



Ethnomedicinal Study and Phytochemistry Analysis of Antihypertensive and Anticholesterol Plants in Sukaharja Village, Lebak Regency, Banten Province, Indonesia

<https://tamandigital.faperta.unand.ac.id/index.php/deskripsi/tanaman/sirsak-annona-muricata-l>

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ABSTRACT

Medicinal plants are still used by people to treat many diseases, such as to lower cholesterol levels and hypertension. In Sukaharja Village, Lebak Regency, Banten Province, Indonesia, for example, the Baduy tribe still believe in practicing traditional medication. The village location is far away from the nearest health facilities. This ethnomedicinal study was conducted in the year 2023 by applying a quantitatively descriptive method. Data were collected by interviewing several respondents selected by purposive and snowball sampling methods. The list of plants was then verified by field observation, followed by species authentication, and use value (UV) calculation. Fresh samples of plants were made into simplicia and underwent phytochemical screening, including a TLC test, in a laboratory. From 50 respondents, we obtained information on 21 plant species used to lower cholesterol levels and for treating hypertension. The highest UV of plants used to lower cholesterol levels were *Peperomia pellucida* (0.56) and *Annona muricata* (0.52), while for treating hypertension were *A. muricata* (0.60), followed by *Allium sativum* (0.56). All of the plants used contained flavonoids, and selected plants examined by TLC test were revealed to be similar to quercetin.

Traditional medication practices need to be preserved, which is as important as conserving medicinal plants that are precious biodiversity in Indonesia.

Keywords: Banten, cholesterol, ethnomedicine, hypertension, phytochemistry





<https://www.gardeningknowhow.com/houseplants/peperomia/peperomia-pellucida-from-seed.htm>

INTRODUCTION

Indonesia has one of the highest biodiversity, with many unique ecosystem and high endemicy. Ethnomedicine is a branch of ethnobotany or health anthropology that study about traditional medication which passed down for generations. This also leads to an opportunity in finding new source of medicine (Saranani *et al.*, 2021). Ethnomedicinal study is among solutions to conserve high biodiversity of medicinal plants in Indonesia.

Secondary metabolites contained in plants have pharmacological effects and, thus, have been used to treat many diseases. Hypercholesterolemia and hypertension are examples of degenerative diseases that have been treated by using medicinal plants based on traditional beliefs.

Phytochemical screening needs to be performed to qualitatively identify the metabolite that might be valued as a medicine in a plant, by using few reagents to identify the presence of flavonoids, saponins, tannins, steroids, terpenoids, and alkaloids contained in medicinal plants (Agustina *et al.*, 2016).

Thin Layer Chromatography (TLC) analysis can be used to confirm the content of secondary metabolites in a plant. Maceration is among method for plant extraction that needs to be performed before running a TLC test.

Maceration is carried out by using a solvent without a heating process. The principle of the TLC test is to separate the identical compound in a mixture from the extract.

Sukaharja is one of villages located in Cikulur District, Lebak Regency of Banten Province (Fig. 1), where Baduy as a traditional tribe is originated and very famous. Most of Baduy tribe work as farmers. Baduy tribe still believes in using plants to perform traditional medication, such as to treat high cholesterol levels and hypertension. Due to the village location that is far away from the nearest health facility, Baduy tribe practices traditional medication by using plants. In Sukaharja Village, we found 25 species of medicinal plants used for treating gastritis and gout arthritis (Rindita *et al.*, 2023). The latest study was carried out in Cihanjuang Village of Banten Province, in which we found 24 plant species used for curing stomach aches (Rahmadini *et al.*, 2022). This study aimed to complete the list of plants used to treat high cholesterol levels and hypertension, including the parts of plants used, the use value of the plants, and how to consume them as traditional medicine. In addition, the secondary metabolites were examined through qualitative methods, including TLC.

MATERIALS AND METHOD

Time and Location

This research was conducted in Sukaharja Village, Cikulur District, Lebak Regency, Banten Province from March until July 2023. Phytochemical screening, including TLC analysis, was performed in the Phytochemistry Laboratory in the Faculty of Pharmacy and Science, Universitas Muhammadiyah Prof. Dr. HAMKA.





Figure 1 Description of the location and situation during research in Sukaharja Village

Tools and Materials

Fieldwork: questionnaire sheets, camera for documentation, herbarium-making tools.

Phytochemical examination: analytical scale (OHAUS), test tubes and other glasswares (IWAKI, Pyrex), aluminum foil sheets, TLC plates, filter papers, hot plate (Oxone), blender (Philips), waterbath (H-WBE-8L), chamber, spray reagents for TLC, UV light (CAMAG), distilled water, Mayer reagent, Dragendorff's reagent, Bouchardat's reagent, Wagner's reagent, 70% ethanol solvent, magnesium powder, HCl 2N, quercetin, 5% FeCl₃, citroborate, and toluene.

Population and Samples

The population of Sukaharja Village was 3,641 people. Sampling of population taken by using the Isaac and Michael formula (Sugiyono, 2013), obtained 50 respondents, including 9 key respondents. Sampling method for collecting respondents was conducted by using purposive and snowball sampling methods (Masturoh & Anggita, 2018). There were two types of respondents, i.e., (a) key respondent that is a person who is believed to have an ability for treating patients, and (b) common or general respondent which is the patient. The chosen respondent must be a native resident of Sukaharja Village with minimum age of 17 years old, must be healthy and communicative. There were also respondents who were previously chosen and included as respondents, but were not able to be interviewed when the interview was performed (Witjoro *et al.*, 2016).

Data Collection

The data were collected by using structured interview supported by validated questionnaire (Sugiyono, 2013). The ethical clearance was also registered to support data collection. Before the interview began, the respondents were provided with an informed consent. From the interview, the local names of

plants were obtained and then confirmed with field observation and morphological identification. The field identification was documented and sent to a botanist for authentication.

Use Value Analysis

The confirmed list of plants was then being analyzed by using value calculation (Gazzaneo *et al.*, 2005). Use value is a parameter which shows the important value of each plant that has been used by local people as a traditional medicine. Use value was calculated as follows:

$$UV = \Sigma U/n$$

where: UV = use value of plant species;

U = number of citations per plant species;

n = number of respondents

Phytochemical Screening

All of the plants were sampled, dried, and processed to be made as simplicia powder (Wahyuni *et al.*, 2014). The powder was then examined by phytochemical screening process by using standard method to detect flavonoids, alkaloids, phenols, terpenoids, steroids, saponins, and tannins (Hanani, 2015; Malik *et al.*, 2016; Shaikh & Patil, 2020).

TLC Test

Only selected plants were chosen to be extracted for the TLC test. The TLC test was performed to determine one metabolite that existed in all samples. Plants chosen for the TLC test was based on UV and literature study. Maceration method was used to extract metabolites from the plants. Maceration produced crude or thick extract which was then used for TLC test (Handayani, 2016). Thick extract from plant was made by macerating 5 g of plant sample, then adding 50 mL of 70% ethanol solvent with a ratio of 1: 10, followed by soaking process for 6 hours with occasional stirring

and being left for 18 hours. The macerate was filtered and separated in another bottle. Then, the remaining residue was remacerated with 50 mL of 70% ethanol for 24 hours and stirred occasionally. The macerates were put together and then concentrated in a 50 °C waterbath to obtain a thick extract.

After obtaining a thick extract, the next step was to confirm the flavonoids compound by using TLC with quercetin as a comparison (Ladeska & Maharadingga, 2019). This step used a stationary phase in the form of silica gel GF254 plates and a mobile phase of toluene : ethyl acetate with a ratio of 3 : 7 (Maulana, 2018).

The mobile phase was introduced into the chamber with the specified comparison and was then waited until the saturation process occurs using filter paper. Condensed plant extracts and the quercetin was dissolved to obtain a concentration of 1,000 ppm. The silica gel GF254 plate was marked with an upper and lower border of 1 cm each. After the saturation process occurred in the mobile phase, the silica gel GF254 plate was stained with each sample, marked by numbers, with a comparator located on the left side. The silica gel plate was then inserted into the chamber, and the migration of eluent from the lower to the upper limit was then observed. The silica gel GF254 plate was left in place until dry and sprayed using citroborate

spray reagent to qualitatively identify flavonoid group compounds. The appearance of citroborate spots is characterized by yellow, blue, or green fluorescence under UV light 366 nm and UV254 nm.

RESULTS

Total Species Found

In this study we identified 21 plant species that have been utilized by people in Sukaharja Village to treat high cholesterol levels and hypertension. These 21 plant species belonged to 17 families. Of all the species, 13 were used to lower cholesterol levels, the other 13 were used to treat hypertension, while 5 species were used for both (Table 1).

Use Value Analysis Results

A use value analysis was carried out on all species of medicinal plants used to treat high cholesterol levels and hypertension (Table 2). Leaves of soursop (*A. muricata*) and garlic bulb (*A. sativum*) showed the highest UV in treating hypertension, while the lowest UV was shown in ginger rhizome (*Zingiber officinale*). *P. pellucida* leaves and *A. muricata* leaves had the highest UV for treating high cholesterol, while *Solanum torvum* leaves had the lowest UV. Part of plants most widely used for traditional medicine was the leaves.

Table 1 Plant species, local name, family, and the utilization

No.	Species Name	Local Name	Family	Anticholesterol	Antihypertensive
1.	<i>Allium sativum</i>	Bawang putih	Amaryllidaceae	-	√
2.	<i>Annona muricata</i>	Sirsak	Annonaceae	√	√
3.	<i>Apium graveolens</i>	Seledri	Apiaceae	-	√
4.	<i>Centella asiatica</i>	Pegagan	Apiaceae	-	√
5.	<i>Polyscias scutellaria</i>	Mamangkokan	Araliaceae	√	-
6.	<i>Gymnanthemum amygdalinum</i>	Sambung nyawa	Asteraceae	√	√
7.	<i>Carica papaya</i>	Pepaya	Caricaceae	√	-
8.	<i>Artocarpus altilis</i>	Sukun	Moraceae	√	-
9.	<i>Moringa oleifera</i>	Kelor	Moringaceae	-	√
10.	<i>Syzygium polyanthum</i>	Salam	Myrtaceae	√	√
11.	<i>Pandanus amaryllifolius</i>	Pandan	Pandanaceae	√	-
12.	<i>Phyllanthus debilis</i>	Meniran	Phyllanthaceae	-	√
13.	<i>Peperomia pellucida</i>	Cacabea	Piperaceae	√	-
14.	<i>Piper betle</i>	Sirih	Piperaceae	√	-
15.	<i>Cymbopogon nardus</i>	Sereh	Poaceae	√	√
16.	<i>Gardenia jasminoides</i>	Kaca piring	Rubiaceae	-	√
17.	<i>Physalis angulata</i>	Cecenet/ciplukan	Solanaceae	√	-
18.	<i>Solanum torvum</i>	Takokak	Solanaceae	√	-
19.	<i>Phaleria macrocarpa</i>	Mahkota dewa	Thymelaeaceae	-	√
20.	<i>Curcuma zanthorrhiza</i>	Temulawak	Zingiberaceae	-	√
21.	<i>Zingiber officinale</i>	Jahe	Zingiberaceae	√	√
Total				13	13

Table 2 Part of plants and use value of antihypertensive plants and anticholesterol plants

No.	Antihypertensive plants	Part of plants	UV	Anticholesterol plants	Part of plants	UV
1.	<i>Annona muricata</i>	Leaves	0.60	<i>Peperomia pellucida</i>	Leaves	0.56
2.	<i>Allium sativum</i>	Bulb	0.55	<i>Annona muricata</i>	Leaves	0.52
3.	<i>Syzygium polyanthum</i>	Leaves	0.51	<i>Cymbopogon nardus</i>	Stem	0.50
4.	<i>Apium graveolens</i>	Leaves	0.44	<i>Zingiber officinale</i>	Rhizome	0.48
5.	<i>Moringa oleifera</i>	Leaves	0.37	<i>Syzygium polyanthum</i>	Leaves	0.42
6.	<i>Cymbopogon nardus</i>	Stem	0.31	<i>Gymnanthemum amygdalinum</i>	Leaves	0.36
7.	<i>Gardenia jasminoides</i>	Leaves	0.26	<i>Artocarpus altilis</i>	Leaves	0.34
8.	<i>Curcuma zanthorrhiza</i>	Rhizome	0.24	<i>Physalis angulata</i>	Leaves	0.30
9.	<i>Phaleria macrocarpa</i>	Leaves	0.20	<i>Piper betle</i>	Leaves	0.24
10.	<i>Centella asiatica</i>	Leaves	0.17	<i>Polyscias scutellaria</i>	Leaves	0.18
11.	<i>Phyllanthus debilis</i>	Leaves	0.11	<i>Pandanus amaryllifolius</i>	Leaves	0.14
12.	<i>Gymnanthemum amygdalinum</i>	Leaves	0.06	<i>Carica papaya</i>	Leaves	0.08
13.	<i>Zingiber officinale</i>	Rhizome	0.04	<i>Solanum torvum</i>	Leaves	0.06

Phytochemical Screening Results

Table 3 shows phytochemical screening results from the 21 plant species used. Flavonoids were the positive compounds in all samples, while other compounds showed varying results.

Table 3 Phytochemical screening of medicinal plants in Sukaharja Village

No.	Species Name	Alkaloids	Terpenoids	Saponins	Phenols	Flavonoids	Tannins	Steroids
1.	<i>Allium sativum</i>	-	+	+	-	+	-	-
2.	<i>Annona muricata</i>	+	-	-	+	+	+	+
3.	<i>Apium graveolens</i>	-	+	-	-	+	+	+
4.	<i>Centella asiatica</i>	-	+	+	-	+	+	-
5.	<i>Polyscias scutellaria</i>	-	+	+	+	+	-	+
6.	<i>Gymnanthemum amygdalinum</i>	-	+	-	+	+	+	-
7.	<i>Carica papaya</i>	+	-	-	+	+	-	+
8.	<i>Artocarpus altilis</i>	-	+	-	-	+	-	-
9.	<i>Moringa oleifera</i>	-	-	-	+	+	+	+
10.	<i>Syzygium polyanthum</i>	-	+	+	+	+	+	-
11.	<i>Pandanus amaryllifolius</i>	-	+	-	-	+	-	+
12.	<i>Phyllanthus debilis</i>	-	+	-	-	+	-	+
13.	<i>Peperomia pellucida</i>	+	-	-	+	+	-	+
14.	<i>Piper betle</i>	-	+	-	+	+	-	-
15.	<i>Cymbopogon nardus</i>	-	+	-	+	+	-	-
16.	<i>Gardenia jasminoides</i>	-	+	-	+	+	+	+
17.	<i>Physalis angulata</i>	+	+	+	-	+	-	+
18.	<i>Solanum torvum</i>	+	+	+	+	+	-	+
19.	<i>Phaleria macrocarpa</i>	-	+	-	+	+	+	+
20.	<i>Curcuma zanthorrhiza</i>	+	-	-	-	+	-	-
21.	<i>Zingiber officinale</i>	+	+	-	-	+	-	-

Notes: + = contains metabolites; - = does not contain metabolites.



TLC Analysis Results

The 5 selected plants produced green and after being sprayed with citroborate spray reagent, produced a blue color (Fig. 2) with the Rf value of quercetin of 0.66 (Table 4).

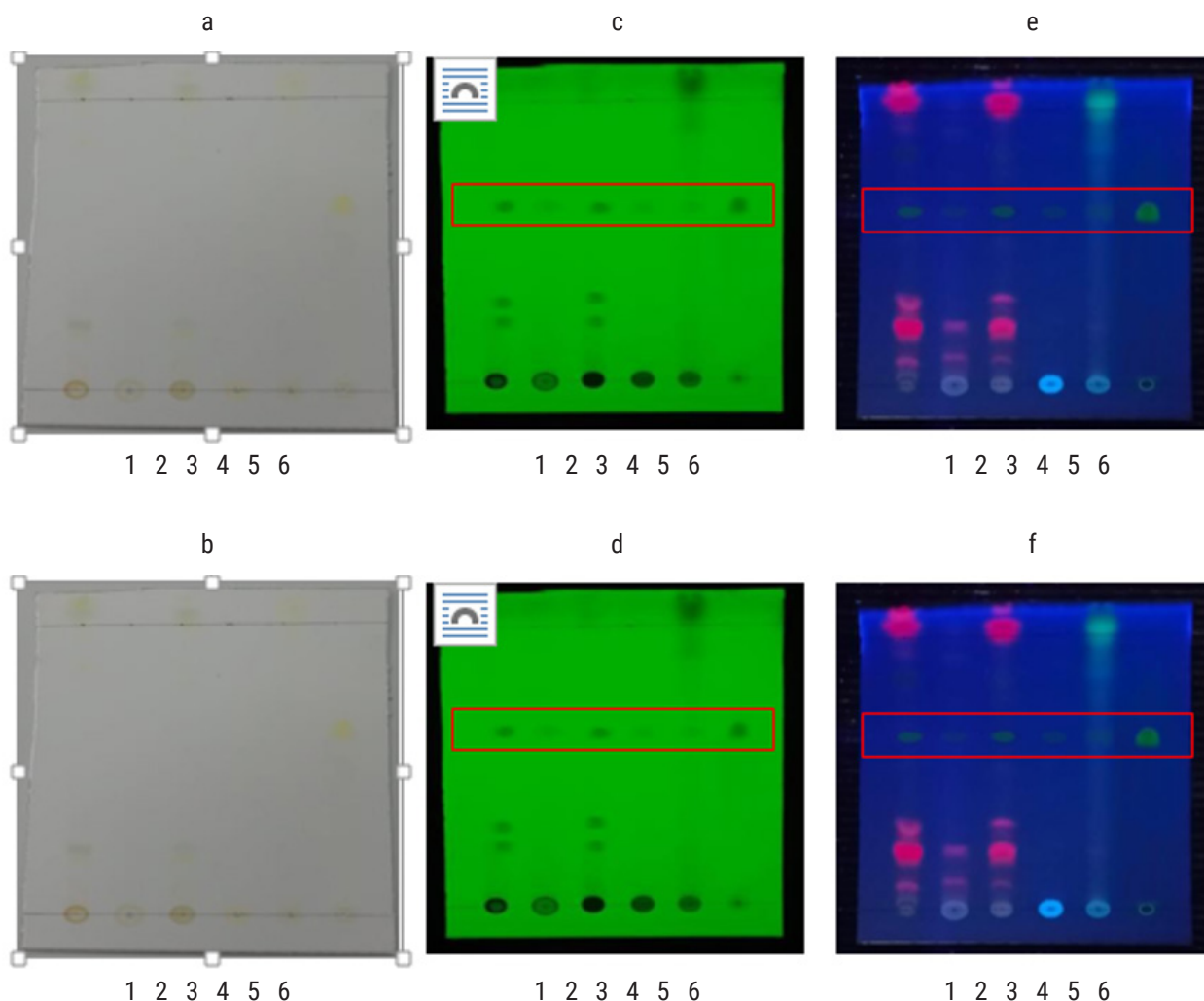


Figure 2 Results of TLC analysis

Notes: (a) Visible light before being sprayed with citroborate; (b) Visible light after being sprayed with citroborate; (c) UV254 nm before spraying with citroborate; (d) UV254 nm after spraying with citroborate; (e) UV366 nm before spraying with citroborate; (f) UV366 nm after spraying with citroborate; Spot 1: Soursop leaves; Spot 2: Garlic bulb; Spot 3: Gardenia leaves; Spot 4: Sambung nyawa leaves; Spot 5: Ginger rhizome

Table 4 The Rf value of TLC analysis result

No	Sample	Rf value	Comparator	Nilai Rf
1.	Soursop leaves (<i>Annona muricata</i>)	0.65		
2.	Garlic bulb (<i>Allium sativum</i>)	0.64		
3.	Gardenia leaves (<i>Gardenia jasminoides</i>)	0.65	Quercetin (standard)	0.66
4.	Sambung nyawa leaves (<i>Gymnanthemum amygdalinum</i>)	0.65		
5.	Ginger rhizome (<i>Zingiber officinale</i>)	0.64		



<https://www.suttons.co.uk/potatoes-onions-garlic/garlic-bulbs/autumn-planting-garlic>

DISCUSSION

The interview process with 50 respondents revealed that there were 21 medicinal plant species in Sukaharja Village used to treat hypertension and high cholesterol (Table 1). Cacabean (*P. pellucida*) and soursop (*A. muricata*) were the plants most often used by people in Sukaharja Village for cholesterol-lowering medicine (Table 2). Plant part mostly used was the leaves. In general, the medicinal concoction was made by boiling 5 to 7 leaves, then filtered, let cool, and drunk as medicine. Lemongrass stems (*Cymbopogon nardus*) also showed high UV. All of these three plants have been studied as having anticholesterol properties (Mazroatul *et al.*, 2016; Iswadi, 2019; Siagian *et al.*, 2022). As an antihypertensive, soursop leaves (*A. muricata*) had the highest UV, followed by garlic bulbs (*A. sativum*) and bay leaves (*Syzygium polyanthum*). The soursop leaves were used by residents, both as anticholesterol and

antihypertensive. According to Suhandi *et al.*, (2022), soursop has the capability to lower blood pressure in people with hypertension. Based on previous research, those three plant species are also known to have antihypertensive properties (Suhandi *et al.*, 2022; Nwokocha *et al.*, 2011; Ismail *et al.*, 2018). To treat high cholesterol, people rarely use papaya (*Carica papaya*) and takokak leaves (*S. torvum*). However, research has been conducted on the anticholesterol properties of papaya leaves (Ademuyiwa *et al.*, 2023), while for takokak, only the fruit part is known to have the capability of lowering cholesterol (Harahap *et al.*, 2022). The plant species that are least used by residents to treat hypertension are *Gymnanthemum amygdalinum* leaves and ginger rhizomes (*Z. officinale*). However, research on these two species as antihypertensive medicinal plants has been carried out (Setiani *et al.*, 2022; Aulena *et al.*, 2021).

Use Value (UV) is an index to determine plants that have the highest use value in a community. Plants with high UV indicate a high level of confidence in the properties of these plants (Yusro *et al.*, 2020). The higher the number of people use the plant, the higher the UV has, and vice versa.

The results of phytochemical screening showed that all plant samples contained flavonoids. Testing for flavonoids using concentrated Mg powder and HCl produced yellow, blue, orange, or red colors which indicated they were positive for flavonoids (Hanani, 2015). Terpenoid compounds were also detected in most of plant samples. The test for terpenoids using chloroform and concentrated H₂SO₄ produced golden yellow, purple brown and brown rings.

For the confirmation test via TLC, this flavonoid compound was chosen because it is known to function as a cholesterol-lowering agent. According to Rachmawati *et al.*, (2019), flavonoids are known to lower cholesterol, triglyceride, and LDL levels and increase HDL levels by inhibiting 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase which functions as a catalyst in the formation of cholesterol. The comparator used is quercetin because this compound is a group of flavonoids that are often found in plants and are known to have many biological activities, such as antioxidants (Illing *et al.*, 2023).

The extraction method used in this research was the maceration method. Maceration is a method of separating compounds by immersing them in an organic solvent at room temperature. From research by Maryam *et al.*, (2023), the content of flavonoid compounds in the maceration method is higher than the other three extraction methods, namely reflux, soxhletation, and percolation. The solvent used is ethanol, because it can attract more active compounds compared to other types of organic solvents. Research carried out by Riwanti *et al.*, (2020) showed that the highest total flavonoid levels were found in 70% ethanol extract. This is thought to be influenced by the polarity of the solvent associated with research which states that the highest flavonoid content is in solvents with medium polarity. The 70% ethanol is a solvent that is more polar than 96% ethanol and more non-polar than 50% ethanol, so flavonoid compounds that are polar in nature will tend to dissolve more in 70% ethanol.

The principle of the TLC test is separation based on the distribution of two phases, namely the mobile phase and stationary phase, that follow the mobile phase polarity. The stationary phase used was silica gel GF254 plates which was able to fluorescence well in UV light with a wavelength of 254 nm (Fitriandini & Jayadi, 2021). The mobile phase is a mixture of ethyl acetate and toluene in a ratio of 3 : 7 (v/v). According to Maulana,

(2018), toluene : ethyl acetate as an eluent in flavonoid compounds produces the most stable compounds. The samples were spotted on the plate, then eluted with an eluent. Stains were detected using 254 nm and 366 nm UV lamps and also using citroborate reagent. Citroborate showed a color of bright yellow at UV 366 nm for flavonoids detection. Citroborate spray reagent is a specific reagent with high sensitivity for detecting flavonoids and is specific for the ortho-dihydroxy group (Murwanto & Santosa, 2012).

CONCLUSIONS

The 50 informants used 21 plant species belonging to 17 families to treat high blood pressure and high cholesterol. The most widely used plant part was the leaves. The highest UV values for anticholesterol plants were shown by *P. pellucida* leaves and *A. muricata* (soursop) leaves, while antihypertensive plants were soursop leaves and garlic bulbs (*A. sativum*). Flavonoids was present in 5 plants having the potential to treat high blood pressure.

ACKNOWLEDGEMENT

I express my gratitude to the people in Sukaharja Village for kindly helping with this research by giving information. Thank you to Lemlitbang Universitas Muhammadiyah Prof. DR. HAMKA for the funding. Lastly, thank you to Lana Maulana for authenticating the plant specimens.

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