# Characteristicsof Chemical Compound Content in Meniran Herb Extract and Miana Leave Extract Based On Phytochemical Screening and Thin Layer Chromatography

**Sesilia Rante Pakadang[1*], Jumain[2*], St. Ratnah[3*], Alfrida Monica Salasa[4*]**
[1,2,3,4]Pharmacy Department, Health Polytechnic of Makassar, Indonesia
sesilia@poltekkes-mks.ac.id

## *ABSTRACT*

*Meniran herbs and miana leaves have been scientifically proven as immunostimulant, antibacterial and antituberculosis. The pharmacological activity of herbs is determined by the content of chemical compounds. The purpose of identifying chemical compounds in meniran herb extract and miana leaf extract based on phytochemical screening and thin layer chromatography (TLC). The extraction method is by maceration using 96% ethanol as solvent. Phytochemical screening includes alkaloids, tannins, saponins, flavonoids, phenols, glycosides, steroids, terpenoids, phlobatins and anthraquinones. TLC identification uses 4 eluent compositions, namely: Chloroform: Methanol: Water = 15:6:1; Ethyl acetate : ethanol : water = 16:5:1; Benzene : ethyl acetate = 7:3 and N hexane : ethyl acetate = 8:2. Phytochemical screening results showed that Meniran herb extract contained alkaloids, tannins, saponins, flavonoids, phenols, steroids and terpenoids. Miana leaf extract contains alkaloids, tannins, saponins, flavonoids (flavones), phenols, steroids, terpenoids, phlobotanins and anthraquinones. The results of thin layer chromatography showed that the meniran herb extract identified 1 polar compound, 17 semi-polar compounds and 7 non-polar compounds. Miana leaf extract identified 1 polar compound, 21 semi-polar compounds and 17 non-polar compounds.*

***Keywords:*** *Meniran, miana, characteristics, phytochemicals, TLC*

## ABSTRAK

Herba meniran dan daun miana telah terbukti secara ilmiah sebagai imunostimulan, antibakteri dan antituberculosis. Aktivitas farmakologi herbal ditentukan oleh kandungan senyawa kimia. Tujuan melakukan identifikasi senyawa kimia dalam ekstrak herba meniran dan ekstrak daun miana berdasarkan skrining fitokimia dan kromatografi lapis tipis (KLT). Metode ekstraksi dengan cara maserasi menggunakan pelarut etanol 96%. Skrining fitokimia meliputi senyawa alkaloid, tannin, saponin, flavonoid, fenol, glikosida, steroid, terpenoid, phlobatanin dan antraquinon. Identifikasi KLT menggunakan 4 komposisi eluen yaitu : Kloroform : Metanol : Air = 15:6:1; Etil asetat : etanol : air = 16:5:1; Benzene : etil asetat = 7:3 dan N hexane : etil asetat = 8:2.. Hasil skrining fitokimia menunjukkan bahwa Ekstrak herba meniran mengandung senyawa alkaloid, tannin, saponin, flavonoid, fenol, steroid dan terpenoid. Ekstrak daun miana mengandung senyawa alkaloid, tannin, saponin, flavonoid (flavon), fenol, steroid, terpenoid, phlobotanin dan antrakuinon. Hasil kromatografi lapis tipis menunjukkan bahwa ekstrak herba meniran teridentifikasi 1 senyawa polar, 17 senyawa semi polar dan 7 senyawa non polar. Ekstrak daun miana teridentifikasi 1 senyawa polar, 21 senyawa semi polar dan 17 senyawa non polar.

**Kata kunci:** Meniran, miana, karakteristik, fitokimia, TLC

## INTRODUCTION

Meniran herb (Phylanthus niruri L) is an herb that has been widely known in Indonesia and has even provided phytopharmaceutical products from meniran extract. Miana leaves (Coleus scutellarioides (L) Benth) are popular in the people of South Sulawesi, especially the Toraja ethnicity as a cough medicine. Meniran herbs and miana leaves have been widely studied as immunostimulants in various diseases, especially tuberculosis infection (Zulkifli 2005; Pakadang, 2015).

The pharmacological mechanism of plants is largely determined by the active substances contained in a plant. Based on observations from various plants that have been carried out, it is found that the secondary metabolites of a plant are alkaloids, flavonoids, phenols, glycosides, tannins, steroids, saponins. Flavonoids are metabolites that are very popular as antioxidants and antibacterials. Research that has been published includes flavonoids which function as immunostimulants (Muthukrishnan dan

Sivakkumar, 2018). Meniran which has been produced as a stimuno phytopharmaca preparation has been identified as containing flavonoids (Kemenkes FHI, 2018).

The initial method to identify the compound content in an herb is phytochemical screening and identification by thin layer chromatography (TLC) and High Performance Liquid Chromatography (HPLC) (V., T. M. I., et al. 2020). Phytochemical evaluation based on chromatography and pharmacognostics will help to ensure the purity, safety, activity and effectiveness of medicinal plants. (Yadav et al, 2019). Phytochemical screening is a way to identify chemical compounds of plant extracts based on the color reaction of the reagents used. Thin layer chromatography (TLC) identifies the amount of chemical compounds from plant extract fractions based on the results of the separation of chemical compounds from the elution process. The results of phytochemical screening and TLC will be the identity of a plant extract fraction such as essential oils from the Coleus aromatica family Lamiaceae which have been identified to contain 26 types of chemical compounds (Weli et al, 2011). The function of TLC is to detect chemical compounds from an extract (Yahaya et al., 2018) and analyze the type and composition of the solvent used to extract simplicia (Gonzales et al., 2019). TLC can also be a reference for standardization of an extract (Issa et al., 2020).

Phytochemical screening of various plants including meniran and miana, however, the content of chemical compounds in a plant is different. This is caused by differences in the variety or growth factor in which the plant lives and the extraction method used to extract plant chemical compounds. So it is important to identify the chemical compounds in the extract fraction to relate to the pharmacological mechanisms of the plant.

## MATERIAL AND METHOD

Meniran herb simplicia was obtained from Yogyakarta and miana leaves were obtained from Tana Toraja Regency, South Sulawesi. Extraction of meniran herb simplicia and miana leaves was carried out by maceration method using 96% ethanol as solvent. The ethanol extract was further fractionated into ether extract (as the non-polar fraction) and n-butanol (as the polar fraction).

Phytochemical screening identified alkaloids, tannins, saponins, flavonoids, phenols, glycosides, steroids, terpenoids, phlobatanins and anthraquinones.

**Alkaloids**

Extract 3 ml + 5 ml HCl 1% then heated for 20 minutes. After cold filtered. 1 ml filtrate + picric acid, a cloudy precipitate or solution is formed (Enerijiofi & Isola, 2019).

Extract + NH4OH until it becomes alkaline then + 10 ml (chloroform: water = 1:1) and shaken. A layer of chloroform + 3 drops of Wagner P formed a red-brown precipitate (Hanani, 2017).

Extract + NH4OH until it becomes alkaline then + 10 ml (chloroform: water = 1:1) and shaken. Chloroform + Mayer P layer formed a white precipitate (Hanani, 2017).

**Tannins**

Extract 1 ml + 3 drops of FeCl3 a green-blue black precipitate is formed (Hanani, 2017)

**Saponins**

Extract 1 ml + 10 ml of water then shaken vigorously to form a stable foam. Add 1 drop of HCL 2 N foam does not disappear (FI ed VI, 2020)

**Flavonoids**

Extract 3 ml + 1 ml NaOH 10% formed yellow color (Enerijiofi & Isola, 2019)

Extract + HCl then + beaten Mg powder will form a yellow-orange-red-purple color (FI ed VI, 2020) Formation of orange to red color indicates the presence of flavones, red to bright red indicates flavanols, bright red to purplish red indicates flavanones (Hanani, 2017).

**Phenols**

Extract 1 ml + 2 drops of $FeCl_3$ a green-blue black precipitate is formed (Hanani, 2017)

**Glycosides**

Extract 1 ml + 10 ml H2SO4 50% then heated for 15 minutes. Next + Fehling's solution and heated until a brick red precipitate is formed (Enerijiofi & Isola, 2019).

**Steroids**

Extract 1 ml + 10 ml concentrated H2SO4 will form a reddish solution (Enerijiofi & Isola, 2019).

**Terpenoids**

Extract 1 ml + 2 drops of acetic acid + 1 drop of concentrated H2SO4 to form a purple red - blue green color (Enerijiofi & Isola, 2019).

**Phlobatannins**

Extract 1 ml + HCL 1% formed a red precipitate (Enerijiofi & Isola, 2019).

**Anthraquinones**

Extract + benzene 10 ml then filtered. Filtrate + 0.5 ml of ammonia. The mixture is shaken vigorously, a purple color will be formed in the layer phase (Enerijiofi & Isola, 2019).

The identification of the number of chemical compounds was carried out using the thin layer chromatography (TLC) method. Silica gel plate G60F254 was prepared with a size of 2 x 5 cm, activated in an oven with a heating of $105^0$C. The elution liquid (eluent solvent) used 4 compositions, namely: eluent 1, Chloroform: Methanol: Water = 15:6:1; eluent 2, Ethyl acetate : ethanol : water = 16:5:1; eluent 3, Benzene : ethyl acetate = 7:3 and eluent 4, N hexane : ethyl acetate = 8:2.

The extract fraction was spotted on a silica gel plate and then eluted in the eluent until the elution limit was determined. Observations included the number of compounds identified as colored stains with different Rf values. The Rf value is calculated by the formula: Rf = compound elution distance (cm) / eluent liquid elution distance (cm).

## RESULT AND DISCUSSION

**Table 1.** The Yield of Miana Leaf Extract and Meniran Herb Extract

| Name of Simplicia | Wet Simplicia | Dry Simplicia | Extract | Yield |
|---|---|---|---|---|
| Meniran Herb | - | 1.000 grams | 123,25 grams | 12,33% |
| Miana Leaf | 6.260 grams | 1.000 grams | 301,86 grams | 30,19% |

**Table 2.** Results of phytochemical screening of meniran herb extract (EHM) and miana leaf extract (EDM)

| No. | Identification of Compounds | Meniran Herb Extract (EHM) | Miana Leaf Extract (EDM) | Results |
|---|---|---|---|---|
| 1 | Alkaloids | cloudy solution | cloudy solution | EHM and EDM contain alkaloids |

| | | | | |
|---|---|---|---|---|
| | |  |  | |
| | Alkaloids | Brown red precipitate  | Brown red precipitate  | EHM and EDM contain alkaloids |
| | Alkaloids | White precipitate  | White precipitate  | EHM and EDM contain alkaloids |
| 2 | Tannins | Black blue precipitate  | Black blue precipitate  | EHM and EDM contain tannin |
| 3 | Saponins | Permanent foam  | Permanent foam  | EHM and EDM contain saponins |
| 4 | Flavonoids | Formation of yellow orange color  | Formation of yellow brown color  | EHM and EDM contain flavonoids |
| | Flavonoids | Yellow | Orange to Red | EHM and EDM contain flavonoids |

| | | | | |
|---|---|---|---|---|
| | |  |  | (flavones) |
| 5 | Phenols | Black blue precipitate  | Dark green precipitate  | EHM and EDM contain phenol |
| 6 | Glycosides | Brown green precipitate  | Dark green precipitate  | EHM and EDM do not contain glycosides |
| 7 | Steroids | Brown red solution  | Bright red solution  | EHM and EDM contain steroids |
| 8 | Terpenoids | Red purple solution  | Green blue solution  | EHM and EDM contain terpenoids |
| 9 | Phlobatannins | Green solution  | Red precipitate  | EHM contains phlobatanin |
| 10 | Anthraquinones | A white precipitate is formed | A purple/pink layer forms on the base | EHM contains anthraquinone |

| | | | | |
|---|---|---|---|---|
| | |  |  | |

**Table 3**. Results Identification of Chemical Compounds Based on Thin Layer Chromatography (TLC)

| Eluent | Extract | Fraction | Rf Value | Stain Color |
|---|---|---|---|---|
| Eluent 1 | Meniran Herb | Ethanol | 0,94 | Green |
| | | | 0,9 | Green |
| Kloroform : | | | 0,86 | Green |
| Metanol : Air | | | 0,28 | Yellow |
| = 15:6:1 | | | 0,16 | Yellow |
| | | | 0,1 | Brown |
| | | N butanol | - | |
| | Miana Leaf | Ethanol | 0,98 | Green |
| | | | 0,96 | coklat |
| | | | 0,84 | Yellow |
| | | | 0,8 | Yellow |
| | | | 0,72 | Yellow |
| | | | 0,68 | Green |
| | | | 0,62 | Green |
| | | | 0,2 | Yellow |
| | | | 0,12 | Yellow |
| | | N butanol | - | |
| | Quercetin | | 0,86 | Yellow |
| Eluen 2 | Meniran Herb | Ethanol | 0,88 | Green |
| | | | 0,8 | Green |
| Etil asetat : | | | 0,6 | Yellow |
| etanol : air = | | | 0,56 | Brown |
| 16:5:1 | | N butanol | 0,9 | Yellow |
| | Miana Leaf | Ethanol | 0,94 | Green |
| | | | 0,8 | Brown |
| | | | 0,28 | Brown |
| | | N butanol | 0,9 | Yellow |
| | Quercetin | | 0,88 | Brown |
| Eluen 3 | Meniran Herb | Ethanol | 0,3 | Yellow |
| | | | 0,84 | Yellow |
| Benzene : etil | | | 0,66 | Yellow |
| asetat = 7:3 | | | 0,96 | Green |
| | | Ether | 0,32 | Pink |
| | | | 0,9 | Green |
| | | | 0,96 | Green |
| | Miana Leaf | Ethanol | 0,4 | Yellow |
| | | | 0,8 | Green |
| | | | 0,88 | Green |
| | | | 0,94 | Green |
| | | Ether | 0,4 | Brown |
| | | | 0,52 | Brown |
| | | | 0,62 | Brown |
| | | | 0,66 | Green |
| | | | 0,72 | Green |
| | | | 0,86 | Green |
| | | | 0,94 | Green |
| | | | 0,98 | Green |

| | | | | |
|---|---|---|---|---|
| | Quercetin | | 0,24 | Yellow |
| Eluen 4 | Meniran Herb | Ethanol | 0,94 | Yellow |
| | | | 0,76 | Yellow |
| N hexane : | | | 0,72 | Yellow |
| etil asetat = | | Ether | 0,98 | Yellow |
| 8:2 | | | 0,9 | Green |
| | | | 0,82 | Green |
| | | | 0,54 | Yellow |
| | Miana Leaf | Ethanol | 0,86 | Yellow |
| | | | 0,76 | Green |
| | | | 0,68 | Green |
| | | | 0,4 | Green |
| | | | 0,2 | Brown |
| | | Ether | 0,98 | Yellow |
| | | | 0,9 | Green |
| | | | 0,78 | Green |
| | | | 0,7 | Green |
| | | | 0,54 | Green |
| | | | 0,46 | Brown |
| | | | 0,4 | Brown |
| | | | 0,32 | Brown |
| | | | 0,2 | Brown |
| | Quercetin | | 0,88 | Brown |

Table 1 data shows that the yield of meniran herb extract is 12.33%, while the yield of miana leaf extract is 30.19%. Extraction using 96% ethanol solvent with maceration method. Based on the observation of the extract form, it was seen that the extract of the meniran herb was denser than the miana leaf extract. The nature of miana leaf extract is more hygroscopic so that it is easily suspended in CMC Sodium. The high yield allows the use of fewer natural ingredients in the preparation of doses for pharmacological activities. The amount of extract yield can be influenced by the size of the simplicia and the type of solvent used to extract the compound in the simplicia. The addition of a solubility enhancer is very influential to improve the transport properties of substances in cells. The study conducted by Aris et al. (2018) proved that the addition of supercritical carbon dioxide (SC-CO2) was able to penetrate solid particles thereby increasing the solubility and total yield of *Momordica charantia* extract. This study extracted meniran herbs and miana leaves with 96% ethanol solvent which is semipolar at room temperature with repeated remaceration processes. The solvent and the extraction temperature affect the amount of the extraction yield. Prasad et al. (2012) investigated the effect of ethanol concentration, extraction temperature and solvent ratio on simplicia and The result is that the optimal conditions that produce the optimal yield of *Brown Mango* are 54% ethanol, temperature $50^0$C and solvent/simplicia ratio 42.4 mL/g. Extraction method, proper solvent composition, co-solvent material and particle size have been proven to play an important role in the extraction process so as to produce high yields (Singh et al., 2018; Dhanani et al., 2015; Qomaliyah et al., 2019; Desmiaty et al., 2019; Li et al., 2014).

Table 2 data shows that the meniran herb extract contains alkaloids, tannins, saponins, flavonoids, phenols, steroids and terpenoids. Miana leaf extract contains alkaloids, tannins, saponins, flavonoids

(flavones), phenols, steroids, terpenoids, phlobotanins and anthraquinones. The ethanol solvent used in this study has been shown to be able to extract secondary metabolite chemical compounds from meniran herb simplicia and miana leaves. Ethanol extract is semi-polar so it is able to filter out most of the chemical compounds in plants. The results of previous research on *Coleus aromaticus* (Coleus family) also found alkaloids, carbohydrates and tannins with ethanol solvent (Bole et al., 2014). Aslam et al. (2020) found high amounts of alkaloids, flavonoids, terpenoids, saponins and phenolics when separating the ethanolic extract of *Viola odorata* leaves by high performance liquid chromatography (HPLC). Several studies have yielded similar results, namely extracting different plants with ethanol as a solvent to extract other plants also produces the same chemical compounds namely phenolics, flavonoids, carbohydrates, glycosides, tannins, steroids, alkaloids, saponins, tannins, triterpenoids (Yelwa et al., 2018; Jeba Malar et al., 2020; Dwira et al., 2020).

The data in table 3 shows that the meniran herbal extract identified 1 polar compound, 17 semi-polar compounds and 7 non-polar compounds. Miana leaf extract identified 1 polar compound, 21 semi-polar compounds and 17 non-polar compounds. The number of compounds found with the same eluent will be different for each extract. This is determined by the difference in the level of polarity of the chemical compound. The ethanol extract in this study provided a higher number of chemical compounds than the ether and n butanol fractions because the ethanol solvent was able to extract semi-polar compounds (polarity levels between non-polar to polar). The number of chemical compounds in the fraction can be identified by TLC or HPLC. Colored spots indicate the presence of chemical compounds dissolved by the eluent used. The effect of the extraction solvent has also been proven by Bhanumathi (2018) who found a higher number of compounds in the extract from the ethanol fraction than the acetone fraction when extracting *Heldigardia populifolia*. The TLC profile can provide clues to find the required chemical compounds from a plant so that the active substance filtering is more efficient. Sakti et al. (2019) has proven that the TLC profile can indicate the presence of catechin compounds in the ether fraction. Similarly, the TLC profile found several good quality flavonoid compounds from *Euphorbia thymifolia* extract (Vaid et al., 2018). Detection of alkaloids, flavonoids and phenol compounds can be detected in different extracts using TLC (Yahaya et al., 2018). The TLC method can also be used to analyze the composition of the extraction solvent to produce the optimal number of compounds as expected (Gonzales et al., 2019; Kerrouri et al., 2016). Meniran herbal extract standardization has been determined based on flavonoid content (Kemenkes FHI, 2018), so that TLC is also potentially used as a means of identification and standardization of miana leaf extract. Issa et al. (2020) have used TLC to standardize the phytochemical compounds of the roots of *Senna occidentalis*.

The success of TLC to identify chemical compounds from plant extracts easily and efficiently has made the TLC method of choice for many researchers and laboratory analysts. Phytochemical screening provides an overview of the active compound content of the extract and identification of the number and type of active compounds is efficiently traced based on TLC.

## CONCLUSION

1. The results of phytochemical screening showed that Meniran herb extract contained alkaloids, tannins, saponins, flavonoids, phenols, steroids and terpenoids. Miana leaf extract contains alkaloids, tannins, saponins, flavonoids (flavones), phenols, steroids, terpenoids, phlobotanins and anthraquinones.
2. The results of thin layer chromatography showed that the meniran herb extract identified 1 polar compound, 17 semi-polar compounds and 7 non-polar compounds. Miana leaf extract identified 1 polar compound, 21 semi-polar compounds and 17 non-polar compounds.

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