

**HASIL PENILAIAN SEJAWAT SEBIDANG ATAU PEER REVIEW
KARYA ILMIAH : PROSIDING SEMINAR INTERNASIONAL**

Judul Karya Ilmiah (Artikel)	: Characteristics of Chemical Compound Content in Meniran Herb Extract and Miana Leave Extract Based On Phytochemical Screening and Thin Layer Chromatography	
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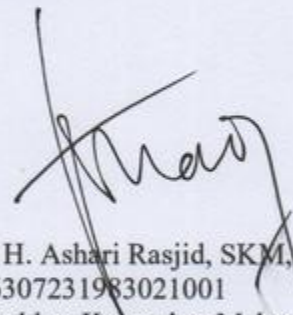
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Table of Contents

Articles

Family Satisfaction in Emergency Patient Services PSC SIGA 119 Kabupaten Mamuju <i>Edi Purnomo, Andi Nasir, Firdaus Syafii</i>	PDF
Family Is A Supporting Factor Of Adolescent Resilience In Facing The Covid-19 Pandemic <i>Hardiyati Hardiyati, Masnaeni Ahmad, Rachmawati Rahim, Musdalifah Musdalifah</i>	PDF
Establishment Of A Covid-19 Administration (Jucovid) As A Strategic Measures For The Protection Of Family From Covid-19 Transmission; A Library Study <i>Malik Saepudin</i>	PDF
Patterns of Using Masks in Prevention of Covid-19 Transmission in Makassar, Indonesia <i>M. Askar M. Askar, Ernawati Ernawati, Wa Mina La Isa, Muhammad Nur</i>	PDF
Prevalence of Reactive HBsAg in Pregnant Women at Binanga Health Center, Mamuju Regency <i>Abbas Mahmud, Nurdiana Nurdiana, Riski Dyah Haninggar, Irmawati Irmawati</i>	PDF
Formulation And Physical Stability Test Of Celery Leaf Extract Gel (Apium graveolens L.) With Variations Concentration Of Hydroxy Propyl Methyl Cellulose And Carbopol <i>Agust Dwi Djajanti, Irene Nopi Praja Sumule, Firmansyah Firmansyah, Rusli Rusli</i>	PDF
Formulation High Fiber Cookies Using Modified Banana Flour (Musa paradisiaca) <i>Firdaus Syafii, Hasmar Fajriana</i>	PDF
Age And Occupation Related To The Event Of Dementia in The Elderly in Binanga Community Health Centers <i>Rachmawati Rahim, Irma Muslimin</i>	PDF
Overview Of The Specific Weight And Composition Of Waste In Offices (Case Study In Governor Of West Sulawesi Province Office Areas) <i>Siti Rahmah, Miftah Chairani Hairuddin</i>	PDF
Study Of The Quality Of Life On Patients With Type 2 Diabetes Mellitus <i>Alfi Syahar Yakub, Dyah Ekowatiningsih, Irma Dama Yanti</i>	PDF
Breastfeeding Education on Mother about Exclusive Breastfeeding in Mamuju Regency, West Sulawesi Province <i>Dina Mariana, Idayati Idayati, Satriani G Satriani G</i>	PDF
Efforts to Prevent Sexually Transmitted Infections (HIV/AIDS) in Wakatobi District, Southeast Sulawesi Province <i>Andi Asrina, Muhammad Ikhtiar, Fairus P. Idris</i>	PDF
UTILIZATION OF BIDARA LEAF (Ziziphus mauritiana L.) EXTRACT AS A NATURAL LARVICIDE <i>Askur Askur, Ridhayani Adiningsih, Abdul Ganning</i>	PDF
Magnesium Intake and Stunting were Associated with Obesity among Adolescent Girls <i>Sitti Patimah, Septiyanti Septiyanti, Sundari Sundari, Andi Imam Arundhana</i>	PDF
Medicines As An Alternative Therapy For Covid-19 <i>Nur Islami Fahmi, Santi Sinala, Ida Adhayanti, Sisilia Teresia Rosmala Dewi</i>	PDF
Characteristics of Chemical Compound Content in Meniran Herb Extract and Miana Leave Extract Based On Phytochemical Screening and Thin Layer Chromatography <i>Sesilia Rante Pakadang, Jumain Jumain, St Ratnah, Alfrida Monica Salasa</i>	PDF
Relationship between the Role of Health Cadres with Immunization of Tetanus Toxoid (TT) in Women of Childbearing Age in the Work Area of Mangasa Health Center of Makassar <i>Ambo Dalle, Sukriyadi Sukriyadi, Ningsih Jaya, Sukma Saini, Sudirman Sudirman</i>	PDF
Women Behavior in Nutrition Compliance for Toddlers During the COVID-19 Pandemic in Coastal Areas of Bantaeng Regency in South Sulawesi Province <i>Sri Ningsih, Erniwati M Erniwati M, Rismayanti Rismayanti, Sulastris Sulastris, Sumarni Sumarni</i>	PDF
Implementation Of Health Protocol In Prevention Of Covid-19 In Students <i>Zaki Irwan, Hasmar Fajriana, Andi Salim, Irma Muslimin</i>	PDF
Levels of Particulate Matter 2.5 (Pm2.5) on Potential Respiratory Disorders in Traders Around the Road of Sultan Alauddin Makassar City <i>Abdur Rival, Hamsir Ahmad, Rasman Rasman, Inayah Inayah, Febriyanti Febriyanti</i>	PDF
The Physical Activity of Patients Diagnosed with Diabetes Mellitus: A Basis for A Counseling Program <i>Amriati Mutmainna, Rusni Mato, Sitti Nurbaya, Dahrianis Dahrianis</i>	PDF
Comparison of Iron (Fe), Calcium (Ca) and Magnesium (Mg) Qualitative Test on Yellow and Black Raisins	PDF

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Comparison of Snacks (100% Cornmeal) and Mungbean (100%) Consumption Between Urban and Rural Areas

Ratnasari Dewi, Hendra Stevani, Nurisyah Asyhari, Tajuddin Abdullah, Djuniasti Karim, Mulyadi Mulyadi

A Study on the Social Support Approach in Overcoming Drop-Out (DO) and Multi Drug Resistant (MDR) Patients of Tuberculosis

Simunati Simunati, Sudirman Sudirman, Abd. Hady J, Andi Asrina

The Mckenzie Exercise Methods For Prevent Text Neck Syndrome Due to Gadget Overused

Desti Kurniawati

Analysis of Examination Results of Ast (Aspartate Aminotransferase) and Alt (Alanine Aminotransferase) Levels in Covid-19 Patient in RS TK II Pelamonia Makassar

Andi Favian Orvala Ruhban, Syahida Djasang, Rahman Rahman

Analysis of Factors Affect the Incidence of Chronic Energy in Pregnant Women in Puskesmas Samata Gowa

Nurul Indah Sari, Ros Rahmawati, Suriani Suriani

Evaluation Of The Phbs Program For 2018, 2019, And 2020 In Masalle Village, Enrekang Regency

Angghi Pertiwi, Muh. Ikbal Arif, Haderiah Haderiah

The Relationship Between Behavior of Hospital Officers with the Use of PPE to Prevent Nosocomial Infections in Thalia Irbam Hospital Kab. Gowa

Lutfiah Amanda Harris, Andi Ruhban, Muh. Ikbal Arif

The Effectiveness of Bobath Exercises on the Ability to Walk and Leg Spasticity of Stroke Patients

Suharto Suharto, Arpandjam'an Arpandjam'an, Abd Rahman, Suriani Suriani

Acceptance and Analysis of Protein and Carbon Content in Quinoa Flour Substituted Cookies and Dragon Fruit Flour

Lydia Fanny, Nur Indah Purnamasari

Risk Factors of Preeclampsia in RSKDIA Pertiwi Makassar

Andi Zulfaidawaty, Djuhadiah Saadong, Theresia Limbong, Indriani Indriani, Maria Sonda

Negative Correlation between the scoring prevention policy with stunting prevalence in South Sulawesi: Cross-Sectional Study

Agustian Ipa, Sirajuddin Sirajuddin, Rudy Hartono

Effect of Precipitation Time of Moringa Seed Powder (Moringa Oleifera) and Tamarind Seed (Tamarindus Indica L) as Coagulant in Reducing Bod and TSS of Domestic Wastewater

Syamsuddin S, Ashari Rasjid, Wahyuni Sahani, Rafidah Rafidah, Nur Humayrah. M.S

Formulation And Physical Quality Of Effervescent Granules Containing Rambutan (Nephelium lappaceum L) Peel Dried Extract

Arisanty Arisanty, Dwi Rachmawaty Daswi

The Effectiveness of Deep Breathing Against Blood Pressure Reduction

Agussalim Agussalim, Muhammad Asikin, I Takko Podding, M. Nasir M. Nasir, Syarifuddin Syarifuddin

Instrument of Inequality in Accessibility of Maternal and Child Health Services, for early detection of stunting: Cross-Sectional Study

Sirajuddin Sirajuddin, Trina Astuti, Ulty Desmarnita, Sitti Saharia Rowa

Acceptance and Iron Content in Amplang with Flour Spinach Substitution (Amaranthus gangeticus)

Fatmawaty Suaib, Sopia Natalia Hangin2, Adriyani Adam, Mustamin Mustamin

Content of Essential Fatty Acids in Polymeric Formula for Stunting Prevention

Hendrayati Hendrayati, Adriyani Adam, Laras Budyghifary

The Effect of God's Crown Fruit Extract and Cinnamon Extract On Decrease Total Cholesterol Levels In Rats White Male

Sainal Edi Kamal, Zulfiah Zulfiah, Rina Asrina, Herman Herman, Gerfan Patandung, Alfreds Roosevelt, Muh. Farid, Megawati Megawati, Sulfiyana H. Ambo Lau, Muhammad Taufiq Duppa, Syachriyani Syachriyani, Firmansyah Firmansyah, Agust Dwi Djajanti, Rusli Rusli

Acceptance and Content of Macro Nutritional Instant Baby Porridge Red Rice Flour and Soy Beans With Substitute of Moringa Leaf Flour

Zakaria Zakaria, Hikmawati Mas'ud, Sunarto Sunarto, Nursalim Nursalim, Nur Fajri Amalia

Literature Study of Mental Health Issues In Families (Communities) During The Covid-19 Pandemic

Bahrudin Bahrudin, Natsir Natsir, Rahman Abd, Abidin Abidin

Analysis of Kelch-Like Ech-Associated Protein (Keap 1) Receptors in Improving Physical Fitness (VO2max) of Indonesian Hajj Healthcare Personnel Candidates

Ismail Ismail, Alfi Syahar Yakub, Muhammad Basri

Impact of the Covid-19 Pandemic on Blood Glucose Management in Diabetes Mellitus Patients

Ridha Dwi Reski N, Muhammad Ardi, Ningsih Jaya, Ruslan Hasani, Sri Wahyuni Awaluddin

Different Effect of Lateral Glide Mulligan and Ventral Glide Kaltenborn on Changes in Lumbal Range of Motion and Functional Capacity on Chronic Low Back Pain

Sudaryanto Sudaryanto, Tiar Erawan, Desti Kurniawati

Effectiveness Combination Muscle Energy Technique and Strain Counterstrain Lumbar and Functional Changes to The Range of Motion in Patients With Non Specific Low Back Pain

Tiar Erawan, Sudaryanto Sudaryanto, Mar'a Nur

Nursing Strategy to Protect Post-Partum Mothers from Covid-19 at RS TK II Pelamonia

Suhartatik Suhartatik, Hasriana Hasriana, Karolina M.D

Quick Response Code Osteoarthritis Exercise to Improve Life Quality of the Elderly in Covid-19 Pandemic

Lisda Oktaviani, Andi Alya Amalia Yusuf, Fathur Rahma Bahtiar

Analysis of the Relationship of Drug Side Effects and Tuberculosis Patient's Compliance After Treating With Drug Synthesis and Herbal Medicine

Rusli Rusli, Rusdriaman Rusdriaman, Raymondus Chaliks, Rudy Hartono, Zizka Zizka, Sainal Edi Kamal, Zulfiah Zulfiah, Rina Asrina, Agust Dwi Djajanti, Ananda Ramadhani

Antioxidant Compound Profile and Total Flavonoid Levels of Ethanolic Extract 70% and 96% Cinnamon (Cinnamomum Burmannii)

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- The Construction a Model of the Community Empowerment to Prevention, Preparedness and Response Disaster Emergency** PDF
Abd Hady J, Naharia Laubo, Firdaus W Firdaus W, Suhaeb Suhaeb, Hariani Hariani
- Analysis of Levels Blood Cholinesterase and Factors of Defects of Pesticides in Farmer Spraying of Rice in the Pakalu Village Kallabirang District Bantimurung** PDF
Haderiah Haderiah, Mulyadi Mulyadi, Lataha Lataha
- A Method of Stunting Reduction Intervention Based on Community and Local Culture** PDF
Hariani Hariani, Ramlah D Ramlah D, Abd. Hady J, Rahmatiah Rahmatiah
- Analysis Affecting the Event of Low Birth Weight Babies in Aisyiyah St. Khadijah Hospital Pinrang Regency on 2020** PDF
Nur Ilmi, Ros Rahmawati, Subriah Subriah, Zulaeha A. Amdadi, Asmawati Gasma
- Knowledge of Prevention Tuberculosis Disease in Makassar City of South Sulawesi Province** PDF
Herman Herman
- Acceptability and Protein Levels of Bassang with Addition of Tempe** PDF
Suriani Rauf, Rudy Hartono, Ruhul Amin, Rizka Ramadani Yusuf
- The Comparison Between Extracts of Basil Leaves (*Ocimum sanctum*) and Papaya Leaves (*Carica papaya*) in Killing *Aedes Aegypti* Mosquitoes** PDF
Zaenab Zaenab, Wahyuni Sahani, Maulidyah Maulidyah
- The Different Effect of Hold Relax and Contrax Relax on Pain and Range of Motion in Knee Joint Osteoarthritis** PDF
Andi Halimah, Sitti Muthiah
- The Comparison Results of Examination of Platelet Counts using EDTA Blood and Sodium Citrate Using Hematology Analyzer** PDF
Artati Artati

Characteristics of Chemical Compound Content in Meniran Herb Extract and Miana Leaf Extract Based On Phytochemical Screening and Thin Layer Chromatography

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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, phlobotanin 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 antraquinon. 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 (*Phyllanthus 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 FeCl₃ 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 105⁰C. 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 R_f values. The R_f value is calculated by the formula: R_f = compound elution distance (cm) / eluent liquid elution distance (cm).


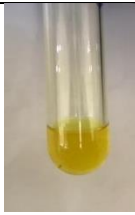



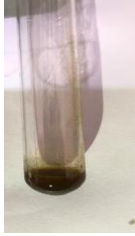






RESULT AND DISCUSSION

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

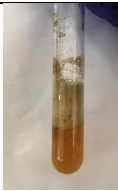











					(flavones)
5	Phenols	Black precipitate 	blue	Dark green precipitate 	EHM and EDM contain phenol
6	Glycosides	Brown precipitate 	green	Dark green precipitate 	EHM and EDM do not contain glycosides
7	Steroids	Brown red solution 		Bright red solution 	EHM and EDM contain steroids
8	Terpenoids	Red purple 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 Kloroform : Metanol : Air = 15:6:1	Meniran Herb	Ethanol	0,94	Green			
			0,9	Green			
	Miana Leaf	N butanol	0,86	Green			
			0,28	Yellow			
		Ethanol	0,16	Yellow			
			0,1	Brown			
			-				
			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						
Eluen 2 Etil asetat : etanol : air = 16:5:1	Quercetin	N butanol	-				
	Meniran Herb	Ethanol	0,86	Yellow			
			0,88	Green			
	Miana Leaf	N butanol	0,8	Green			
			0,6	Yellow			
		Ethanol	0,56	Brown			
			0,9	Yellow			
			0,94	Green			
			0,8	Brown			
			0,28	Brown			
			N butanol	0,9	Yellow		
			Eluen 3 Benzene : etil asetat = 7:3	Quercetin	N butanol	0,88	Brown
				Meniran Herb	Ethanol	0,3	Yellow
						0,84	Yellow
Miana Leaf				Ether	0,66	Yellow	
	0,96	Green					
	Ethanol	0,32		Pink			
		0,9		Green			
		0,96		Green			
		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 : etil asetat = 8:2			0,72	Yellow	
			Ether	0,98	Yellow
				0,9	Green
				0,82	Green
				0,54	Yellow
				0,86	Yellow
	0,76	Green			
	Miana Leaf	Ethanol	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°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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