

A COMPARATIVE STUDY OF THE SECONDARY METABOLITE FROM *Talinum triangulare* (Jacq.) Willd. METHANOLIC EXTRACT FROM MALANG AND KEDIRI, EAST JAVA**STUDI KOMPARATIF SENYAWA METABOLIT SEKUNDER *Talinum triangulare* (Jacq.) Willd. DI MALANG DAN KEDIRI, JAWA TIMUR DENGAN EKSTRAK METHANOL**Sarah Fadilah Budiarti^{1, 2)}, Fatchiyah Fatchiyah^{1, 2)*}

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How to cite:Budiarti, SF, F Fatchiyah. 2022. A comparative study of the secondary metabolite from *Talinum triangulare* (Jacq.) Willd. Methanolic Extract from Malang and Kediri, East Java. *Journal of Tropical Biology* 10 (2): 146-153.**ABSTRACT**

Talinum triangulare is a medicinal plant that has been used broadly by the Indonesian community because of its properties. Several studies have identified the components of secondary metabolites in *T. triangulare*. However, the content of secondary metabolites in each part of *T. triangulare* is not clearly understood. Therefore, this study aimed to analyze the types of secondary metabolites of leaf, stems, and roots of *T. triangulare*. The plant from Malang was chosen as the sample source because it is a highland suitable for the life of *T. triangulare*. The plant from Kediri was chosen as a lowland location. Samples of leaves, stems, and roots of *T. triangulare* were extracted by the maceration method. Phytochemical analyses included phenolics, flavonoids, alkaloids, tannins, saponins, and terpenoids were undertaken by spectrophotometer. The absorbance values were measured at different wavelengths. The leaf and stems of *T. triangulare* contain flavonoid compounds, tannins, phenolics, alkaloids, saponins, and terpenoids with higher levels of secondary metabolites in the leaf. While the roots have the lowest secondary metabolite was only containing steroids. These results indicate that the leaf metabolite secondary of *T. triangulare* has high potential health benefits for regulating molecular and cellular metabolism. The differential levels and types of secondary metabolites samples obtained from Kediri tend to be lower than samples obtained from Malang.

Keywords: leaf, root, secondary metabolite, stem, *Talinum triangulare***ABSTRAK**

Talinum triangulare merupakan tanaman obat yang dimanfaatkan secara luas oleh masyarakat Indonesia karena khasiatnya. Beberapa penelitian membuktikan komponen metabolit sekunder pada *T. triangulare*. Namun, kandungan metabolit sekunder dari setiap bagian *T. triangulare* masih belum diketahui secara pasti. Oleh karena itu, tujuan penelitian ini untuk menganalisis jenis metabolit sekunder pada daun, batang, dan akar *T. triangulare*. Malang dipilih menjadi daerah pengambilan sampel karena merupakan daerah dataran tinggi yang cocok untuk *T. triangulare*, sedangkan Kediri dipilih karena merupakan lokasi dataran rendah. Sampel daun, batang, dan akar dari *T. triangulare* diekstrak dengan metode maserasi. Analisis fitokimia meliputi fenolik, flavonoid, saponin, dan terpenoid. Nilai absorbansi diukur pada panjang gelombang berbeda. Daun dan batang *T. triangulare* mengandung senyawa flavonoid, tannin, fenolik, alkaloid, saponin, dan terpenoid kadar metabolit sekunder pada daun lebih tinggi dibandingkan padabatang. Sedangkan akar yang memiliki metabolit sekunder paling rendah hanya mengandung steroid. Perbedaan kadar dan jenis metabolit sekunder yang diperoleh dari sampel Kediri cenderung lebih rendah dibandingkan dengan sampel dari Malang. Hasil penelitian ini mengindikasikan bahwa metabolit sekunder daun *T. triangulare* memiliki potensi manfaat kesehatan yang tinggi untuk regulasi molekuler dan metabolisme seluler.

Kata kunci: akar, batang, daun, metabolit sekunder, *Talinum triangulare***INTRODUCTION**

The medicinal plant is a type of plant that is widely used because of its pharmacological value. Its therapeutic efficacy comes from the chemical compounds or secondary metabolites which are divided into several classes with different pharmacological effects. Nowadays, treatment using medicinal plants tend to be chosen because it can reduce the side effect of synthetic drugs

consumption [1]. Indonesian communities have been using medicinal plants because they are safer and have minimal side effects than synthetic drugs. Indonesian people have widely used Javanese ginseng or also often called som Jawa. People in Langkat, North Sumatra, often consume boiled water of Javanese ginseng root to reduce fever [2]. Malaysia in Nort Kayong Regency uses Javanese ginseng roots to maintain their immune system by boiling it first [3]. People in Serawai, Bengkulu use

the leaves of Javanese ginseng to treat ulcers by cooking or eating the leaves directly, and the boiled water of Javanese ginseng root is used to increase stamina [3].

Talinum paniculatum (Jacq.) Gaertn. and *Talinum triangulare* (Jacq.) Willd. are two medicinal plants from the same genus known as Javanese ginseng by Indonesian people. These plants look similar. Among these plants, the most widely used Javanese ginseng is *T. triangulare*. However, some morphological characteristics have differences. *T. paniculatum* inflorescence is in the terminal with flowering time at 4 pm. While *T. triangulare* inflorescence is in the terminal and axillar with flowering time at 10 am, and its peduncle form is triangular. The tip of leaves of *Talinum triangulare* is obtuse compared with *T. paniculatum*. The petal color of *T. triangulare* is darker and larger in size than *T. paniculatum* [4].

Previous research showed that *Talinum triangulare* has many secondary metabolites. *T. triangulare* has a high content of flavonoids, saponins, alkaloids, and low tannin content [5]. Sunday et al. [6] reported the highest content of secondary metabolites from the qualitative test of *T. triangulare* leaf were alkaloids and saponins. In general, studies that have been carried out state that the leaf, stem, and root of *T. triangulare* contain many secondary compounds that can treat disease and improve health [6].

Environmental factors significantly affect the secondary metabolite content in the plant. Some of these include altitude, temperature, and sunlight irradiation. These environmental factors are different in each location. Vu et al. [7] stated that a highland is a suitable place for the growth of perennial plants, such as *T. triangulare*. Malang is a location classified as highland for chosen as the sampling location, and the lowland that will be selected is Kediri [3, 7].

Research about secondary metabolite content in *T. triangulare* has been carried out, but the content in its parts is still not clear. The parts of *T. triangulare*, especially the leaves, have been widely used for traditional medicinal purposes. The roots also have been utilized for treatment and repellent. In contrast, the stem is not utilized yet. This makes *T. triangulare* less attention to its pharmacological value in modern medicine. Therefore, it is necessary to determine the type of secondary metabolite in the leaf, stem, and root of *T. triangulare*. In addition, the geographic characteristic may have an impact on the *T. triangulare* secondary metabolite contents. Therefore, in this study, we compare the secondary metabolites of *T. triangulare* from Malang (highland) and Kediri (lowland).

METHODS

Plant identification. Plant specimens were identified to confirm the species. Identification was made by observing the parts of the plant (leaf, stem, root). The identification process was carried out at The Laboratory of Taxonomy, Structure, and Plant Development, Department of Biology, University of Brawijaya and Purwodadi Botanical Garden.

Plant sample extraction. The samples were extracted using the maceration method [8]. Fifty grams for each fresh sample (leaves, stems, and roots) of *T. triangulare* were mashed using mortar. The refined sample was macerated in 250 mL of methanol solvent with solid : solution ratio was 1:5 for 24 hours. The extract was filtered using filter paper. Residue produced from the filtration process was macerated in 250 mL methanol for 1 hour and then was filtered. Both filtrates were combined and evaporated at 50°C.

Test for phenolics (Ferric Chloride Test). One milliliter of the extract was mixed with 0.5 mL of 5% FeCl₃. The presence of phenolic compound is indicated by a color change to blue or black [9]. The absorbance value was measured by a spectrophotometer at 280 nm [10].

Test for flavonoids (Alkaline Test). One milliliter of the sample was treated with 0.5 mL of 10% NaOH. Changes in color to reddish yellow indicate the presence of xanthenes and/or flavones, dark orange-brown indicates the presence of flavonols, reddish-purple indicates the presence of chalcone, and blue indicates the presence of anthocyanins [9]. The absorbance of the sample was measured by a spectrophotometer at 430 nm [10].

Test for tannins (Ferric Chloride Test). One milliliter of the extract was taken and added with 0.5 mL of 1% FeCl₃. The formation of blue or black color is a positive indicator of tannins. The absorbance value of this test was measured by a spectrophotometer at 700 nm [10].

Test for saponins (Foam Test). One milliliter of the extract was taken and diluted with 1 mL of distilled water. The sample contains saponins if the foam is present after shaking [4]. The absorbance was measured by a spectrophotometer at 435 nm [11].

Test for terpenoids (Salkowski's Test). One milliliter of sample was added with 0.5 mL of chloroform and 1 mL of concentrated H₂SO₄. If terpenoids are present in the sample, it generates brown red ring. The sample was measured by a spectrophotometer for the absorbance value at 538 nm [12].

Test for alkaloids (Wagner's Test). One milliliter of the plant extract was acidified with 1 mL of 1:1 HCl and warmed for 20 minutes. The extract was added with 0.5 mL of Wagner reagent

after cooling down. The positive indicator showed by the forming of brownish-red precipitation [9]. The absorbance value was measured by a spectrophotometer at 470 nm [10].

RESULTS AND DISCUSSION

Type of secondary metabolite in *T. triangulare* leaf. The result of phytochemical tests on leaves of *T. triangulare* leaves from Malang and Kediri showed a slight difference (Figure 1). While the absorbance value can be seen in Table 1.

A positive result on flavonoid test was indicated by a change of the extract color to yellowish-brown for Malang sample and yellowish-green for Kediri samples. The presence of tannin was indicated by the formation of brown color and brownish-green precipitate of Malang sample. While Kediri sample showed positive results only by a change color to brownish. Based on this result, Malang sample has a higher concentration of tannin compared Kediri sample. These results correspond with the absorbance value. Both extracts of samples positively contain phenolic due to the formation of brown color. The alkaloid test on both samples showed positive results indicated by a change color to reddish-brown. Malang sample showed positive results on the saponin test, which was marked by the foam formation after the test. The last test is terpenoid in which both extracts contained

terpenoids because of the forming reddish-brown ring.

Type of secondary metabolite in *Talinum triangulare* stem. The results of the phytochemical test on *T. triangulare* stem gave slightly different results between Malang and Kediri samples (Table 2). A qualitative phytochemical test of *Talinum triangulare* stem can be seen in Figure 2.

Flavonoid test on both samples showed a change in color to yellow without any precipitation. The tannin test on the Malang sample showed an appearance of a color change to brownish-yellow and brownish precipitate. Meanwhile, on Kediri sample only gave a change in color to greenish-brown, indicating low intensity. These results correspond with the absorbance value. The phenolic test revealed Malang sample generated green sediment and changed in color to greenish-brown. While the Kediri sample only gave a change in color to brown. Based on the color density and the presence of sedimentation, it is known that the Malang sample has a higher phenolic content than the Kediri sample. *T. triangulare* leaves contain alkaloid compounds, which were performing reddish-brown solution. In the saponin test, only *T. triangulare* performed a positive result. However, both samples generated a brown ring color and indicated terpenoid compounds.

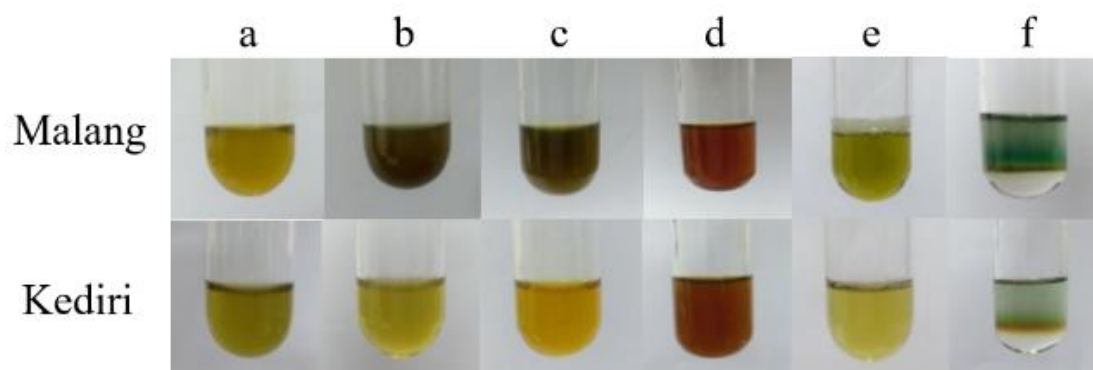


Figure 1. Qualitative phytochemical screening of *T. triangulare* leaves from Malang and Kediri. (a) Flavonoid, (b) Tannin, (c) Phenol, (d) Alkaloid, (e) Saponin, (f) Terpenoid/Steroid

Table 1. Secondary metabolites compound identification of *Talinum triangulare* leaves

Secondary metabolites compound		Malang	Kediri
Flavonoid (Alkaline Test)	Appearance	Fawn color	Yellowish-green
	Absorbance (A)	4	3.319
	Qualitative	++++	+++
Tannin (Ferric Chloride Test)	Appearance	Brown color and greenish-brown sediment	Greenish-brown
	Absorbance (A)	1.302	0.623
	Qualitative	+++	+
Phenol (Ferric Chloride Test)	Appearance	Brown color	Brown color
	Absorbance (A)	4	4
	Qualitative	++++	+++
Alkaloid (Wagner Test)	Appearance	Reddish - Brown	Reddish - Brown
	Absorbance (A)	4	4
	Qualitative	+	+

Secondary metabolites compound		Malang	Kediri
Saponin (Foam Test)	Appearance	Foam	No Foam
	Absorbance (A)	2.732	1.328
	Qualitative	+	-
Terpenoid/ Steroid (Salkowski Test)	Appearance	Brown ring	Brown ring
	Absorbance (A)	1.783	0.599
	Qualitative	Terpenoid (++)	Terpenoid (+)

Note: (-) not detected, the secondary metabolite intensity were depicted in following various plus symbols, (+) low, (++) moderate, (+++) high, (+++++) very high.

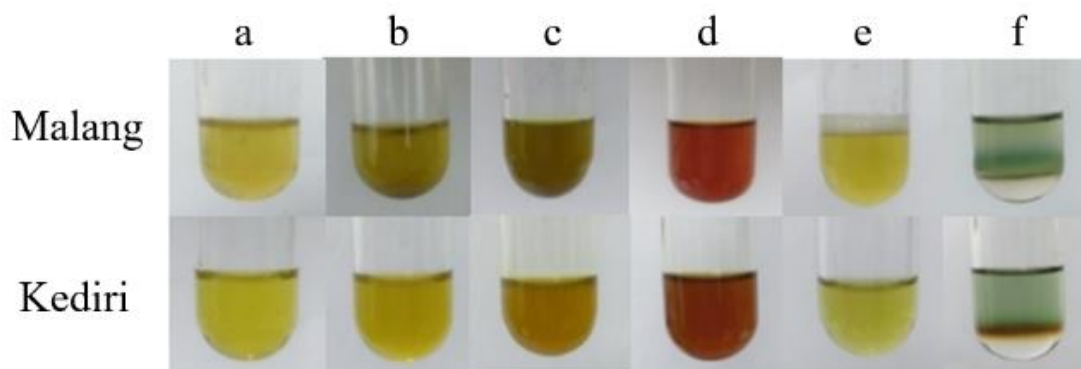


Figure 2. A qualitative appearance of secondary metabolites screening in *Talinum triangulare* (a) Flavonoid, (b) Tannin, (c) Phenol, (d) Alkaloid, (e) Saponin, dan (f) Terpenoid/Steroid

Table 2. Secondary metabolites compound identification of *Talinum triangulare* stem

Secondary metabolites compound		Malang	Kediri
Flavonoid (Alkaline Test)	Appearance	Yellow color	Yellow color
	Absorbance (A)	2.457	1.589
	Qualitative	+++	++
Tannin (Ferric Chloride Test)	Appearance	Tawny color and brown sediment	Greenish-brown
	Absorbance (A)	0.957	0.402
	Qualitative	+++	+
Phenol (Ferric Chloride Test)	Appearance	Greenish - Brown color and green sediment	Brown color
	Absorbance (A)	4	4
	Qualitative	+++	++
Alkaloid (Wagner Test)	Appearance	Reddish - Brown	Reddish - Brown
	Absorbance (A)	4	4
	Qualitative	+	+
Saponin (Foam Test)	Appearance	Foam	No Foam
	Absorbance (A)	1.776	0.897
	Qualitative	+	-
Terpenoid/ Steroid (Salkowski Test)	Appearance	Brown ring	Brown ring
	Absorbance (A)	1.683	1.375
	Qualitative	Terpenoid (++)	Terpenoid (++)

Note: (-) not detected, the secondary metabolite intensity were depicted in following various plus symbols, (+) low, (++) moderate, (+++) high, (+++++) very high.

Type of secondary metabolite in *Talinum triangulare* root. The phytochemical result of *T. triangulare* roots (Figure 3) showed a slight difference. Table 3 showed the results of the absorbance value of the phytochemical test on the roots of *T. triangulare*. Flavonoids contained in the roots of *T. triangulare* showed a change in color to slightly yellowish in both samples. In the tannin test, the Malang sample showed a positive result indicated by a formation of brownish-yellow color. Meanwhile, the Kediri sample showed a negative result because the extract did not give a brownish color. *T. triangulare* from Malang and Kediri

presented phenol and alkaloid compounds, which showed fawn color and reddish-brown colors. The saponin test indicated that both samples were negative saponins because the samples did not produce foam after the test. *T. triangulare* also showed terpenoid or steroid compounds in both Malang and Kediri samples.

Comparison of secondary metabolite content of leaf, stem, and root of *Talinum triangulare* from Malang and Kediri. The results showed that the leaf and stem of *T. triangulare* have the same type of secondary metabolites (Table 4). There were flavonoids, tannins, phenolics, alkaloids,

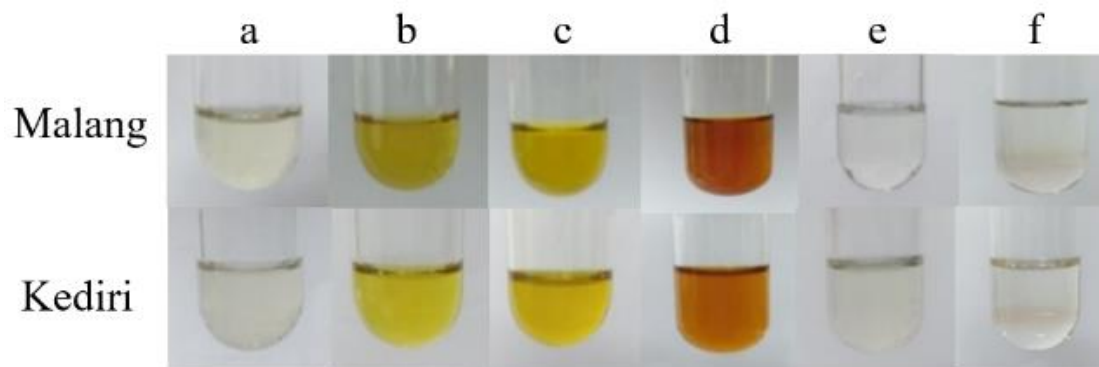


Figure 3. Secondary metabolites screening appearance of *Talinum triangulare* roots from Malang and Kediri, (a) Flavonoid, (b) Tannin, (c) Phenol, (d) Alkaloid, (e) Saponin, dan (f) Terpenoid/Steroid

Table 3. Secondary metabolites compound identification of *Talinum triangulare* roots

Secondary metabolites compound		Malang	Kediri
Flavonoid (Alkaline Test)	Appearance	Yellow color	Yellow color
	Absorbance (A)	0.243	0.355
	Qualitative	+	+
Tannin (Ferric Chloride Test)	Appearance	Tawny color	Yellow color
	Absorbance (A)	0.202	0.025
	Qualitative	+	-
Phenol (Ferric Chloride Test)	Appearance	Fawn color	Fawn Color
	Absorbance (A)	4	4
	Qualitative	+	+
Alkaloid (Wagner Test)	Appearance	Reddish - Brown	Reddish - Brown
	Absorbance (A)	4	4
	Qualitative	+	+
Saponin (Foam Test)	Appearance	No Foam	No Foam
	Absorbance (A)	0.133	0.128
	Qualitative	-	-
Terpenoid/ Steroid (Salkowski Test)	Appearance	Reddish - Brown ring	Reddish - Brown ring
	Absorbance (A)	0.333	0.34
	Qualitative	Terpenoid (+); Steroid (+)	Terpenoid (+); Steroid (+)

Note: (-) not detected, the secondary metabolite intensity were depicted in following various plus symbols, (+) low, (++) moderate, (+++) high, (+++++) very high.

Table 4. Comparison of the secondary metabolites intensity of *Talinum triangulare* based on the plant tissues and locations

Secondary metabolites compound	Leaves		Stem		Roots	
	Malang	Kediri	Malang	Kediri	Malang	Kediri
Flavonoid	++++	+++	+++	++	+	+
Tannin	+++	+	+++	+	+	-
Phenol	++++	+++	+++	++	+	+
Alkaloid	+	+	+	+	+	+
Saponin	+	-	+	-	-	-
Terpenoid	++	+	++	++	+	+
Steroid	-	-	-	-	+	+

Note: (-) not detected, the secondary metabolite intensity were depicted in following various plus symbols, (+) low, (++) moderate, (+++) high, (+++++) very high.

saponins, and terpenoids. However, the content of those secondary metabolites tends to be higher in the leaf than stem, especially flavonoids and phenolics. The root of *T. triangulare* has the lowest content of secondary metabolites and does not contain saponins. In contrast, root is the only part of *T. triangulare* that contains steroids. Based on the location, several secondary metabolites in the

Malang sample have higher content than Kediri, such as flavonoids, tannins, phenolics, saponins, and terpenoids (Table 4).

The *T. triangulare* from Malang and Kediri contained several secondary metabolites. The flavonoids test gave a positive indicator by changing color to yellow after being treated with NaOH 10% [13]. The positive result of the tannin

test is the formation of a green precipitate [14] and brown color, which indicated the presence of pseudo tannins [15]. Based on this, samples that formed green precipitate after the test were classified as having a higher tannin content. In the phenolics test, a positive test showed a color change to brown. In this test, all samples had an absorbance value of 4. Therefore, the results determination was based on the formation of green precipitate and the density of the darker color. A positive result of the alkaloid test was represented by the formation of a reddish-brown precipitate [16]. However, *T. triangulare* extracts that have been tested for alkaloids only gave a color change to reddish-brown, indicating low intensity. Saponin was positive test, when the Malang samples produced foam in reaction solution [16, 17]. Meanwhile, the roots Malang and Kediri samples did not show froth. Thus, roots Malang and Kediri samples were categorized in negative even its absorbance value was 4. The measured absorbance value was predicted due to the color of the extract which was only added with distilled water.

As a part of the plant, the leaf is the most exposed part to UV irradiation. If the UV exposure is too high, the tissue exposed will be damaged. Increased UV radiation interfered with the photosynthesis process, plant water metabolism, and free radical production [17]. Therefore, the leaf produced more secondary compounds as a protection system. High levels of UV-B irradiation are directly proportional to the altitude of a location [18]. In the current study, *T. triangulare* from Malang presented high intensity secondary metabolites, which higher altitude against Kediri, 445 and 67 meters above sea level, respectively [19, 20]. Saponins, phenols, flavonoids, and tannins are secondary metabolites that act as protective agents against UV irradiation [21, 22]. These secondary metabolites were found higher in leaf samples. Saponins could prevent cell apoptosis induced by UV-B exposure by lowering the level of caspase involved in intrinsic apoptosis, preventing the release of cytochrome c from mitochondria, and preventing DNA fragmentation [21, 22]. Saponins in *Talinum triangulare* can be used as an antiinflammatori and antiviral for human [17, 23]. Flavonoids are one of secondary metabolites in plant act as plant defense against free radicals formed during metabolism processes or radiation [18]. Besides that, flavonoids also play a role in attracting pollinators [24, 25, 26]. Phenol compound performed plant protection effects by preventing lipid peroxidation inhibiting free radical diffusion, suppressing active peroxidations, and stabilizing cell membrane [22, 27].

Terpenoids detected in stems were slightly higher than in Kediri leaf sample. These metabolites act as defense mechanisms against insects, fungi, and nematodes. It also plays a role in plant growth and development. Although the stems of *Talinum triangulare* have the same type of secondary metabolites as the leaves, no research states that the stems of *Talinum triangulare* are consumed by the people like the leaves. That probably *T. triangulare* stem is slightly woody [28, 29].

The root of *T. triangulare* has the lowest content of secondary metabolites. It did not contain saponins, indicated by the absence of froth after the test. The absence of saponins in the roots is thought to be related to the function of saponins as plant protection against UV-B irradiation [30]. While the root is the part of the plant that does not receive UV-B radiation. The root is the only part that contains steroids. Root steroids can play an important role in root growth and development [26, 27]. The biological function of steroids inside plant promotes antitumor, antibacterial, and immunosuppressant. However, the low steroid content in the roots will increase root growth through cell elongation and differentiation [31]. Overall, the highest secondary metabolite content was found in the leaves of *T. triangulare* from Malang.

CONCLUSION

In summary, from both cities showed the leaf, stem, and root of *T. triangulare* contain differential level of flavonoids, tannins, phenolics, alkaloids, saponins, and terpenoids, which are may have potential biological function as healthy food. Overall, we found that he secondary metabolites of *T. Triangulare* from Malang city were higher than that of Kediri.

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