AGRIVITA Journal of Agricultural Science. 2018. 40(2): 212-221

AGRIVITA
Journal of Agricultural Science

Dominant Understorey Plants Producing Herbal Medicine Materials on Homegarden Agroforestry System in Menoreh Hills, Kulon Progo District

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ARTICLE INFO

Keywords:
Active compounds
Agroforestry
Homegarden
Understorey

Article History:
Received: September 14, 2015
Accepted: July 21, 2017

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ABSTRACT

Priority products in homegarden agroforestry systems are still dominated by wood. Understorey is an important component of the homegarden community, but not a priority in its management because there is not much information about its benefits. Undestorey has several benefits such as food sources, food crops and medicinal materials. Research aimed to know the diversities of understorey species, the dominant of understorey species and the content of compounds in dominant understorey in the homegarden agroforestry system in Menoreh hill, Kulon Progo District. The study sites were categorized into 3 zones based on elevation, i.e. zone 1 (< 300 m above sea level (asl)), zone 2 (301-600 m asl) and zone 3 (> 600 m asl). This research method used inventory and identification of understorey species, and identification the content of dominant understorey compound by TLC method. The results showed that there were 41 species of understorey, the dominant species in zones 1, 2 and 3 were *Curcuma mangga* Val. Zone 3 produced the highest diversity. Based on the similarity index, there was a difference between the homegarden groups in zones 1, 2 and 3. Phenols and terpenoids were identified as in *C. mangga.*

INTRODUCTION

Communities in Kulon Progo district have adopted an agroforestry system in the form of home estate, which is a combination of multi canopy that has a close relationship, consisting of a number of trees and crops around the house, the livestock may be present and may not (Nair, Gordon, & Mosquera-Losada, 2008). The homegarden in Indonesia has a variety of names, such as *karang kitri* (East and Central Java), *buruan* (Sunda), and *kinta* (Timor). The homegarden in the Javanese community has the function as a source of high nutrient foods (protein, vitamins and minerals), medicinal plants, herbs, firewood, forage crops and also timber (Kumar & Nair, 2004). So, farmer can take multiple benefits from agroforestry (Supriyadi, Hartati, Machfiroh, & Ustiatik, 2016). Classifications of agroforestry based on vegetation components, functions, levels of management input and environmental conditions and ecological suitability (Atangana, Khasa, Chang, & Degrande, 2014), agroforestry is classified into complex and simple agroforestry (Nair, 1993). A simple agroforestry is a mix of one perennial or woody plant with one or several types of crops, with the example *tumpang sari* in Perum Perhutani. Complex agroforests are special type of agroforestry system characterized by a forest-like structure and significant plant diversity in which useful trees and tree crops species attain substantial greater density, compared to natural forest, through planting, selection, and management of useful species from spontaneous regeneration (Asase & Tetteh, 2010). The example of complex agroforestry can be found in the Menoreh hills in Kulon Progo district, in which the areas are planted with two or more woody plants (sengon, mahogany, teak), estate crops (cloves, cocoa tree and coffee) and agricultural crops (*empon-empon*, cassava and herbs) (Hani, Indrajaya, Suryanto, & Budiadi, 2016).

ISSN: 0126-0537 Accredited by DIKTI Decree No: 60/E/KPT/2016

Cite this as: Widyastuti, S. M., Hadi, E. E. W., & Wahyuono, S. (2018). Dominant understorey plants producing herbal medicine materials on homegarden agroforestry system in Menoreh hills, Kulon Progo District. *AGRIVITA Journal of Agricultural Science, 40*(2), 212–221. http://doi.org/10.17503/agrivita.v40i0.657

Table 1. Agroclimatic condition at research site

Factor	Agroclimatic condition		
	Zone 1	Zone 2	Zone 3
Type of soil	Alluvial and Regosol	Latosol	Latosol
Slopes	< 2º	15-40º	> 40º
Rainfall	114.08 mm $month^{-1}$	160.92 mm $month^{-1}$	160.92 mm $month^{-1}$
Temperature	27.57 ºC	25.025 ºC	25.025 ºC

Remarks: zone 1 (< 300 m asl), zone 2 (301-600 m asl) and zone 3 (> 600 m asl)

Homegarden is a form of a complex agroforestry system which is a mixture of various elements that provides a variety of products (multi-products), has the structure and the dynamics of ecosystems that are similar to natural forests with a relatively high diversity of flora and fauna. The floral diversity is not just limited to woody plant species, but also on various types of understorey vegetation. The research problem is understorey species known as weed and not fully utilized. The research question was, whether dominant understorey species in the homegarden has potential as an antimicrobial and what an active compound content of these capabilities?

The studies on ethnobotany and local wisdom revealed that people utilize the understorey plants around them to fulfill their daily needs. The further developed studies implied that some compounds of the plants can be used for medical purpose. The use of herbal medicine with scientific responsibility can be accomplished by understanding the compounds content. It is important to provide the correct and effective utilization of the plants for herbal medicine. The purpose of this study was to determine the dominant understorey species in homegarden, knowing its potential as an antimicrobial and the amount of active compound that has the potential.

MATERIALS AND METHODS

The Site of Study

Observations were carried out in the homegarden of communities in Kulon Progo on the three different zones based on the elevation, i.e. zone 1 (< 300 m asl), zone 2 (301-600 m asl) and zone 3 (> 600 m asl) (Fig. 1). Zones 1, 2 and 3 have different agroclimate conditions shown in Table 1. Further study to determine the compound content of the dominant understories was conducted in the Laboratory of Pharmaceutical Biology, Faculty of Pharmacy Gadjah Mada University of Yogyakarta.

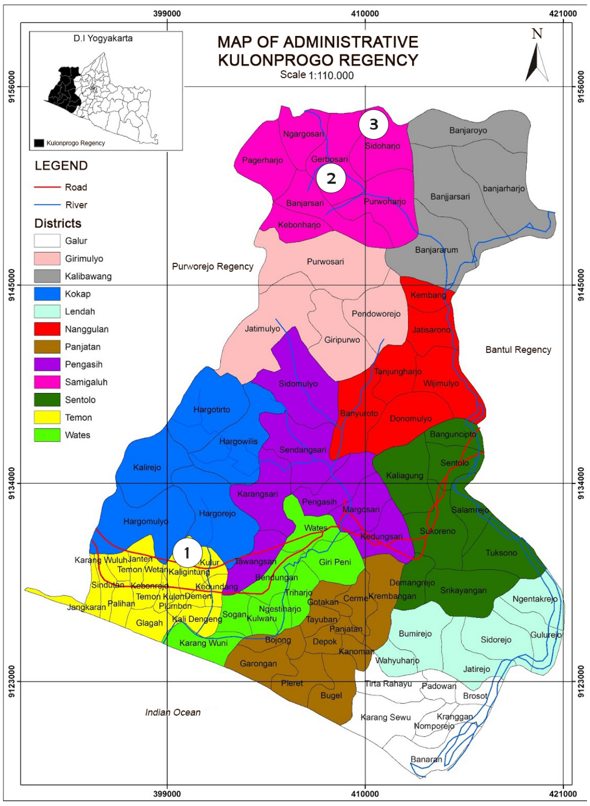


Fig. 1. Map of geographic distribution of three different zones (code 1, 2 and 3 illustrate the zones 1, 2 and 3)

Vegetation Data Collection

The plots were set up at each location with a size of 20 x 20 m to determine the main wooden trees. In this 20 x 20 m plots, another smaller plots measuring 1 x 1 m were set up to invent the understorey plants. The sampling plots were laid using regular sampling method, which were placed on the intersection between the horizontal line and the vertical line. The distance between each collection tracks was 5 m, and the distance between plots in the same tracks was 10 m. Importance Value Index (IVI) was used to specify the type of dominance against other species. IVI is the total of Relative Density (RD) and Relative Frequency (RF) (Soerianegara & Indrawan, 2005). Diversity of species (H1) assessed based on an index of species richness (R1) and evenness index (E), this is the general formula for vegetation analysis that can be used to obtain these values, using the following formula by Odum (1993). The index of species diversity is classified in the value between 0-1, if the value equal to 1 it could be interpreted that the species diversity between communities are relatively same

Selection of Understorey Plant Samples

The most dominant understorey species (highest IVI score) that being utilized as herbal medicine according to the interview with local community by using a snow ball method. Interview was conducted with sources who know and understand the use of plants as a source of herbal medicine at research site, preliminary information came from village government. The number of informant was different at each location.

Sample Preparation and Chemical Extraction

Rhizome of *C. mangga* was harvested from each site of approximately 3 kg of wet samples. Samples were rinsed under running water then cut into slices with a thickness of 2-7 mm, and dried by oven at 40-50 ºC. Once dried, the samples were grinded into fine powder and stored as dry powder samples prior to the chemical extraction.

The extraction was conducted by maceration method, which was filtrating the active compounds by suspending the powder samples in 70 % ethanol. Two hundred grams of powder samples were suspended with 70 % ethanol and then soaked in a solution of 70 % ethanol for at least 24 hours with occasionally stirred. The suspensions were filtered using a Buchner funnel. Each extract sample produced from maceration then concentrated by keping them at hot water in a water bath until all the solvent evaporated. The thickened extracts were removed into glass bottles and stored in a refrigerator before further analysis.

Identification of Compounds Content

The compounds content was identified from the extract of dominant understorey plants using Thin Layer Chromatography (TLC). The separation of chemical components was based on the principles of adsorption and partition, which is determined by the stationary phase (adsorbent) and the mobile phase (eluent). The identification of compounds group were conducted in the following procedures: (1) The thickened extracts were diluted with the mixture of chloroform:methanol, 2:1 (v/v) into a concentration of 100 mg mL^{-1}; (2) Approximately 2 mL of suspension were removed using a micropipette, then spotted on the 60 F254 silica gel plate, once dried, it was eluted with a mobile phase of n-hexane:ethyl acetate, 3:1 (v / v); (3) The mobile phase was run until the eluent reaches the end of the silica plate and then removed with a pipette for drying.

The class of active compounds can be determined by color testing, determination of solubility, the Rf numbers and the characteristics of the UV spectrum. Phenolic compounds were identified using $FeCl_3$ or with ammonia vapor, while the alkaloid compounds were identified by Dragendorf reagent spray (Harborne, 1987). Terpenoid compounds were detected using solution of 0.2 % $KMnO_4$ in water, and Vanillin sulphuric acid reagent (vanillin-H_2SO_4) in chloroform, while 131 Cerium (IV) sulfate was used to detect the presence of common organic compounds (Table 2). Each reagent gave its unique response to each compounds class.

Table 2. Spot visualization and the compounds group interpreted in the study

No	Spot visualization	Compounds group	Positive interpretation
1.	Cerium (IV) sulphate	Terpenoid	Brown spot, purple, brownish purple
2.	Lieberman-Buchard (*)	Triterpenoid	Bluish dark spot
3.	Vanilin sulfat (**)	Terpenoid	Purple spot, brown
4.	Sitroborat	Flavonoid	Yellow spot
5.	Dragendorf	Alkaloid	Orange spot
6.	Ammonium vapor	Phenols	Yellow spot
7.	$FeCl_3$	Polyphenols	Greenish blue spot
8.	$AlCl_3$ 5 %	Flavonoid	Yellow spot

Remarks: (*) Plate was heated at 85-90 °C for 15 minutes after spraying with Lieberman-Buchard (LB) reagent 377;
(**) Plate was heated at 100 °C for 15 minutes after spraying with Vanillin sulphuric acid reagent

RESULTS AND DISCUSSION

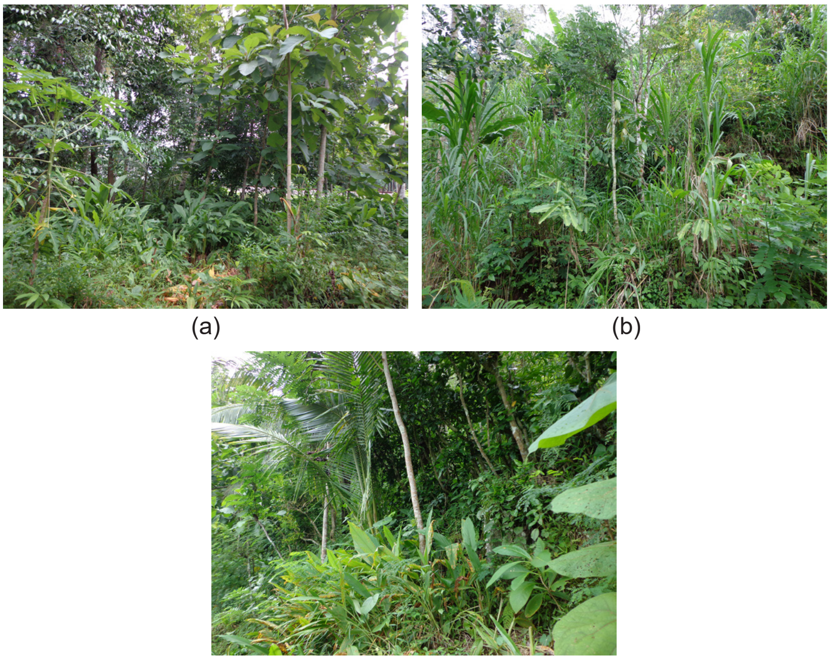
Understorey Plant Species

In Kulon Progo, timber tree plantations are established in nearly all of area with wide range of elevation, due to their high economical value (Table 3). Socio-cultural and economic characteristics of gardeners are important for explaining plant diversity in homegardens (Díaz-Reviriego et al., 2016). The fruit trees are also planted in the wide range of area as a source of food for the family. Forage crops are grown to meet the needs of people who have farm animal or livestock (in zone 3). Estate crops such as clover, grown only in zones 2 and 3, as these types are less suitable to be grown in zone 1. All types of plants that require intensive maintenance are planted in the homegarden in order to facilitate proper maintenance, both short and long term. Timber trees tend to be planted as a border (Fig. 2) and serves as the boundary between the private lands. The homegarden trees tend to be more diverse with the increasing of elevation. Environmental factors that are influential on the distribution of tree species is altitude, geographical distance and slope (Abebe, Sterck, Wiersum, & Bongers, 2013). Altitude alterations cause local microclimate changes such as light intensity, temperature and humidity. Environmental factors play roles in physiological processes of plants. All physiological processes influenced by environmental temperature and some of the processes are also depend on the presence of light.

Table 3. Tree species of the homegarden agroforestry system in Kulon Progo

Zone	Site	Shade level	The benefit and tree species	
1	Kulur	87.78	Wood	*Tectona grandis, Swietenia macrophylla, Albizzia chinensis*
			Food	*Cocos nucifera, Gnetum gnemon.*
			Wood	*T. grandis*
2	Gerbosari	71.49	Food	*Artocarpus heterophyllus, C. nucifera, Carica papaya, Musa sp.*
			Estate Crop	*Theobroma cacao, Syzygium aromaticum*
3	Sidoharjo	62.53	Wood	*S. macrophylla, T. grandis,* and *A. chinensis*
			Food	*A. heterophyllus, C. nucifera*
			Forage Crop	*Leucaena leucocephala*
			Estate Crop	*T. cacao, S. aromaticum*

Remarks: zone 1 = elevation < 300 m asl, zone 2 = elevation 301-500 m asl, zone 3 = elevation > 500 m asl



(a) (b) (c)

Fig. 2. The condition of homegarden in zone 1 (a), zone 2 (b) and zone 3 (c)

Table 4. Five understorey plant species with highest importance value index (IVI) of agroforestry system in zone 1, 2 and 3

No	Kulur (zone 1)		Gerbosari (zone 2)		Sidoharjo (zone 3)	
	Species	IVI	Species	IVI	Species	IVI
1.	*C. mangga*	55.49	*C. mangga*	60.15	*Chrysanthemum morifolium*	37.89
2.	*Oplismenus burmanii*	23.81	*S. nodiflora*	23.67	*Unidentified*	27.59
3.	*Zingiber zerumpet*	21.25	*B. pandurata*	17.58	*C. esculenta*	26.38
4.	*Oxalis barrelieri*	14.83	*M. pudica*	11.40	*C. asiatica*	15.55
5.	*Curcuma urpurascens*	13.19	*O. barrelieri*	11.40	*B. laevis*	14.44

Remarks: Source from proceed observation data

Homegarden is an agroforestry practice widely adopted by the community in Kulon Progo to provide the basic needs. The understorey species found in the homegarden of the zone 1 was less diverse than zones 2 and 3 (Table 4). The understorey species of homegardens with different sites have different composition. The number of understorey species different between zones 1, 2 and 3 (Table 5), it increases with the rising of elevation and decreasing shade intensity. The dominant understorey species in all area was *Curcuma mangga*, a member of family Zingiberaceae. Most of the species of Zingiberaceae family are economically important, since they are being used in the treatment of various ailments, culinary uses and as ornamental plants (Ounjai, Osathanunkul, Madesis, & Osathanunkul, 2017). Tushar, Basak, Sarma, & Rangan (2010) confirmed that the family Zingiberaceae, cure to myriad ailments such as gastro intestinal disorder, chest and lungs related problems, an antidote to snake venom. *C. mangga* has the ability to grow at wide range of area from the open area to the shaded area offer a large potential for its development in an agroforestry system. Zingiberaceae can survive from the lowlands to an elevation of 2000 m above sea level (asl), especially in an area with high rainfall. In zone 1, local communities utilize them as a traditional medicine ingredient but they will be cleared up during the rainy season as it is getting denser and become a mosquito breeding nest. Zingiberaceae species are also grown in zone 3, but less utilized as the areas are considerably far from the village and zone 1 has provided enough yields. The difference elevation of study sites was assumed to affect the composition of understorey in homegarden (Zhu et al., 2009), because the factors affecting the structure and the development of the homegarden vegetation are climate, edaphic quality and local socio-cultural.

Table 5. Species richness index (R’), evenness index (E’) and species diversity index (H’) of understorey plants in Kulon Progo homegarden.

Zone	R’	E’	H’
1	2.88	0.80	2.23
2	3.04	0.77	2.12
3	3.35	0.85	2.45

Understorey species is one of the components of the forest ecosystem. Mohri et al. (2013) mentioned that the homegarden is an area surrounding a residence that provides various goods and services to members of the household. Aguilar-Støen, Moe, & Camargo-Ricalde (2009) classified the types of plants in the homegarden to eight groups according to the benefit, i.e. vegetables, ornaments, foods, fruits, herbs, medicines, construction materials and other benefits. In addition to provide food for livestock, traditional medicines and source of material genetic diversity, the understorey species also play an important role in annual nutrient cycling. Understorey species also can supply organic material and create the appropriate conditions for soil microbes (Supriyadi, Hartati, Machfiroh, & Ustiatik, 2016). The litters returned to the soil contain high nutrients.

Diversity of Understorey Plant Species

To determine the diversity of species and the similarity between communities in zones 1, 2 and 3, the diversity index and evenness index of understorey plants in the homegarden were calculated. The value of species richness index (R'), evenness index (E') and the diversity index (H') of understorey plants in homegarden is corresponding to the increase in elevation, homegarden with the highest species richness and diversity were found in zone 3 (Fig. 3). The understorey species diversity is strongly influenced by environmental factors such as light, moisture, soil pH, canopy cover and the competition ability level of each species, it is in accordance with the statement by Loidi et al. (2015) that many recent studies have shown that factors such as elevation, precipitation, temperature, isolation and bedrock greatly influence species richness. The plant composting the homegarden communities are the shade-tolerant species and the shade covering of the homegarden are relatively large (60-80 %). Light quality and intensity has a profound effect on plant growth. Understorey vegetation in a tropical rain forest is less diverse than the vegetation in the relatively open area, and most likely belongs to a single family. This is different with the understorey vegetation in the slopes area with more intense light that allow more species to grow and promote more diverse vegetation (Sari, Periadnadi, & Nasir, 2013). Suranto, Tedianto, Purwanto, Setyono, & Mahadjoeno (2015) mentioned that the local temperature has a relationship with the elevation and finally affect the growth of the plant. Temperature is a primary factor affecting the rate of plant development (Hatfield & Prueger, 2015).

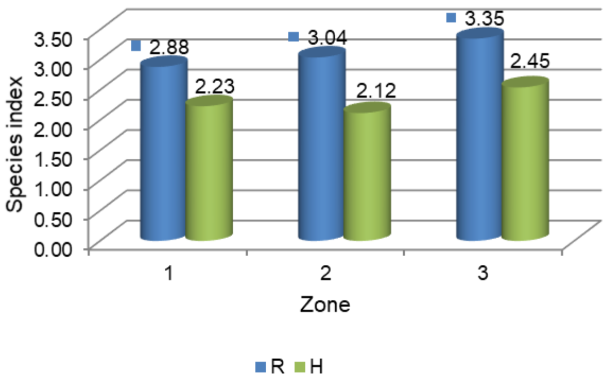
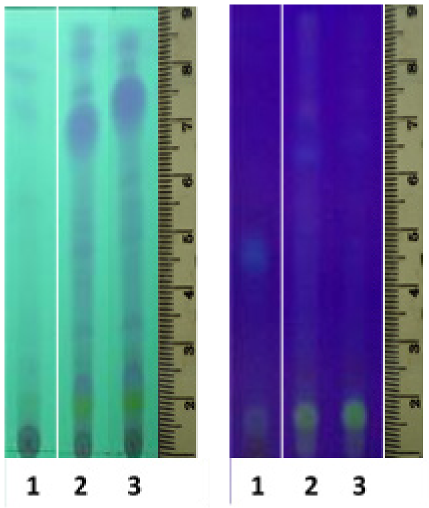


Fig. 3. Species richness index (R') and species diversity index (H') of understorey communitie

Curcuma mangga Extract Compounds

Thin Layer Chromatography (TLC) Test

The chromatography results using 254 nm UV light showed that three samples extract of zone 1, 2 and 3 tend to have similar pattern. However chromatograms of extracts of zone 1 showed less obvious separation. This is probably caused by lesser compound contents of zone 1 samples extract than the zones 2 and 3 samples. Using a 366 nm UV light, extracts from all zones had visible yellow spots. It shows that all extracts contain the same compounds although have different concentration/ amount of content, given by different appearance of the spots (Fig. 4).



Remarks:
1 = extract of *C. mangga* in zone 1, 2; 2 = extract of *C. mangga* in zone 2; 3 = extract of C. mangga in zone 3

Fig. 4. TLC Chromatogram with UV light 254 nm (a), with 366 nm UV light (b)

Different samples from different sites showed variations on spot visualization. Accumulation of chemical compounds in plants are influenced by genetic (Martinez-Villaluenga et al., 2010) and environmental factors (Aires et al., 2011). The height of the growing place is an environmental condition that includes various conditions that can limit or support plant growth (Duryat, 2008). The results of Guerrero-Chavez, Scampicchio, & Andreotti (2015) study showed that strawberry phenolic compounds and specific fruit quality traits were different at each altitude and season. The height of the growing place is one of the environmental factors that can affect plant conditions in morphology and physiology (Coomes & Allen, 2007). The height of different growing places leads to differences in the availability of nutrients, moisture, sunlight intensity and temperature. This difference can directly or indirectly affected the metabolism of plants, especially medicinal plants as they relate to the active substance.

The shade coverage rate at the study sites ranged from 60-90 %. Zone 3 with a 60-70 % shade level has a very bright dyeing point on the chromatogram, while zone 2 with a shade level above 70 % has a dyeing point and a light 1 zone with a fate rate of > 80 % has an unclear visualization.

The height of different growing places leads to differences in the availability of nutrients, moisture, sunlight intensity and temperature. Shading percentage is an implementation of the light intensity associated with the process of photosynthesis. The less than perfect process of photosynthesis affects the secondary metabolite levels produced by plants as a result of research by Chen et al. (2010) who stated that the higher the rate of photosynthesis, the higher the caffeine produced.

The production of active compounds of medicinal plants were determined by crop varieties, cultivation and environmental conditions for growth (Widodo, Subositi, & Supriyati, 2011) In the same plant species, content of active compounds different from one region to another (Chan et al., 2008). The quality and quantity of good active compounds can be obtained by optimizing the external factors of selection of suitable plant sites for certain medicinal plants, each plant requires certain environmental conditions to produce well, because the different places of growth might affect the quality of an extract (Chan et al., 2008). Plants can produce more active compounds when exposed to elevated light conditions (Frankel & Berenbaum, 1999). Therefore, the development of medicinal plants directed at the cultivation activities to obtain the optimal quality of plant active ingredients.

Compounds Extract of *C. mangga*

Rhizome *C. mangga* is used in Java as a food spice and treatment of abdominal pain, fever and cancer-related diseases (Malek et al., 2011). Some research results show *C. mangga* has the ability as analgesic and anti inflammatory (Ruangsang, Tewtrakul, & Reanmongkol, 2010), and also anti allergy (Tewtrakul & Subhadhirasakul, 2007). In addition, *C. mangga* is one of the species of Zingiberaceae that has high antioxidant (Chan et al., 2008) and has activity Glutation-Stransverase (GST) is an enzyme that plays a role in the detoxification of foreign compounds in the body, and able to suppress the occurrence of oxidative stress (Tedjo, Sajuthi, & Darusman, 2010).

Test results using spray ammonia vapor and Cerium (IV) sulfate showed that the same compound content in the three extracts tested, however there was a difference in sighting from the resulting spot (Table 6). The visible variations of the resulting spot might be due to differences in environmental conditions from the site where it is grown. Secondary metabolism is produced by certain organisms under certain conditions (Tyc, Song, Dickschat, Vos, & Garbeva, 2017) for example through the treatment of fertilization and maintenance. Land maintenance was done intensively by landowners in zones 2 and 3 rather than in zone 1, landowners in zone 1 do not undertake intensive care or maintenance, only when there is spare time as most owners are employees. Pal et al. (2015) mentioned that the secondary metabolite of the stevia can be improved through the selection of appriate growing locations and proper nutrient management.

Table 6. Active compounds in *C. mangga* extract

Number of spot	Location		
	Zone 1	Zone 2	Zone 3
Total	5	10	9
Active	1 (Rf = 0.78)	5 (Rf = 0.12; 0.21; 0.38; 0.75; 0.84)	3 (Rf = 0.12; 0.21; 0.81)

The identification *C. mangga* extract compounds resulting two different classes of compounds, i.e. phenols and terpenoids. Both classes of compounds act as antibacterial and usually found in disinfectant. Mechanism of action of phenolic compounds in killing bacterial cells is by denaturizing the protein of bacterial cells (Kusdarwati, Sari, & Mukti, 2010). Hartati, Suganda, & Fidrianny (2014) stated that the general secondary metabolites content of Zingiberaceae were flavonoids, phenols, terpenoids and essential oils. Secondary metabolites produced by Zingiberaceae are generally have ability to inhibit the growth of pathogens that harm human life, including *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and fungi such as *Neurospora* sp., *Rhizopus* sp. and *Penicillium* sp.

CONCLUSION

There were 41 species of homegarden understorey species in Kulon Progo. The dominant species in zones 1, 2 and 3 was *C. mangga*. The number of understorey species tends to increase as the elevation increase, but the abundance of each species tends to decrease, as the results of variation in elevation and climate. The compounds of C. mangga extracts were identified as phenols and terpenoids.

ACKNOWLEDGEMENT

The research was funded by DPP Faculty of Forestry grants in 2013 to the first author. Thank you to I. Riastiwi and D. Anita for technical assistance in preparing of this manuscript.

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