Lieberman Burchard | Formation of green, yellow, or red | Negative |

**Table 2.** Rf value of vetiver oil elution results with phase

| Compound | Rf |
|---|---|
| Terpenoid | 0,54 |
| Steroids | 0,9 |

| Compound | (a) | (b) | (c) | (d) |
|---|---|---|---|---|
| Alkaloid |  |  |  |  |
| Flavonoid |  |  |  |  |
| Tannins |  |  |  |  |
| Steroids |  |  |  |  |
| Terpenoid |  |  |  |  |

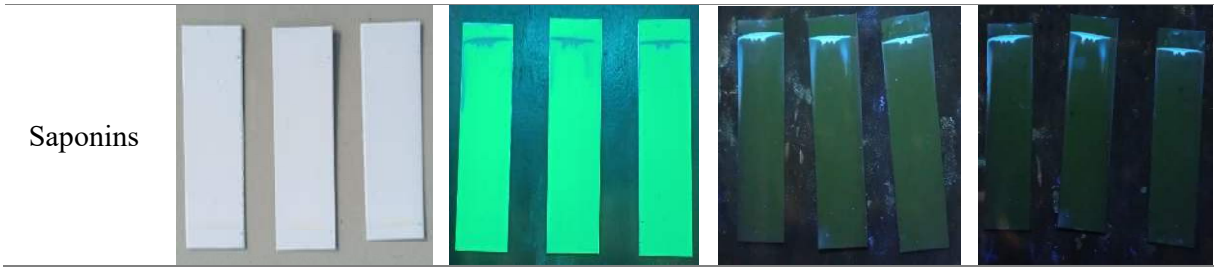



**Figure 1.** KLT results of secondary metabolite compounds. The results were observed in (a) visible light (b) UV light 254 nm (c) UV light 366 nm (d) UV light 366 nm after being sprayed with color reagent

## DISCUSSION

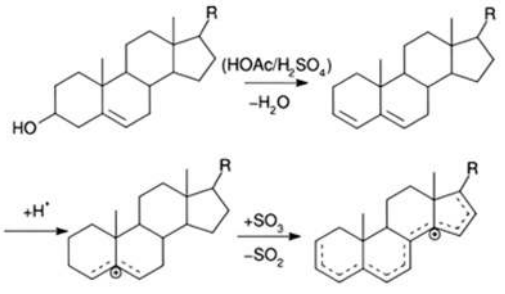
In this research, the initial stage was plant determination. The plants used in this study were vetiver plants obtained from Sukakarya Village, Samarang District, Garut Regency. The results of the determination carried out at Herbarium Jatinangor, Biosystematics and Molecular Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjajaran University with determination letter number 323/LBM/IT/II/2024 show that the plants used in this study are true vetiver with the scientific name *Chrysopogon zizanioides* (L.) Roberty synonym *Vetiveria zizanioides* (L.) Nash which comes from the *Poaceae* family.

Furthermore, the next stage is the identification of secondary metabolite compounds in vetiver essential oil using Thin layer chromatography. Thin Layer Chromatography (KLT) is a method of separating components based on differences in the level of interaction between the two phases of the separating material. KLT is a method of separating components based on differences in the level of interaction between two separating phases. KLT can be used to determine the compounds contained in the mixture qualitatively by comparing the Rf of the standard with the Rf of the sample. Identification of secondary metabolite compounds by KLT uses silica gel 254 nm as a polar stationary phase where the plate is cut to a size of 7x2 cm then activated by means of warming in the oven at 120˚C for 15 minutes. As for the solution phase, various eluents are used which have different polarity properties. Vetiver essential oil is bottled on the activated plate and then inserted into the chamber containing the saturated eluent. Spotted plates were identified under UV light 254 nm and 366 nm after complete elution. Testing using thin layer chromatography was carried out on 6 compounds, namely alkaloids, flavonoids, tannins, steroids, terpenoids, and saponins. Analysis of the first test is the identification

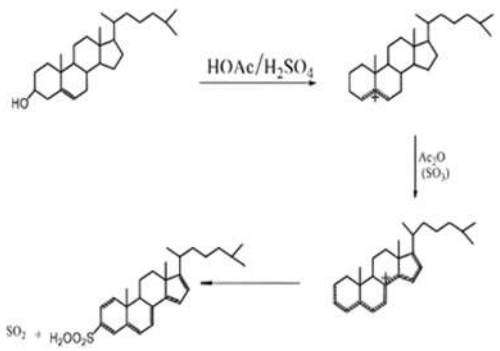
of alkaloid compounds with eluent ethyl acetate: methanol: water (100: 13.5: 10) after the plate is sprayed with dragendroff reagent and observed under UV light 366 nm resulting in blue color while positive alkaloid if formed brown or orange color so that vetiver essential oil does not contain alkaloids. The second is the identification of flavonoid compounds with the eluent n-butanol: acetic acid: water (4: 1: 5) after the plate is given ammonia vapor and observed under UV light 366 nm resulting in a light blue color while positively to flavonoids if an incentive yellow stain is formed so that the essential oil of vetiver does not contain flavonoids. Furthermore, the third test is the identification of tannin compounds with methanol: water (6:4) eluent after the plate is sprayed with FeCl 5% and observed under UV 366 nm produces a blue color while positively contains flavonoids if a black stain is formed so that vetiver essential oil does not contain tannin. The fourth test is the identification of steroid compounds with chloroform eluent: methanol (9: 1) after the plate is sprayed with Lieberman Burchard reagent and observed under UV light 366 nm, resulting in a blue stain with an Rf value of 0.9. Positive contains steroids if a blue-green stain is formed. According to research by Pratiwi et al, the Rf value of steroid standards ranges from 0.57-0.97. Thus, the Rf value of the sample falls within the range of standard Rf values of steroid compounds, so it can be said that vetiver essential oil is positive for steroids. Identification of terpenoid compounds with n-hexane: chloroform (2:7) eluent after the plate was sprayed with Lieberman Burchard reagent and observed under UV light 366 nm produced a blue stain with an Rf value of 0.54. Positively containing steroids if a blue-violet or red-violet stain is formed. According to research published by Taupik et al, the standard Rf value of terpenoid compounds ranges from 0.2-0.8. Thus, the Rf value of the sample is included in the range of standard Rf values of terpenoid compounds, so it can be said that vetiver essential oil positively contains terpenoids. The last test is the identification of saponin compounds with eluent chloroform: methanol: water (13:7:2) after the plate is sprayed with Lieberman Burchard reagent and observed under UV light 366 nm produces a light blue color. Meanwhile, it is positive for saponins if a green, yellow, or red color is formed so that vetiver essential oil does not contain saponins.

In research published by Tutik with the title "Effectiveness Test of Ethanol Extract of Fragrant Root (*Vetiveria zizanoides* L.) as an Antifeedant Against Cabbage Pest *(Plutella xylostella*)" the vetiver used comes from Yogyakarta contains secondary metabolite compounds, namely terpenoid compounds. While, the

results of this study show that vetiver essential oil contains secondary metabolite compounds, namely steroid and terpenoid compounds. Steroid and terpenoid tests use Lieberman Burchard spray reagent. Lieberman Burchard reagent is a mixture of anhydrous acetic acid and concentrated $H_2SO_4$. When the KLT plate is sprayed with Lieberman Burchard reagent, the anhydrous acetic acid reacts with the acid so that the C atom on the anhydrous forms a carboxylation. In steroid compounds, the formation of this carboxyation along with the release of $H_2O$ and its electrons, results in double bonds moving. The formation of carboxy causes electrophilic addition followed by the release of hydrogen, resulting in a green-blue stain (Nurjannah et al., 2022). The reaction equation of the steroid test is as follows:




The formed carboxylation reacts with the O atom in the -OH group in the terpenoid compound to produce a blue-violet or red-violet stain (Takaeb & Leo, 2023). The reaction equation of the terpenoid test is as follows:




Discoloration formed is based on the ability of steroid and terpenoid compounds to form colors by $H_2SO_4$ in anhydrous acetic acid solution (Takaeb & Leo, 2023). The results of this study are different from the results of previous studies, the difference in these results can be caused by several factors including differences in the area where the sample grows, each area with different altitudes will produce different temperatures. This causes a series of metabolic processes in plants to be disrupted, so that the compounds produced from the process will be different in each region (Katuuk et al., 2018). Then it can also be caused by the method or method of treatment, where in the previous study the sample used vetiver ethanol extract while in this study the sample used vetiver essential oil so the method is different, in the previous study used a cold extraction method, namely maceration while in this study using the distillation method. Then it can also be caused by differences in the eluent used so that it can affect the number of interested components (Usman & Muin, 2023).

## CONCLUSION

The essential oil of vetiver contains secondary metabolite compounds, namely steroid compounds characterized by the discovery of blue stains with Rf of 0.9 and terpenoid compounds characterized by the presence of bright blue stains with Rf of 0.54. Suggestions in this study need further research to test the antibacterial activity of vetiver essential oil (*Vetiveria zizanioides* (L.) Nash) from Garut Regency.

## REFERENCE


Banu, R.H., dan Nagarajan, N. (2014). TCL and HPTLC Fingerprinting pf leaf Extracts of Wedelia Chinensis (Osbeck) Merrill. *Journal of Pharmacognosy an Phytochemistry*, 2(6):29-23.

Hanani, E. (2015). Analisis Fitokimia. Penerbit Buku Kedokteran EGC.

Katuuk, R. H. H., Wanget, S. A., & Tumewu, P. (2018). The Effect Of Differences In Site Height On The Content Of Secondary Metabolites Of Babadotan Weeds (Ageratum conyzoides L.).

La Jawa, E. O., Sawiji, R. T., Yuliawati, A.N. (2020). Skrining Fitokimia Dan Analisis Kromatografi Lapis Tipis Ekstrak Etanol Kulit Buah Naga Merah (Hylocerues polyrhizus). *Indonesian Journal of Pharmacy and Natural Product*, 3(1):48-49.

Mainawati, D., Brahmana, E. M., dan Mubarrak, J. (2019). Uji Kandungan Metabolit Sekunder Tumbuhan Obat yang Terdapat di Kecamatan Rambah Samo Kabupaten Rokan Hulu. Universitas Pasir Pangaraian.

Nuramalina, P. W., Kiki M. Y., dan Kodir R. A. (2019). Karakterisasi Akar Wangi (*Vetiveria zizanioides* (L.) Nash) yang Ditanam di Dua Daerah Berbeda di Kawasan Kabupaten Garut. Universitas Islam Bandung.

Nurjannah, I., Ayu, B., Mustariani, A., & Suryani, N. (2022). Skrining Fitokimia Dan Uji Antibakteri Ekstrak Kombinasi Daun Jeruk Purut (*Citrus hystrix*) Dan Kelor *(Moringa oleifera* L.) Sebagai Zat Aktif Pada Sabun Antibakteri. Spin, *4*(1), 23–36. https://doi.org/10.20414/spin.v4i1.4801

Pratiwi, S. A., Februyani, N., & Basith, A. (2023). Skrining dan Uji Penggolongan Fitokimia dengan Metode KLT pada Ekstrak Etanol Kemangi (Ocium basilicum L) dan Sereh Dapur (Cymbopogon ciratus). Dalam *Pharmacy Medical Journal*, 6(2).

Ramadhan, A. D., Hakim, A. R., Byna, A. (2023). Identifikasi Senyawa Terpenoid dari Ekstrak Etanol Daun Karinat (*Rubusmoluccanus* L) dengan Metode Kromatografi Lapis Tipis. Jurnal Farmasi Syifa, 1(1):17-19.

Sahabur, J. T. (2019). Tinjauan Metabolit Sekunder dan Skrining Fitokimia. Universitas Islam Indonesia. Buku Ajar. 52-57.

Takaeb, M. J., & Leo, M. I. (2023). Identifikasi Metabolit Sekunder pada Sopi Kualin (SOKLIN) yang Dibuat Dengan dan Tanpa Fermentasi di Desa Kualin Nusa Tenggara Timur. *Jurnal Sains dan Edukasi Sains*, *6*(2), 111–116. https://doi.org/10.24246/juses.v6i2p111-116

Taupik, M., Suryadi, A. M. A., Kilo, J. L., Uno, W. Z. & Badjeber, S. B. (2022).

Identifikasi Senyawa Metabolit Sekunder Daun Spigelia anthelmia L. dan Uji Aktifitas Antioksidan Menggunakan Metode DPPH (1,1-Diphenyl-2-Picrylhidrazy). *Journal Syifa Sciences and Clinical Research (JSSCR)*, *4*(3). https://doi.org/10.37311/jsscr.v4i3.15927

Tutik, T. (2020). Uji Efektivitas Ekstrak Etanol Akar Wangi (*Vetiveria zizanoides L.*) Sebagai Antifeedant Terhadap Hama Kubis-Kubisan (*Plutella xylostella*). Universitas Malahayati.

Usman, Y., & Muin, R. (2023). Uji Kualitatif Dan Perhitungan Nilai Rf Senyawa Flavonoid Dari Ekstrak Daun Gulma Siam. *Journal of Pharmaceutical Science and HerbalTechnology*, 1(1).

Zahoor, S., Shahid, S., and Uroj, F. (2019). Review of Pharmacologcal Activities of *Vertiveria zizanioides* (Linn) Nash. University of Management & Technology: Pakistan.