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Harvest methods determine the yield and quality of *Sonchus arvensis* accessions cultivated in organic way

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Abstract. *Raisawati T, Melati M, Aziz SA, Rafi M. 2023. Harvest methods determine the yield and quality of* Sonchus arvensis *accessions cultivated in organic way. Biodiversitas 24: 4110-4119.* Sowthistle (*Sonchus arvensis* L.) is a wild plant species with high potential for medicinal uses. Nonetheless, the understanding of agronomical aspect of this species is still limited, hindering the large-scale cultivation efforts. This species has unique traits in term of leaves morphology in which it has rosette (old) leaves and upper (inflorescence) leaves which might require particular harvesting method to obtain optimal yield and quality. This study aimed to evaluate the effect of harvest methods on leaf production, total flavonoid content, antioxidant activity and flavonoid profiles of three accessions of sowthistle. The experiment used randomized complete block design with three replications. The first factor is harvesting methods namely gradual and simultaneous harvest of lower and upper leaves of sowthistle. The second factor is three accessions of *S. arvensis* namely Lembang, Tawangmangu, and Banjararum. The results showed that the simultaneous harvest resulted in higher leaf number by 65%, fresh weight by 55%, and dry weight by 60% compared to those of gradual harvest. However, the difference between fresh weight and dry weight of upper leaf was more determined by the accessions with Tawangmangu had the highest weight. Antioxidant activity of sowthistle in this study is not only caused by total flavonoid. Tawangmangu accession gradually harvested produced high lower leaf antioxidants, while those harvested simultaneously produced the highest total flavonoid contents and antioxidant activity of upper leaves. The results of HPLC analysis showed the flavonoid profiles of *S. arvensis* leaves which contain rutin, myricetin, luteolin, quercetin, kaempferol and apigenin. Kaempferol was the highest content of flavonoid in rosette and upper leaves. The findings of this study suggest that simultaneous harvesting is recommended to obtain high sowthistle production and quality.

Keywords: HPLC, kaempferol, luteolin, rosette leaf, upper leaf

INTRODUCTION

Sowthistle (*Sonchus arvensis* L.) is one of the wild plants that can be consumed as a vegetable. This plant also has the potential for medicinal purposes which is often used as a component in at least eleven herbal remedies in traditional medication by community including to treat kidney stones, obesity, uric acid and cholesterol (Ministry of Health of the Republic of Indonesia 2013). *S. arvensis* has soft and thin leaves similar to its close relative *S. oleraceus* but it differs from *S. asper* which has thick leaves (Mejías et al. 2018). The leaves of *S. arvensis* are lanceolate and arranged alternately on the inflorescence in the generative phase (Wahyuni et al. 2019). The leaves grow in a rosette with a circular arrangement near the ground because the stems are short. Hasan et al. (2017) reported that the development of sowthistle leaves occurs in two stages, namely old (lower) leaves or rosette leaves which grow above the ground surface, and young leaves or upper leaves which grow on stems.

There are several bioactive compounds contained in *S. arvensis*. The total flavonoid content in sowthistle leaves is generally calculated as quercetin, while the Ministry of Health of the Republic of Indonesia (2008) determined the identity compound of sowthistle to be luteolin with a content of 0.06%. Khan (2012) reported the profile of the flavonoids in sowthistle, namely apigenin, kaemferol, quercetin, orientin, rutin, hyperoside, and catechins. Gomaa et al. (2015) reported the profile of flavonoids in *S. oleraceus* L., namely apigenin, daidzein, kaemferol, luteolin, myricetin, quercetin, daidzin, genistin, hesperidin, hyperoside, kaemferol-7-O-glucoside, naringin and rutin. Based on research by Khan (2012), Gomaa et al. (2015) and the Ministry of Health of the Republic of Indonesia (2008) above, the standards of flavonoid profile include rutine, myricetin, quercetin, kaempferol which belong to the flavonol class and apigenin and luteolin which belong to the flavon class.

Cultivation and harvesting methods can affect crop quality including bioactive compounds. Unlike food crops, medicinal plants are usually cultivated in an organic system or by minimizing the use of chemicals. Ibrahim et al. (2013) reported on *Labsiah pumila* Benth that organic fertilization can increase the phenolic content, flavonoids and total dissolved sugar content compared to chemical fertilization. Several studies have shown that cultivating sowthistle outside its natural habitat produces different growth responses while the application of manure can increase its growth and yield. Organic cultivation uses natural materials, such as manure, to support plant growth. Manure as a source of soil organic matter can improve physical properties and increase soil fertility. The results of previous experiments on ten accessions of *S. arvensis* showed that with organic cultivation, Lembang, Tawangmangu and Banjararum accessions resulted in higher leaf production, total flavonoid content and antioxidant activity compared to other accessions (Raisawati et al. 2020).

Harvesting sowthistle is generally done before the flowers bloom by cutting the base of the stem, thus the rosette leaves and upper leaves are harvested simultaneously (Nurhayati 2016; Raisawati et al. 2018). This method creates a problem since there is a difference in optimum development time for the lower and upper leaves. Old leaves growing above the soil surface (rosette leaves) experienced senescence when sowthistle entered the generative phase. Therefore, it is necessary to adjust the method or time of harvest for the rosette leaves and upper leaves of sowthistle. The research of Hasan et al. (2017) showed that the method of harvesting the rosette leaves and upper leaves could increase the total flavonoid content of sowthistle leaves. The highest total flavonoid content was obtained from the upper leaves if the rosette leaves and upper leaves were harvested at the same time (Hasan et al. 2017).

In addition to the effect of harvesting methods, the production of plant bioactive compounds may differ among accessions. Variations in total flavonoid content and antioxidant activity were also found in ten accessions of sowthistle cultivated outside its natural habitat and harvested simultaneously, resulting in total flavonoid content and antioxidant activity (IC_{50}) in the range of 0.40-1.01 mg g^{-1} and between 246.45-490.19 µg mL^{-1} respectively (Raisawati et al. 2020). Until now, the knowledge of cultivation technique of sowthistle is limited. This study aimed to evaluate the effect of harvesting methods on leaf production, total flavonoid content, antioxidant activity and flavonoid profile of three accessions of *S. arvensis* in organic cultivation. This research is the first study to report the profile of flavonoids of different sowthistle accessions in Indonesia in relation to the method of harvest.

MATERIALS AND METHODS

Experimental design

The experiment was conducted at the experimental organic farm of Institut Pertanian Bogor, Cikarawang, Bogor, Indonesia (6°30’-6°45’ S, 106°30’-106°45’ E) which is located 190 meters above sea level. Soil pH was 5.76 and during the experiment, the average of monthly rainfall, relative humidity, daily temperature and light intensity were 419.7 mm, 84.2%, 26.3°C, and 105148.9 lux.day^{-1}, respectively. The research used randomized complete block design with two factors and three replications. The first factor was *S. arvensis* accessions, consisting of Lembang, Tawangmangu, and Banjararum accessions while the second factor was harvesting methods i.e., gradually harvested and simultaneously harvested of rosette and upper leaves. In total, there were 18 experimental units (2 harvesting methods x 3 accessions x 3 replicates) with 50 plants for each experimental unit. Thus, the present study involved 900 sowthistle plants.

Plant cultivation procedure

The seeds of each accession were sowed in planting box filled with rice-hull charcoal and chicken manure. At eight weeks after sowing, 50 healthy seedlings of each accession were transplanted to 3×2 m plot with planting distance of 30x40 cm. The land preparation used a no-tillage system. The soil in the experimental site was applied with 20 tons ha^{-1} chicken manure, 1 ton ha^{-1} rice-hull charcoal, and 1 ton ha^{-1} lime one week before planting. Irrigation was not applied during the experiment due to the abundance of rainfall. Weeding was done manually once a month. Plant protection used natural pesticide namely turmeric solution in a ratio of 1:10 (v/v). Gradual harvesting for rosette leaves was carried out when the sowthistle began to show stem elongation of ±6-8 cm (bolting), i.e., when 3 upper leaves had formed, while the upper leaves were harvested at the blooming phase. In simultaneous harvest, the rosette and upper leaves were taken at the blooming phase, but the rosette and upper leaves were separated for measurement (Figure 1). The period from seedling to harvest ranged between 16-18 weeks with seedling phase around 8 weeks, transplanting to bolting phase around 6-8 weeks and bolting to blooming phase around 2-4 weeks.

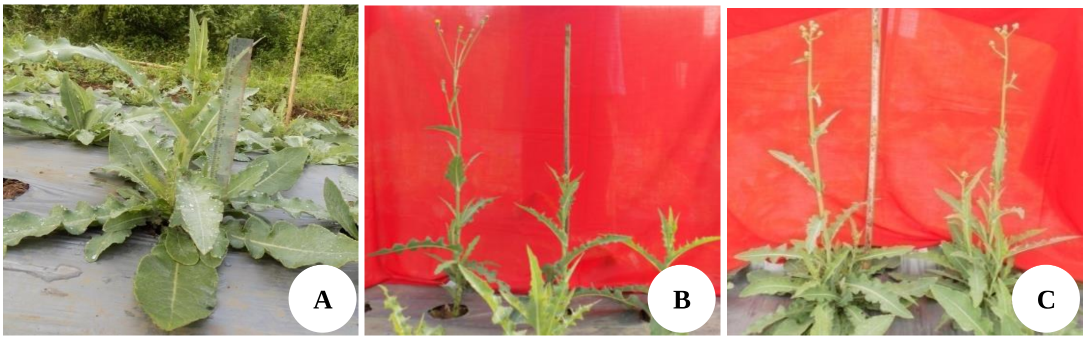

Figure 1. Conditions for gradual and simultaneous harvest of sowthistle. A: shows harvesting gradually when rosette leaves harvested in bolting phase, B: shows harvesting gradually when upper leaves (without rosette leaves) harvested in blooming phase, C: shows harvesting simultaneously when rosette and upper leaves harvested simultaneously in the blooming phase

The performance and number of sowthisthle rosette and upper leaves harvested simultaneously is shown in Figure 2, whereas Figure 3 shows the performance and number of sowthisthle leaves harvested gradually.

Flavonoid analysis (HPLC analysis)

Fresh leaves were cleaned, stored at room temperature for 3 days and dried in oven at 40°C for 48. The dried leaves were crushed to a 30 mesh powder. The dried powder was stored at -20°C. Flavonoids (flavonols rutin, quercetin, kaempferol, myricetin and flavones apigenin and luteolin), tert-butylhydroquinone (TBHQ) were purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile, methanol, HCl and formic acid were obtained from E-Merck (Darmstadt, Germany).

The flavonols quercetin, kaempferol, myricetin and flavones apigenin and luteolin in the leaves of three accessions of *S. arvensis* were analyzed according to the method by Andarwulan et al. (2010). The dried powder of sample (0.5 g) was extracted for 1 h at 50°C in 50% aqueous methanol containing 1.2 M HCl and 0.5 g/L TBHQ. The extract was allowed to cool and the solution was made up to 100 mL with methanol. Approximately 2 mL was filtered through a 0.45 µm filter (PTFE; P/N E252, Whatman, Buckinghamshire, Inggris). The HPLC (Shimadzu, Japan) instrument was equipped with a model series LC-20 AD pump, DGU-20As degasser, FCV-10ALVP, CBM-20A system controller, SIL-20A autosampler, CTO-20AC column oven, and a SPD-M20A Diode array detector. System control and data analyses were carried out using LC solution software (Shimadzu, Japan). Separation and determination were done on a Shimpack VP-ODS columb (150 mm × 4.6 mm i.d.Shimadzu, Japan) using a one step linear gradient. Mobile phase A (methanol) and B (0.2% formic acid water solution) ratios changed as follows: 0-10 min, 15%-35% A; 10-45 min, 35%-50% A; 45-50 min, 50%-80% A; 50-60 min, 80%-95% A; 60-65 min, 100% A. After each run the chromatographic system was set to 0% B for 5 minutes and equilibrated for 5 minutes. The total run time was 75 minutes at a flow rate of 1 mL/min. The eluent was monitored by a diode array detector, flavonoids were quantified on the basis of comparison to standards at 270 nm for luteolin and kaempferol, 330 for rutin and myricetin and 340 for quercetin and apigenin. The sample injection volume was 10 µL, and the column temperature was set at 35°C.

The flavones and flavonols derivatives were quantified using the linear calibration curves. Standard curve regression equations were y = 12205x+5312, R2 = 0.999 for rutin, y = 31936x-3061, R2 = 0.998 for myricetin, y = 23213x-4353, R2 = 0.999 for luteolin, y = 40737x+1426, R2 = 0.999 for quercetin, y = 31664x-12548, R2 = 0.999 for kaempferol and y = 41007x-2911, R2 = 0.999 for apigenin, where Y was the detector response and X was concentration of standards. Stock solutions of individual standards (10 mg/L) were prepared via dissolution of the appropriate amounts in methanol. The stock solutions were stored at -20°C. Linearity was assessed using seven standard concentrations, 0.156, 0.312, 0.625, 1.25, 2.5, 5 and 10 mg/L. The limits of detection (LOD) and quantitation (LOQ) were calculated using signal-to-noise ratios of 3 and 10, respectively. The limits of detection (in µg/ml on column) were 0.12 for rutin, 0.28 for myricetin, 0.11 for luteolin, 0.07 for quercetin, 0.03 for kaempferol and 0.04 for apigenin. Inter and intra-assay CV for quantification ranged from 2.69 to 5.0 and 3.19 to 4.99, respectively for flavonoid standards.

Figure 2. The performance and number of sowthisthle rosette and upper leaves harvested simultaneously. A-B. The total rosette leaves. C. The upper leaves

Figure 3. Performance and number of sowthisthle leaves harvested gradually. A. Rosette leaves harvested in the bolting phase. B. Upper leaves harvested in the blooming phase

Observed variables

The observations were conducted on agronomical characters of plant (e.g., leaf number, leaf size, fresh and dry weight of leaf per plant), and chemical contents (e.g., Chlorophyll, anthocyanin, and carotenoid) following Budiarto et al. (2019), while vitamin C with AOAC (2006). Other analyses included total flavonoid content (Chang et al. 2002), antioxidant activity (IC_{50}) (Wahyuni et al. 2022), profile flavonoid, concentration of N (Kjeldahl), P (Olsen), K (HCl 25%), and nutrient uptake.

Statistical analysis

Data were analyzed using Minitab 16. Data were presented as the mean values ± standard error (SE) of three replications. Results were analyzed by one-way ANOVA to identify significant differences between the groups and compare the means test analyzed by Tukey with α=0.05.

RESULTS AND DISCUSSION

Soil chemical properties

Following the methods prescribed by the Center for Soil and Agro-climate Research, soil samples for analysis were taken at a depth of 0-15 cm. The results of soil analysis in the experimental area showed a soil pH of 5.76, indicating slightly acidic category. Analysis of soil nutrient status showed a total N value of 0.21% (moderate), a total P of 68.60 mg.100 g^{-1} (very high), a total K of 57.5 mg.100 g^{-1} (high) and an organic C of 1.79% (low). Therefore, fertilization was carried out using goat manure to increase nutrient contents and lime to increase pH. Sowthistle can adapt to soils with a pH range of 5.2 to 7.2 (Nurhayati 2016; Wahyuni et al. 2019).

The addition of goat manure and residue from previous fertilization resulted in sufficient availability of N, P and K contents of nutrients. This can be seen from the N, P and K contents of the leaves. The results of the N analysis of the rosette leaves showed a value of 2.98-3.41%, and P of 0.29-0.33%. Meanwhile, in the upper leaves, the N and P contents of the leaves were 3.31-3.59% and 0.31-0.35%. The adequacy of N nutrients is 2.5-3.5%, and P is 0.2-0.4%. The K content of the analysis results showed a value of 5.81-6.28% for the lower leaves and 4.99-5.96% for the upper leaves. The K value obtained in the study was higher than the K nutrient adequacy value, which was 1-3%.

Leaf growth and production

Rosette leaves

In general, the results showed that leaf growth and rosette leaf production of sowthistle in the simultaneous harvest treatment was better than those in the gradual harvest treatment. The leaf number, leaf fresh and dry weight of sowthistle of different harvest methods and accessions are presented in Table 1. The number of rosette leaves in simultaneous harvest was 65% higher than those in gradual harvest. The findings indicated that when rosette leaves were harvested before the upper leaves, the growth and development of these leaves were not at the maximum stage yet, and they continued to grow even though the plant had entered the generative phase. In line with the leaf number, the fresh weight and dry weight of the rosette leaves in simultaneous harvest were 55 and 60% higher than those in gradual harvest.

The differences in characteristics among accessions were found in leaf size and leaf yield. Tawangmangu accessions had 8 and 9% wider rosette leaves than Lembang and Banjararum, respectively. Weight of fresh leaves were not different between Lembang and Tawangmangu accessions but 6% and 29% higher respectively, than leaf weight of the Banjararum accession (Table 1).

Plant growth is determined by the nutrients absorbed. Simultaneous harvest absorbed higher nitrogen and phosphorus than gradual harvest (Table 2) and can use nitrogen and phosphorus more efficiently to increase the number of leaves, fresh weight, and dry weight of plants. Adequate levels of nitrogen and phosphorus in leaves can increase total chlorophyll. Nitrogen is a constituent of many important molecules, including chlorophyll. Phosphorus is also an important structural component of nucleic acids, coenzymes, nucleotides, phosphoproteins, phospholipids, and sugar phosphates (Elboraie and Kasem 2019).

Nitrogen increases plant growth through photosynthetic activity. The photosynthetic capacity of leaves is related mainly to nitrogen concentration because proteins in the Calvin cycle and thylakoids represent the majority of nitrogen in leaves. Therefore, nitrogen can increase photosynthesis and then increases plant growth. Previous report on broccoli showed that the interaction between N and B was significant on plant height, leaves area, leaves fresh weight, leaves dry matter percent, leaves mineral content such as N, P and K, chlorophyll a, chlorophyll b, carotenoids, vitamin C, T.S.S., N, P, K and nitrate content (Metwaly 2016).

Upper leaves

Gradual harvest of upper leaves affected leaf width. The upper leaves were 30% wider in gradual harvests than that in simultaneous harvest (Table 3). When rosette leaves were harvested prior to the upper leaves, the plant lose its resource for photosynthesis because the rosette leaves are a strong resource. Lack of resource causes the upper leaves, besides as a sink for plants, to expand the leaf surface to increase the capture of sunlight in the photosynthesis processes in which the products will be used for plant recovery, rejuvenation, and development until flowering. The limited resources can inhibit the growth rate and yield of a plant. This can be seen from the number of leaves, fresh weight and dry weight of upper leaves which were not significantly different between upper leaves that were harvested in stages and simultaneously.

Leaf production in term of fresh weight and dry weight of upper leaves was more influenced by accession. The difference between accessions occurred in the fresh weight and dry weight of upper leaves. Tawangmangu accession had leaf fresh weight and leaf dry weight18 and 28%, respectively, higher than Lembang and Banjararum accessions (Table 3).

Tawangmangu accession showed higher production than Lembang and Banjararum accessions. This was presumably because Tawangmangu accessions absorbed significantly higher phosphorus nutrients than Lembang and Tawangmangu accessions (Table 4). The presence of significantly higher phosphorus nutrients was thought to be able to support upper leaf production in Tawangmangu accession.

Table 1. Effect of harvesting methods and accessions on average number, width, fresh weight and dry weight of rosette leaves of sowthistle

Treatment	Leaf number	Leaf area/ plant (cm^2)	Leaf fresh weight/plant (g)	Leaf dry weight/plant (g)
Harvest methods				
Gradual	37.5±2.24[b]	1908.11±45.05	89.64±7.86[b]	8.06±1.46[b]
Simultaneous	61.8±3.52[a]	2005.08±40.55	138.66±7.43[a]	12.90±0.34[a]
Accessions				
Lembang	49.8±1.98	1915.60±17.72[b]	119.21±4.93[a]	10.83±1.30
Tawangmangu	54.8±4.34	2062.23±37.74[a]	125.96±6.78[a]	11.23±1.14
Banjararum	47.7±2.79	1891.95±32.50[b]	97.29±7.76[b]	9.38±1.12
Interaction	ns	ns	ns	ns

Notes: Numbers followed by different letters in the same column show significant differences based on Tukey's test at the level of significance α=5; ns: non-significant; n=3

Table 2. Effect of harvesting methods and accessions on nutrient uptake of rosette leaves of sowthistle

Treatment	Nitrogen/plant (mg)	Phosphorus/plant (mg)	Potassium/plant (mg)
Harvest methods			
Gradual	27.54±3.70[b]	2.71±0.31[b]	48.95±5.70
Simultaneous	38.42±1.08[a]	3.69±0.08[a]	77.50±1.23
Accessions			
Lembang	34.52±3.48	3.27±0.30	65.15±5.36
Tawangmangu	33.06±3.52	3.43±0,25	65.80±5.10
Banjararum	31.37±2.31	2.90±0.28	58.72±4.16

Notes: Numbers followed by different letters in the same column show significant differences based on Tukey's test at the level of significance α=5; n=3

Table 3. Effect of harvesting methods and accessions on average number, width, fresh weight and dry weight of upper leaves of sowthistle

Treatment	Leaf number	Leaf area/ plant (cm^2)	Leaf fresh weight/plant (g)	Leaf dry weight/plant (g)
Harvest methods				
Gradual	17.7±3.53	937.50 ±49.96[a]	37.30±6.41	3.85±0.39
Simultaneous	15.8±2.19	711.48 ±40.59[b]	41.61±4.50	3.94±0.42
Accessions				
Lembang	15.8±1.28	780.43 ±58.06	35.94±2.61[b]	3.78±0.32[b]
Tawangmangu	18.0±1.50	783.29 ±50.15	46.30±1.83[a]	4.59±0.28[a]
Banjararum	16.4±2.79	909.75 ±25.12	36.16±4.93[b]	3.32±0.30[b]
Interaction	ns	ns	ns	ns

Notes: Numbers followed by different letters in the same column show significant differences based on Tukey's test at the level of significance α=5; ns: non-significant; n=3

Table 4. Effect of harvesting methods and accessions on nutrient uptake of upper leaves of sowthistle

Treatment	Nitrogen/plant (mg)	Phosphorus/plant (mg)	Potassium/plant (mg)
Harvest methods			
Gradual	13.32±0.55	1.33±0.05	23.00±2.62
Simultaneous	13.38±1.51	1.21±0.04	19.68±2.05
Accessions			
Lembang	12.88±0.93	1.17±0.06[b]	19.92±1.24
Tawangmangu	15.33±0.17	1.55±0.05[a]	21.21±1.12
Banjararum	11.86±0.90	1.10±0.02[b]	22.87±3.10

Notes: Numbers followed by different letters in the same column show significant differences based on Tukey's test at the level of significance α=5; n=3

Total chlorophyll, carotene and anthocyanin levels

Differences in harvesting methods caused differences in total chlorophyll and anthocyanin levels in rosette and upper leaves (Table 5). Simultaneous harvesting resulted in 17% higher total chlorophyll content than when harvested gradually. The method of harvesting did not affect the anthocyanin levels in rosette leaves, although there was a tendency for simultaneous harvests to produce higher levels of anthocyanins than in gradual harvests. Accessions had no significant difference in total chlorophyll content and lower leaf anthocyanin levels.

Chlorophyll, which functions to capture solar energy, will increase the process of photosynthesis which is the procurement of energy that will be used for the synthesis of macro-molecules in cells, including carbohydrates. The results of this macro-molecular synthesis after several changes will become food reserves and will be accumulated in young tissues that grow like plants that are getting taller, the number of leaves is increasing, the diameter and width of the leaves are increasing.

As with the rosette leaves, the method of harvesting the upper leaves affected the total chlorophyll content but did not cause a difference in the anthocyanin levels. Simultaneous harvesting produced a total chlorophyll content of 22% higher than when harvested gradually. There was no significant difference in the total chlorophyll and upper leaf anthocyanin levels among accessions (Table 5). In both lower and upper leaves, the carotene content was affected by the harvest method, while the inter-accession test had no effect. Simultaneous harvesting produced higher levels of carotene when compared to gradual harvesting (Figures 4 and 5).

The levels of vitamin C in the upper leaves harvested gradually were higher than those harvested simultaneously ($p>0.05$). There was no difference in vitamin C levels between accessions although there was an indication that the value of both variables was higher in Tawangmangu accession (Table 6).

Total flavonoid content and antioxidant activity

Rosette leaves

The high total flavonoid content of rosette leaves in Banjararum accession harvested simultaneously (Figure 6A) was likely because simultaneous harvesting delayed the harvest of rosette leaves for two weeks so that lower leaf growth was still ongoing, and showed maximum growth, so it had more photosynthate to increase secondary metabolism.

The smaller IC50 value of an extract, the better antioxidant potential (Dzoyem et al. 2014). Antioxidant activity in rosette leaves ranged from 35.79 (Tawangmangu accession that was harvested simultaneously) to 83.52 µg mL^{-1} (Banjararum accession that was harvested simultaneously) (Figure 6B). The other studies in callus extract of *S. arvensis* had antioxidant activity around 22.56-145.7 µg mL^{-1} (Wahyuni et al. 2022).

The rosette leaves of the Tawangmangu accession harvested simultaneously showed a low total flavonoid content but high antioxidant activity (Figure 6). In general, it is known that there is a relationship between phenolic levels and antioxidant activity. The high antioxidant activity of the rosette leaves of the Tawangmangu accession harvested simultaneously was thought to be contributed by vitamin C, anthocyanins, and carotenoids apart from total flavonoids. This assumption is that antioxidant activity does not only come from flavonoids. Similarly, Sajirani et al. (2014) stated that anthocyanins, which are part of flavonoid compounds, have antioxidant effects, as well as terpenoids and ascorbic acid. Further Albishi et al. (2013) reported that high levels of phenolic compounds were not accompanied by high antioxidant activity, possibly due to differences in chemical constituents that contributed to the capture activity.

Table 6. Effect of harvesting methods and accessions on vitamin C levels of sowthistle leaves

Treatment	Vitamin C ($mg.100g^{-1}$ FW)	
	Rosette leaves	Upper leaves
Harvest methods		
Gradual	14.84±1.01	19.88±0.85[a]
Simultaneous	14.05±0.48	17.55±1.66[b]
Accessions		
Lembang	14.08±0.71	18.07±0.64
Tawangmangu	14.28±0.42	19.41±0.96
Banjararum	15.00±0.24	18.65±0.60
Interaction	ns	ns

Notes: Numbers followed by different letters in the same column show significant differences based on Tukey's test at the level of significance α=5%, ns: not significant; n=3; FW: fresh weight

Table 5. Effect of harvesting methods and accessions on sowthistle chlorophyll and anthocyanin levels

Treatment	Rosette leaves		Upper leaves	
	Total chlorophyll (mg/g)	Anthocyanin (mg/g)	Total chlorophyll (mg/g)	Anthocyanin (mg/g)
Harvest methods				
Gradual	1.52±0.05[b]	0.04±0.006	1.72±0.18[b]	0.06±0.001
Simultaneous	1.78±0.18[a]	0.06±0.001	2.18±0.27[a]	0.05±0.001
Accessions				
Lembang	1.66±0.13	0.05±0.002	1.98±0.30	0.06±0.009
Tawangmangu	1.66±0.28	0.04±0.006	1.94±0.21	0.06±0.008
Banjararum	1.63±0.09	0.06±0.002	1.93±0.23	0.05±0.007
Interactions	ns	ns	ns	ns

Notes: Numbers followed by different letters in the same column show significant differences based on Tukey's test at the level of significance α=5; ns: non-significant; n=3

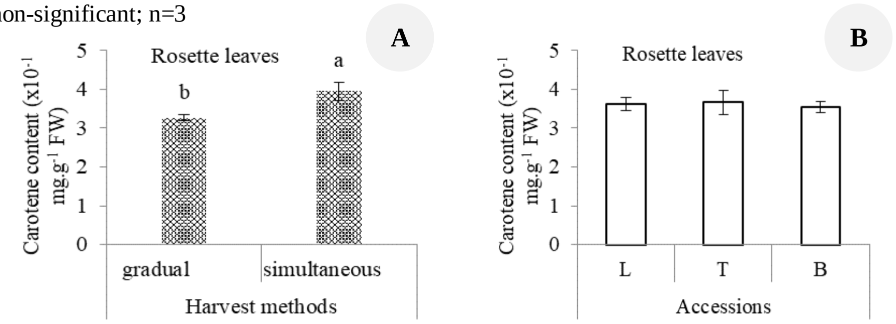

Figure 4. The carotene content of the rosette leaves of sowthistle in different treatments: A. Harvesting method; B. Accession. Notes: L: Lembang; T: Tawangmangu; B: Banjararum. Different letters show significant differences based on Tukey's test at the level of significance α=5%, error bar: standard error, n=3; FW: fresh weight

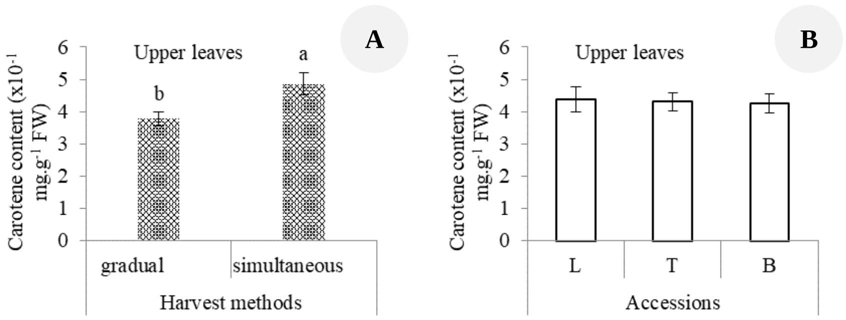

Figure 5. Carotenoid content of sowthistle upper leaf in different treatments: A. Harvesting method; B. Accession. Notes: L: Lembang, T: Tawangmangu, B: Banjararum. Different letters show significant differences based on Tukey's test at the level of significance α=5%; error bar: standard error; n=3; FW: fresh weight

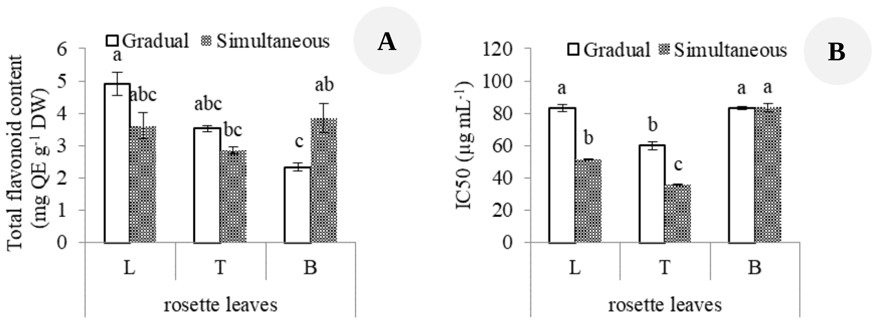

Figure 6. Effect of harvesting methods and accessions on: A. total flavonoid content; B. and antioxidant activity of sowthistle rosette leaves. Different letters show significant differences based on Tukey's test at a significance level of α=5%, error bar: standard error, L: Lembang, T: Tawangmangu, B: Banjararum, QE: Quercetine Equivalent, DW: dry weight

Upper leaves

The upper leaves of Tawangmangu accession harvested simultaneously had high total flavonoid content and high antioxidant activity (Figures 7A and 7B). This indicates that the high antioxidant activity in Tawangmangu accession harvested simultaneously mostly originated from total flavonoids. The total flavonoid content in this study was higher than that of the Lembang, Tawangmangu and Banjarmarum accessions in ex-situ planting, namely 1.01, 0.88 and 0.83 mg $QE.g^{-1}$ DW (Raisawati et al. 2020). According to Alirezalu et al. (2018), in general, differences in phenolic and flavonoid levels in plant species are mainly related to differences in the growing environment and genetic factors. In *Crataegus* spp, total flavonoid levels are influenced by interactions between varieties and plant parts including flowers and leaves (Alirezalu et al. 2018).

The result of antioxidant activity in this study was higher than previous study, which found antioxidant activity in Lembang, Tawangmangu and Banjararum accessions of 318.88, 282.88 and 279.39 µg mL^{-1} (Raisawati et al. 2020). The difference in the results of antioxidant activity and flavonoid levels can be caused by differences in geography, climate, soil conditions, treatment, herbicides and pesticides used. Herlina et al. (2017) reported that in black cumin malondialde-hyde (MDA) levels are influenced by accession and environmental cultivation.

Flavonoid profiles

Rosette leaves

There was an interaction effect between harvesting method and accession on the contents of quercetin and kaempferol. The kaempferol content in Tawangmangu accession was 68% lower by simultaneous harvest than when it was gradually harvested, but the kaempferol content was not significantly different between Lembang and Banjararum accessions. The rosette leaves of Banjararum accession harvested simultaneously had 35% higher quercetin content than those gradually harvested, but in Lembang and Tawangmangu accessions the quercetin content did not differ between harvesting methods (Table 7).

Rutin, myricetin, luteolin and apigenin were not affected by the interaction. Harvesting methods did not cause differences in contents of rutin, myricetin, and luteolin, but significant difference was found in apigenin contents. Simultaneous harvesting caused apigenin contents to be significantly higher by 44% compared to gradual harvesting (Table 8).

Accessions had significant differences in the contents of rutin, myricetin and apigenin, but not in luteolin. When compared to the Banjararum accession, the Tawangmangu accession yielded 79% significantly higher rutin contents, 41% and 50% higher myricetin and apigenin contents but not significantly higher than the Lembang accession. Banjararum accession resulted in significantly lower contents of rutin, myricetin and apigenin (Table 8).

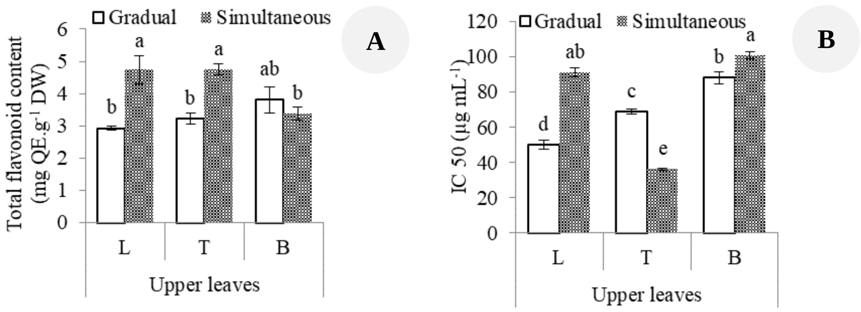

Figure 7. Effect of harvesting method and accession on: A. Total flavonoid content; and B. Antioxidant activity, of sowthistle upper leaves. Different letters show significant differences based on Tukey's test at a significance level of α=5%, error bar: standard error, L: Lembang, T: Tawangmangu, B: Banjararum, QE: Quercetine Equivalent, DW: dry weight

Table 7. Effect of interaction between harvest method and accession on quercetin and kaempferol of sowthistle rosette leaves

Harvest methods	Accessions		
	Lembang	Tawangmangu	Banjararum
	Quercetin (mg g^{-1} DW)		
Gradual	0.51±0.06^{a}	0.59±0.04^{a}	0.43±0.01^{b}
Simultaneous	0.51±0.08^{a}	0.50±0.07^{a}	0.58±0.09^{a}
	Kaempferol (mg g^{-1} DW)		
Gradual	1.07±0.16^{ab}	1.11±0.15^{a}	0.74±0.13^{ab}
Simultaneous	1.04±0.20^{ab}	0.66±0.10^{b}	1.02±0.14^{ab}

Notes: Numbers followed by different letters in the same column show significant differences based on Tukey's test at the content of significance α=5%; ns: non-significant; n=3

Table 8. Effect of harvesting methods and accessions on the contents of rutin, myricetin, luteolin and apigenin of sowthistle rosette leaves

Treatment	Rutin (mg g^{-1})	Myricetin (mg g^{-1})	Luteolin (mg g^{-1})	Apigenin (mg g^{-1})
Harvest methods				
Gradual	0.38±0.04	0.27±0.01	0.50±0.03	0.16±0.01^{b}
Simultaneous	0.44±0.04	0.27±0.02	0.51±0.02	0.23±0.03^{a}
Accessions				
Lembang	0.41±0.05^{b}	0.31±0.02^{a}	0.54±0.04	0.20±0.02^{ab}
Tawangmangu	0.52±0.05^{a}	0.28±0.04^{ab}	0.51±0.03	0.24±0.02^{a}
Banjararum	0.29±0.02^{c}	0.22±0.02^{b}	0.47±0.02	0.16±0.03^{b}

Notes: Numbers followed by different letters in the same column show significant differences based on Tukey's test at the content of significance α=5%; ns: non-significant; n=3

The Tawangmangu and Lembang accessions in general showed results that were not significantly different (Tables 7 and 8). This is thought to be caused by differences in the origin of the accessions and the adaptability of each accession. The Tawangmangu and Lembang accessions were obtained from cultivation environment, so they have genotypes that are more adaptable to cultivation treatments. The Tawangmangu accession originated from plants cultivated at the Center for Research and Development of Medicinal and Traditional Medicinal Plants in Tawangmangu and the Lembang accession grew wild at the Manoko Experimental Garden, Research Institute for Spices and Medicinal Plants in Lembang. Meanwhile, the accession of Banjararum originated from wild plants, so it may experience delays in adaptation when it is cultivated. Banjararum accession required a longer time to adapt, both vegetative growth and generative growth. From the observations, it was found that the bolting time for the Banjararum accession was 10-14 days slower than the Lembang and Tawangmangu accessions. Khan (2012) stated that differences in secondary metabolite biosynthesis can be caused by genetics and plant physiological responses.

The kaempferol content produced by the Tawangmangu accession harvested gradually contributed to 32% of total flavonoids, and 5-7% of apigenin. Andarwulan et al. (2010) reported that in *Talinum triangulare* (Jacq) Wild plants, the largest contribution of flavonoids originated from kaempferol (90%) and the lowest was quercetin. While on *Pluchea indica* Less. the biggest contribution of flavonoids originated from quercetin (81%) while the lowest were from myricetin and kaempferol.

Upper leave

The contents of the six types of flavonoids were influenced by the interaction between harvesting method and accession. The contents of rutin, kaempferol and apigenin in the three accessions did not differ between harvesting methods but the trend was different. There were compounds with higher contents and lower ones when harvested simultaneously. Significant differences between harvesting methods in particular accessions were found in the contents of myricetin, luteolin and quercetin (Table 9).

The contents of rutin, myricetin, luteolin, quercetin, kaempferol and apigenin in the upper leaves were the result of interactions between harvesting method and accession (Table 9). In general, the simultaneous harvest in the Lembang and Tawangmangun accessions showed higher yields than the simultaneous harvest in the Banjararum accession. It is likely that high contents of flavonoids in the simultaneous harvest of Lembang and Tawangmangu accessions are related to total chlorophyll (Table 5). High total chlorophyll in simultaneous harvests indicated that sufficient photosynthate was available for the biosynthesis of flavonoids.

Banjararum accession with gradual harvesting produced high contents of kaempferol (Table 9), indicating that the upper leaves harvested gradually experienced photosynthate deficiency. In gradual harvesting, the rosette leaves were harvested first causing a loss of source so that the upper leaves did not receive photosynthates from the rosette leaves. Injury due to harvesting of the lower leaves caused the plants to recover and rejuvenate new leaves. Rejuvenation due to harvesting of the lower leaves caused the leaves to become strong sink organs.

Another possibility is that the high content of kaempferol in the gradual harvest of Banjararum accession was due to the gradual harvest showing a high content of K nutrients (Table 4) and its interaction with the genotype of Banjararum accession. One of the functions of K is in enzymatic activity in the synthesis of carbohydrates and proteins. The presence of high K nutrients increased enzymatic processes thereby increasing kaempferol biosynthesis.

The kaempferol content produced by Banjararum accession harvested gradually contributed to 37% of the total flavonoid content, while the lowest contribution was apigenin which was produced by Banjararum accession harvested simultaneously, which was 4%. According to Petrussa et al. (2013), biosynthesis of flavonoids is highly correlated with enzymatic pathways and genetic characters in various species. It was found that the contents of kaempferol (1.39±0.10 mg g^{-1}) and rutin (0.81±0.07 mg g^{-1}) were 32 and 43% higher than the results of Khan (2012), on the other hand, the contents of quercetin (0.73±0.03 mg g^{-1}) and myricetin (0.43±0.06 mg g^{-1}) were 7 and 50% lower. The contents of luteolin (0.82±0.03 mg g^{-1}) were 27% higher than the BPPOM standard.

Table 9. Effect of interaction between harvest methods and accessions on rutin, myricetin, luteolin, quercetin, kaempferol and apigenin of sowthistle upper leaves

Harvest methods	Accessions		
	Lembang	**Tawangmangu**	**Banjararum**
	Rutin (mg g^{-1} DW)		
Gradual	$0.54±0.10^{bc}$	$0.61±0.12^{abc}$	$0.47±0.09^{bc}$
Simultaneous	$0.69±0.11^{ab}$	$0.81±0.07^{a}$	$0.37±0.08^{c}$
	Myricetin (mg g^{-1} DW)		
Gradual	$0.27±0.04^{c}$	$0.40±0.03^{ab}$	$0.28±0.04^{bc}$
Simultaneous	$0.41±0.04^{a}$	$0.36±0.04^{abc}$	$0.43±0.06^{a}$
	Luteolin (mg g^{-1} DW)		
Gradual	$0.15±0.\ 02^{c}$	$0.24±0.02^{c}$	$0.27±0.01^{c}$
Simultaneous	$0.78±0.08^{ab}$	$0.82±0.03^{a}$	$0.68±0.08^{b}$
	Quercetin (mg g^{-1} DW)		
Gradual	$0.64±0.12^{a}$	$0.65±0.07^{a}$	$0.71±0.05^{a}$
Simultaneous	$0.65±0.01^{a}$	$0.73±0.03^{a}$	$0.56±0.04^{b}$
	Kaempferol (mg g^{-1} DW)		
Gradual	$0.89±0.13^{b}$	$0.85±0.11^{b}$	$1.39±0.10^{a}$
Simultaneous	$1.08±0.12^{ab}$	$1.10±0.12^{ab}$	$1.09±0.21^{ab}$
	Apigenin (mg g^{-1} DW)		
Gradual	$0.29±0.05^{ab}$	$0.26±0.04^{ab}$	$0.21±0.04^{bc}$
Simultaneous	$0.31±0.05^{ab}$	$0.35±0.02^{a}$	$0.12±0.02^{c}$

Notes: Numbers followed by different letters in the same column show significant differences based on Tukey's test at the content of significance α=5%; ns: non-significant; n=3

In conclusion, our study revealed that simultaneous harvesting resulted in higher fresh weight and rosette leaf dry weight than those gradual harvesting. The Tawangmangu accession produced higher fresh weight and dry weight than the Lembang and Banjararum accessions. Tawangmangu accession harvested simultaneously produced the highest total flavonoid contents and antioxidant activity of upper leaves. The flavonoid profiles obtained on the rosette leaves and upper leaves of sowthistle across different harvesting methods and accessions were rutin, myricetin, luteolin, quercetin, kaempferol and apigenin. Kaempferol showed the highest contents of flavonoids among the six types of flavonoids. Luteolin levels which were higher than the BPOM standard (0.06%) were found in the upper leaves harvested simultaneously. Simultaneous harvesting is therefore suggested to obtain high yield and quality of sowthistle.

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