Borneo Journal of Pharmacy

http://journal.umpalangkaraya.ac.id/index.php/bjop

PHYTOCHEMICAL SCREENING OF SUNFLOWER LEAF (*Helianthus annuus*) AND ANTING-ANTING (*Acalypha indica* Linn) PLANT ETHANOL EXTRACT

Khoirul Ngibad

Universitas Maarif Hasyim Latif, Sidoarjo, East Java, Indonesia

email:
khoirul_ngibad@dosen.umaha.ac.id

Keywords:
Acalypha indica Linn
Helianthus annuus
Ethanol
Phytochemical screening

Abstract

Many local plants in Indonesia were used as traditional medicines, such as sunflower (Helianthus annuus) and anting-anting (Acalypha indica Linn) plants. Both of them can be used as an antimalarial, antibacterial, antifungal, analgesic, and antihyperlipidemic. This study aims to provide an overview of the secondary metabolites groups contained in sunflower leaf and anting-anting plants for the testing of alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and sesquiterpenoids. This study included the extraction of sunflower leaves and anting-anting plants separately using the maceration method for 24 hours with 80% ethanol solvent. A shaker aided the stirring for three hours. Phytochemicals tested each extract with reagents. The results of phytochemical tests with reagents showed that 80% ethanol extract of sunflower leaves contained an alkaloid, tannin, steroid, and sesquiterpenoid compounds whereas anting-anting plants contained an alkaloid, flavonoid, and triterpenoid compounds.

DOI: https://doi.org/10.33084/bjop.v2i1.689.

INTRODUCTION

Many local plants in Indonesia were used as traditional medicine, such as sunflower plants (*Helianthus annuus*) and anting-anting (*Acalypha indica* Linn) plants. The sunflower plants are ornamental plants whereas anting-anting plants are wild plants. Sunflowers are from North America. In 1919, it was planted in Java. These ornamental plants thrive in mountainous areas and areas that have enough moisture and get plenty of direct sunlight. The tree grows in the lowlands to an altitude of 1,500 m above sea level (Dalimartha, 2009). On the other hand, the anting-anting plants grow in several areas known as the following; ceka mas (Malay), lelatang (Jakarta), rumput kokosongan (Sunda), rumput bolong-bolong (Java) and anting-anting (Malang, East Java). These plants are straightforward to maintain and require water like other plants by watering evenly or maintaining soil moisture (Arisandi & Andriani, 2008).

In Indonesia researches about bioassay from sunflower and anting-anting plants already have done. Sunflower extract with a dose of 0.358 g/kg had an analgesic effect on white male mice induced thermally (Masuara, 2016). Other research showed that chloroform and 96% ethanol extract of sunflower leaves had antimalarial activity with IC_{50} of 0.037 µg/mL (very active as antimalarials) and IC_{50} of 17.637 µg/mL (active as an antimalarial), respectively (Ngibad, 2019). On the other hand, 96% ethanol extract of anting-anting plants has also been investigated and can also be used as antibacterial (Cahyaningrum & Artini, 2018) and antifungal (Devi, 2017).

Phytochemical screening is the initial stage in phytochemical research which aims to determine the groups of secondary metabolites found in the plants studied. The phytochemical screening method was carried out by using a reagent that can produce color to show whether there was a group of secondary metabolites (Simaremare, 2014). Therefore, in this study, we will determine the content of alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and sesquiterpenoids in sunflower leaf and anting-anting plants.

MATERIAL AND METHODS

Tools and Materials

Tools used in this study included separating funnels, oven, beaker glass, blender, sieves, analytical scale,

water bath, and stirring rod. Research materials used in included sunflower leaf and anting-anting plant, ethanol, chloroform, purified water, Lieberman-Buchard reagent, Dragendorff reagent, Mayer reagent, acetic anhydride acid, sulfuric acid, chloride acid, Iron (III) and magnesium powder.

Methods

Sample Preparation

All parts of sunflower leaves and anting-anting plant was weighed, washed with clean water, cut into small pieces, the sample was heated indirectly in the hot sun. After that, the sample was blended until it was in powder form and was sifted using 60 mesh sieve size.

Extraction

Extraction was carried out by the maceration method using 80 % ethanol solvent. The second powder of the sample was treated separately. Each was weighed 100 g for the maceration process. Then, it was extracted using 80% ethanol for 24 hours with shaking for three hours using shaker at the speed of 120 rpm. Then, it was filtered into filtrate and pulp. The pulp was dried at room temperature until free from the solvent. The pulp was re-soaked with 150 mL of 80% ethanol, then filtered. Each filtrate was combined and concentrated with rotary evaporator until concentrated extract was obtained. Then, it is poured with N_2 gas to remove the remnants of the solvent and determine the yield of each crude extract (Abu *et al*., 2017).

Phytochemical Screening

Phytochemical Screening with reagents to determine the content of active compounds in 80% ethanol extract of sunflower leaves and anting-anting plants. The phytochemical screening contains the active compound by reagent test of 80% ethanol concentrated extract from sunflower leaves and anting-anting plants (Soebagio & Rusdiana, 2007).

Alkaloid Test

Sunflower leaf and anting-anting plants extracts with concentration of 10000 mg/L were entered in the test tube as much as 0.5 mL, then were added of 0.5 mL 2% HCl and the solution was divided into three test tubes. Test tube 1 solution was added by 0.5 mL of dilute acid solution for comparison, test tube 2 was added by 2-3 drops of Dragendorff reagent and test tube 3 was added by 2-3 drops of Mayer reagent. If the test tube 2 was formed an orange precipitate and in test tube 3 was formed a yellowish-white deposit, the test results were positive for alkaloids.

Flavonoid Test

Sunflower leaf and anting-anting plant extracts with concentration of 10000 mg/L were entered in the test tube as much as 0.5 mL, then were dissolved in 1 – 2 mL of hot 50% methanol. After that, were added by Mg metal and 4-5 drops of concentrated HCl. The red or orange solution formed indicated the presence of flavonoids.

Tannin Test

Sunflower leaf and anting-anting plant extracts with concentration of 10000 mg/L were entered in the test tube as much as 0.5 mL, then were dissolved in 1 – 2 mL of water and were added by 2 drops of 1% $FeCl_3$ solution. The blackish green or dark blue solution formed indicated the precence of tannins.

Saponin Test

Sunflower leaf and anting-anting plant extracts with concentration of 10000 mg/L were entered in the test tube as much as 0.5 mL, then were added by 10 mL of water while were shaked for 1 minute. If a foam was formed, 2 drops of HCl 1 N was added. The foam formed can be stable for 10 minutes with height of 1-3 cm indicated the precence of saponins.

Triterpenoid/Steroid Test

Sunflower leaf and anting-anting plant extracts with concentration of 10000 mg/L were entered in the test tube as much as 0.5 mL. Then, was dissolved in 0.5 mL chloroform, and was added with 0.5 mL anhydrous acetic acid. Then, this mixture was added by 1-2 mL H_2SO_4 concentrated through the test tube wall. If the

result obtained in the form of a brownish or violet ring on the border of the two solvents indicated the precence of triterpenoid whereas if bluish green formed indicated the presence of steroids.

Sesquiterpenoid Test

Sunflower leaf and anting-anting plant extracts with concentration of 10000 mg/L were entered in the test tube as much as 0.5 mL. Then, were added by diethyl ether and were shaken. Then, the ether layer was taken and evaporated with vaporizer cup above the water bath. The filtrate obtained was added with 10% vanillin in H_2SO_4. The blue, green and black solution formed indicated the precence of sesquiterpenoids.

RESULTS AND DISCUSSION

Extraction

The extraction method used in this study was maceration extraction. The maceration is processed to extract simplicia using a solvent with several shuffles or stirring at room temperature. The principle of maceration method is occur sufficient contact time between the solvent and the extracted material and the continuous distribution of organic solvents into plant cells which results in the breakdown of cell walls and membranes so that the active compounds of secondary metabolites in the cytoplasm will be dissolved in organic solvents (Altemimi et al., 2017). The results of maceration of sunflower leaf and anting-anting plant powder was shown in Tables 1 and 2.

Table 1. Results of maceration of sunflower leaf powder

Extract color	Sample weight (g)	Concentrated extract weight (g)	Yield (%) (W/W)
dark green	100	21.76	21.76

Table 2. Results of maceration of anting-anting plant powder

Extract color	Sample weight (g)	Concentrated extract weight (g)	Yield (%) (W/W)
green	100	23	23

Phytochemical Screening

Phytochemical screening is a qualitative test which aims to determine the active compound content of secondary metabolites in plant extracts (Masoko, 2017). The analysis was carried out by taking a sample of 80% ethanol extract from sunflower leaves and anting-anting plants separately in the test tube. Then, adding reagents according to the compounds identified. The phytochemical screening was carried out on alkaloid, flavonoids, tannins, saponins, triterpenoids, and sesquiterpenoids compound groups. The qualitative analysis of the active compounds in 80% ethanol extract of sunflower leaves and anting-anting plants was shown in Tables 3 and 4.

Table 3. Phytochemical screening results of ethanol extract from sunflower leaves

Compound groups	Reagent	Color reaction results	Description
Alkaloid	Dragendorff	Orange deposit	+
	Mayer	No precipitated	-
Flavonoid	Mg metal	Yellow	-
Tannin	1% $FeCl_3$	Blackish green	++
Saponin	Water	Foam	-
Steroid	Lieberman-Burchard	Greenish ring	++
Seskuiterpenoid	vanillin-sulfuric acid	Blue, green and black	++

Description:
Signs (++) : contain more compounds (solid color)
Signs (+) : contained compounds (colored)
Signs (-) : no contained compounds (no color formed) (Sirait, 2007)

Based on Table 3, it can be seen that the class of compounds contained in 80% ethanol crude extracts of sunflower leaves was alkaloids, tannins, steroids, and sesquiterpenoids. The 80% ethanol is polar solvent so that it can extract the polar, semipolar and nonpolar compounds which are bound in their glycosides. Based on Table 4, it can be seen that the class of compounds contained in 80% ethanol crude extract of the anting-anting plant was alkaloids, flavonoids, and triterpenoids.

Table 4. Phytochemical screening results of ethanol extract from anting-anting plants

Compound group	Reagent	Result of reaction color	Description
Alkaloid	Dragendorff	orange deposit	++
	Mayer	no sediment formation	-

Flavonoid	Mg metal	yellow-orange yellow	+
Tannin	1% $FeCl_3$	yellow	-
Saponin	Water	no foaming	-
Triterpenoid	Lieberman-Burchard	violet ring	++
Seskuiterpenoid	Vanillin-sulfuric acid	yellow	-

Description:
Signs (++) : compound more (solid color)
Signs (+) : contained compounds (colored)
Signs (-) : no contained compounds (no color formed)

Alkaloid Test

Qualitative evidence to show the presence of alkaloids can be obtained using Dragendorff and Mayer reagent. The extracts containing alkaloids will form red to orange precipitates with Dragendorff reagents and form yellowish white precipitates with Mayer reagents. The precipitates are formed because of the formation of complex compounds between metal ions from reagents and alkaloid compounds. Furthermore, the purpose of adding 2% HCl in the alkaloid test is to extract alkaloids because alkaloids are alkaline, so they are usually obtained with acid-containing solvents (Harborne, 1998).

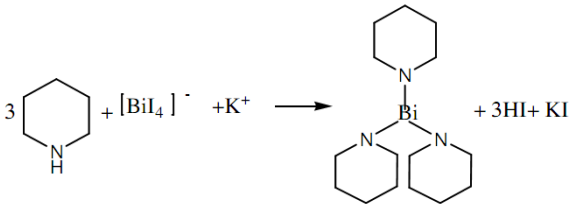
The principle for identifying alkaloids is the precipitation of alkaloids with heavy metals. Dragendorff reagent is used to detect the presence of alkaloids because these reagents contain bismuth which is high atomic heavy metal (Sirait, 2007). In the manufacture of Dragendorff reagent, bismuth nitrate is dissolved in HCl to avoid hydrolysis reactions because bismuth salts are readily hydrolyzed to form bismuthyl (BiO^+), whose result is shown in the reaction below.

$$Bi^{3+} + H_2O. \longrightarrow BiO^+ + 2H^+$$

For Bi^{3+} remained in solution, the solution was added to the acid so that the equilibrium will shift to the left. Furthermore, the Bi^{3+} of bismuth nitrate reacts with potassium iodide to form a precipitate of Bismuth (III) iodide which then dissolves in excess potassium iodide to form potassium tetraiodobismuthate. The estimated reaction that occurs in the alkaloid test is as in the following reaction as shown in Figure 1:

Reagent reaction of Dragendorff

$$Bi(NO_3)_3.5H_2O + 3KI \longrightarrow BiI_3 + 3KNO_3 + 5H_2O$$
$$BiI_3\downarrow + KI \longrightarrow [BiI_4]^- + K^+ \downarrow \text{ (with excess KI)}$$



Metal complex with alkaloid
(orange precipitates)

Figure 1. Suspected reaction between alkaloids and Dragendorff reagent

Alkaloid test result from 80% ethanol extract both sunflower leaves and anting-anting plants shows the formation of orange precipitates when react with Dragendorff reagents and the formation of yellowish white without precipitates when react with Mayer's reagents. So, both extracts contain alkaloids.

Flavonoid Test

Flavonoids are usually founded as flavonoids O-glycosides. The compound contains one hydroxyl group of flavonoids (or more) bounded to one sugar (or more) with a hemiacetal bond (reaction between aldehyde and alcohol group) which is not acid resistant (Kumar & Pandey, 2013). A glycoside will decompose over its components to produce sugar and alcohol by being hydrolyzed by acid. The alcohol produced is called aglycone while the sugar residue from flavonoid glycosides is glucose, rhamnose, galactose, and gentiobiose so that the glycosides are each called glucoside, rhamnoside, galactoside and gentiobioside (Kite & Veitch, 2009).

The addition of concentrated HCl in the flavonoid test was used to hydrolyze flavonoids into their aglycones by hydrolyzing O-glycosyl. H+ from acid will replace glycosyl because of its electrophilic nature. This reduction with concentrated Mg and HCl produces complex compounds that are red or orange in flavonol, flavanone, flavanonol, and xanthone (Tu *et al*., 2017).

The estimated reaction that occurs in the flavonoid test is as in the following reaction as shown in Figure 2:

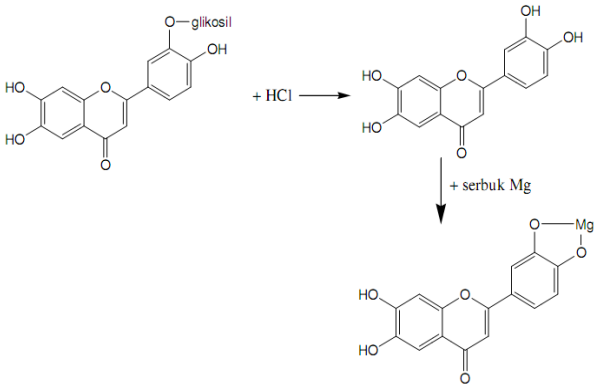


Figure 2. Suspected reaction of flavonoids with Mg powder and concentrated HCl

Flavonoid test result from 80% ethanol extract of sunflower leaves did not contain flavonoids because they do not show color formation red or orange while 80% ethanol extract of anting-anting plant produced yellow-orange color which showed the 80% ethanol extract contained flavonoids.

Tannin Test

The appearance of a blackish green color indicates the presence of tannin compounds. Phytochemical tests using $FeCl_3$ are used to determine whether a sample contains a phenol group. The presumption of the presence of a phenol group is shown in blackish green or blue ink so that if the phytochemical test with $FeCl_3$ gives a positive result, it is possible in a sample there is a phenol compound, and it is likely one of them is tannin because tannins are polyphenol compounds. Blackish green or blue ink is formed on the extract after adding it with $FeCl_3$ because the tannins will form complex compounds with $FeCl_3$ (Singh & Bag, 2013). The tannin test result of 80% ethanol extract from sunflower leaves contained catechol tannin compounds, namely the formation of blackish green color with the addition of $FeCl_3$. While 80% ethanol extract of anting-anting plant did not contain tannin compounds.

The formation of blackish green color on 80% ethanol extract of sunflower leaves is due to the formation of complex compounds between Fe and tannins. Complex compounds are formed due to the coordination of covalent bonds between ions/metal atoms and non-metallic atoms. Polyphenols are class of tannins which have many OH groups so that the O atom in the OH group can act as a coordinated ligand base on Fe^{3+}. Because OH^- is more than one, it is possible to donate both of its O atoms to Fe^{3+} (Dai & Mumper, 2010). The estimated reaction that occurs in the tannin test with $FeCl_3$ is as in the following reaction as shown in Figure 3:

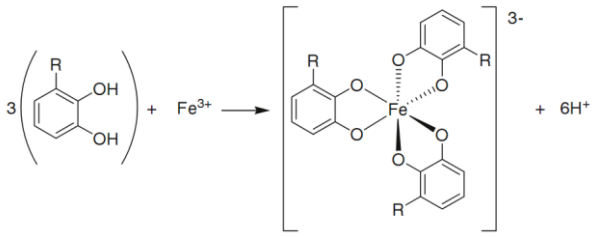


Figure 3. Octahedral geometry coordination in the iron-polyphenol complex (Perron & Brumaghim, 2009)

Triterpenoid/Steroid Test

Triterpenoid/steroid compounds will dehydrate with the addition of strong acids and form salts which provide several color reactions. The addition of chloroform is done to dissolve this compound because it dissolves well in chloroform and does not contain water molecules. Anhydrous acetic acid is used to form the acetyl derivative. If there is a water molecule in the test solution, the anhydrous acetic acid will change to acetic acid before the reaction runs and the acetyl derivative will not be formed. The most widely used test is the Lieberman-Buchard reaction (acetic-anhydride-H_2SO_4), most triterpenes and sterols give a blue-green color (Sheel *et al*., 2014).

The triterpenoid/steroid test results from 80% ethanol extract of Sunflower leaves produced greenish ring colors which indicate that the extract contained steroids. While the results of the triterpenoid test from 80% ethanol extract of the anting-anting plant produced

the color of the brownish violet ring solution which indicated that the extract contained triterpenoids.

Sesquiterpenoid Test

Sesquiterpenoids are part of a class of terpenoid compounds that are built by three isoprene units consisting of the skeleton of an acyclic or bicyclic group with a naphthalene framework. Sesquiterpenoid compounds are one of the ingredients in volatile oils that are volatile. Terpenoid compounds have quite large bioactive, including antifeedant, hormone, antimicrobial, antibiotics and toxins as regulators of plant growth and sweeteners (Yadav *et al*., 2014).

The reagent or color test used in the sesquiterpenoid analysis in this research was vanillin-sulfuric acid, which reacted as color forming. Color testing of a sesquiterpenoid compound was carried out with simplicia extract crushed in a mortar and then was added with ether solution. After that, the ether layer was taken and dried or evaporated and then was added vanillin in concentrated sulfuric acid. The occurrence of colors such as green, blue, red indicated the presence of mono and sesquiterpenoid compounds (Sirait, 2007).

Sesquiterpenoid test results from 80% ethanol extract of sunflower leaves produced colors such as blue, green and black. This shows that the extract positively contains a group of sesquiterpenoid compounds. Whereas 80% of ethanol extracts of anting-anting plants are not colored.

CONCLUSION

Based on the results of this research, it can be concluded that the results of the phytochemical screening test showed ethanol extract of sunflower leaves containing alkaloids, tannins, steroids, and sesquiterpenoids while ethanol extract of anting-anting plant containing alkaloids, flavonoids, and triterpenoid.

REFERENCES

Abu, F., Taib, C.N.M., Moklas, M.A.M., Akhir, S.M. 2017. Antioxidant Properties of Crude Extract, Partition Extract, and Fermented Medium of Dendrobium sabin Flower. *Evidence-Based Complementary and Alternative Medicine*. 2907219.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., Lightfoot, D.A. 2017. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants*. 6(4):42.

Arisandi, Y., Andriani, Y. 2008. *Khasiat Tanaman Obat*. Jakarta: Pustaka Buku Murah.

Cahyaningrum, P.L., Artini, N.P.R. 2018. Uji Aktivitas Antibakteri Serbuk Instan Kombinasi Temu Mangga (Curcuma mangga Val.) dan Daun Anting-Anting (Acalypha indica L.). *Jurnal Kesehatan Terpadu*. 2(1):1-6.

Dai, J., Mumper, R.J. 2010. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules*. 15(10):7313-7352.

Dalimartha, S. 2009. *Atlas Tumbuhan Obat Indonesia*. (6th Ed). Jakarta: Pustaka Bunda.

Devi, S. 2017. Uji Daya Hambat Infusa Tanaman Akar Kucing (Acalypha indica L.) terhadap Jamur Candida albicans. *JAFP (Jurnal Akademi Farmasi Prayoga)*. 2(1):13-18.

Harborne, A. 1998. *Phytochemical methods a guide to modern techniques of plant analysis*. Berlin: Springer Science & Business Media.

Kite, G.C., Veitch, N.C. 2009. Assigning glucose or galactose as the primary glycosidic sugar in 3-O-mono-, di- and triglycosides of kaempferol using negative ion electrospray and serial mass spectrometry. *Rapid Communications in Mass Spectrometry*. 23(19):3125-3132.

Kumar, S., Pandey, A.K. 2013. Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal*. 162750.

Masoko, P. 2017. Phytochemical Analysis, Antioxidant and Antibacterial Properties of Spilanthes mauritiana Used Traditionally in Limpopo Province, South Africa. *Journal of Evidence-Based Complementary & Alternative Medicine*. 22(4):936-943.

Masuara, D. 2016. Uji Efektivitas Ekstrak Bunga Matahari (Helianthus annuus L) sebagai Obat Analgetik secara In Vitro pada Mencit Putih Jantan (Mus muculus). *Pharmacon*. 5(1):192-196.

Ngibad, K. 2019. Efektivitas Kombinasi Ekstrak Etanol Daun Bunga Matahari (Helianthus annuus) dan Tanaman Anting-Anting (Acalypha indica Linn) sebagai Antimalaria Secara In Vivo. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy)*. 5(1):12-19.

Perron, N.R., Brumaghim, J.L. 2009. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochemistry and Biophysics*. 53(2):75-100.

Sheel, R., Nisha, K., Kumar, J. 2014. Preliminary Phytochemical Screening of Methanolic Extract of Clerodendron infortunatum. *IOSR Journal of Applied Chemistry*. 7(1):10-13.

Simaremare, E.S. 2014. Skrining fitokimia ekstrak etanol daun gatal (Laportea decumana (Roxb.) Wedd). *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*. 11(1):98-107.

Singh, K.L., Bag, G.C. 2013. Phytochemical Analysis and Determination of Total Phenolics Content in Water Extracts of Three Species of Hedychium. *International Journal of PharmTech Research*. 5(4):1516-1521.

Sirait, M. 2007. *Penuntun fitokimia dalam farmasi*. Bandung: Institut Teknologi Bandung Press.

Tu, X., Ma, S., Gao, Z., Wang, J., Huang, S., Chen, W. 2017. One-Step Extraction and Hydrolysis of Flavonoid Glycosides in Rape Bee Pollen Based on Soxhlet-Assisted Matrix Solid Phase Dispersion. *Phytochemical Analysis*. 28(6):505-511.

Yadav, N., Yadav, R., Goyal, A. 2014. Chemistry of Terpenoids. *International Journal of Pharmaceutical Sciences Review and Research*. 27(2):272-278.