

Red Guava Leaf Harvesting Impact on Flavonoid Optimization in Different Growth Phases

SANDRA ARIFIN AZIZ*, MUNIF GHULAMAHDHI

*Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University,
Kampus Darmaga, Bogor 16680, Indonesia*

Received February 4, 2011/Accepted July 29, 2011

Harvesting process is a critical time to identify the quality of raw material for traditional medicine. The time and harvesting techniques, drying process after harvesting, and processing to make the *simplicia*, are the crucial role to make the good quality of the natural product. On the other hand, there is a lack of general understanding and appreciation about the processes involved in governing shoot and tree growth and development, i.e. red guava. The research objective was to evaluate the influence of leaf harvesting and growth phases on red guava for flavonoid production as antioxidant. Randomized factorial block design in time were laid out with two factors and followed by Duncan's multiple range test. The treatments were the amount of leaf harvested on tertiary branches (0, 25, 50, and 100%) and growth phases of the plant (vegetative and generative). Leaf harvesting 25% on tertiary branches significantly increased the leaf number (766.3 tree⁻¹) and the number of new quaternary branches, decreasing leaf area index (LAI) and leaf dry weight at the end of the experiment (22 weeks of observation/WO). The highest leaf dry weight (156.94 g tree⁻¹) and LAI (0.47) was found in harvesting 25% tertiary branches. Harvesting 100% leaf on tertiary branches in vegetative phase significantly produced the lowest flavonoid production (7.82 g tree⁻¹). The result suggested that flavonoid production from red guava leaves should be done by harvesting 50% leaf on tertiary branches in generative phase can be used to produce the highest flavonoid (89.90 g tree⁻¹).

Key words: red guava, leaf flavonoid, growth phases, harvest

INTRODUCTION

Harvesting process is a critical time to identify the quality of raw material for traditional medicine. The time and harvesting techniques, drying process after harvesting, and processing to make the *simplicia*, are the crucial role to make the good quality of the natural product. Garcia *et al.* (2003) stated that ineffective handling and the decompartmentation of enzymes or degradation of chemical compound, will change the chemical properties and decline the special quality or even changing the content of secondary metabolite, so its uses as medicine were not achieved.

Lugasi *et al.* (2003) stated that flavonoid as the most important group in plants, has antioxidant properties and can be group further as 13 groups. Chan *et al.* (2010) stated that guava has antioxidant properties (AOP) in total phenolic content, radical-scavenging activity, ferric-reducing power and ferrous ion-chelating (FIC) ability that is comparable to black teas, and generally herbal teas had a lower antioxidant values than teas of *C. sinensis*. Qian and Nihorimbere (2004) stated that total phenolic in guava is 575.3 ± 15.5 and 511.6 ± 6.2 mg equal to galic acid g⁻¹ leaf dried weight. Gutiérrez *et al.* (2008) stated that extracts and metabolites of this plant, particularly those from leaves and fruits possess useful pharmacological activities. *P. guajava* is mainly known for its antispasmodic and

antimicrobial properties (Lutterodt *et al.* 1999), and has also been used extensively as a hypoglycaemic agent. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepatoprotection, anti-allergy, antigenotoxic, antiplasmodial, cytotoxic, cardioactive, anticough (Garcia *et al.* 2003), antidiabetic, antiinflammatory activities, supporting its traditional uses, that suggested a wide range of clinical applications for the treatment of infantile rotaviral enteritis, diarrhoea and diabetes. Garcia *et al.* (2003) stated that other uses of guava leaves extract are as antimutagenic, and as cure for asthma. Cushnie and Lamb (2005) stated that increasingly, this class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral, and antibacterial activity. Moreover, several groups have demonstrated synergy between active flavonoids as well as between flavonoids and existing chemotherapeutics. The extraction of the phenolic fraction from guava seeds (*Psidium guajava* L.) at various operating conditions was explored (Castro-Vargas *et al.* 2010), but not from the leaves.

Leaf harvest will decrease the leaf number per plant, which also is one of pruning product. Dickson *et al.* (2000) found that different growth phases in plant, when the plant was dominated by vegetative growth or when the plant started the generative phase, were one of the factor that influenced the assimilate. Trentacoste *et al.* (2010) showed that on olive tree, the olive oil yield and its components (fruit number, average fruit weight, and fruit

*Corresponding author. Phone/Fax: +62-251-8629353,
E-mail: sandraaziz@yahoo.com

oil concentration) depend on crop load and source-sink ratios as affected by environmental conditions, management and the alternate bearing typical of the species. On olive orchards (Vilalobos *et al.* 2006), the wide variability and complexity makes it difficult to provide solutions to the numerous management questions using a pure experimental approach. Aboveground accumulated biomass was allocated equally to fruits and vegetative growth, which in turn was partitioned into 30% for leaves and 70% for stems, branches and trunk. A model of growth and yield and may be useful not only for evaluating productivity at different scales but also for solving different management problems (nutrient requirements, plant protection, etc.).

This research was aimed to study the influence of plant growth phases and the amount of leaf harvested to the growth and development of red guava and flavonoid production from the leaves.

MATERIALS AND METHODS

The research was carried out on March to November 2006 at Biopharmaca Research Station Bogor Agricultural University and Bogor Agro Lestari Laboratory cooperating with Post Harvest Research and Development Institute Bogor, Indonesia. Four year-old red guava trees from air layering, hand counter, and UV spectrometer were used.

The red guava plants were planted in a terraced land with 30° incline. Pretreatment by picking all the flower and fruit were used to uniform the phases of each of the treatments and given at the same time (0 WO). Liming with 0.5 kg dolomit plant⁻¹ and 10 kg lamb manure plant⁻¹ were given for conditioning, and 90 g Urea: SP-36 : KCl = 1 : 1 : 1 plant⁻¹ were the basic treatment. To induce the new leaf growth, 3 g Gandasil-D 1⁻¹ water were given weekly up to 1 week before the treatments were applied.

Randomized factorial block design in time with Duncan’s multiple range test (DMRT) with α=5% were used in this experiment. The first factor was leaf harvesting (Table 1) and the second factor were: (i) vegetative (all flower and fruit picked at the pretreatment and fruit picked up to ± 1 cm in diameter, to observe the generative organ development) and (ii) generative phase (all flower and fruit picked at the pretreatment and all flower and fruit remained intact throughout the experiment).

The first harvest at the beginning of the experiment was done when 50% of the plants flowered from the

generative phase treatment (0 WAP). The fully opened leaves were handpicked from the tip to the base of the tertiary to pentanary branches, without the leaf bud. If there is no leaf on the tertiary branch, then the leaf from the tertiary lateral branches will be harvested. The second harvest was at the end of the experiment (22 WO). Rutin (molecules weight = 610.53 dalton) were used as standard for quantitative analysis of total flavonoid.

Variables observed from 2-22 WO were: the leaf number increase, tertiary and quartenary branches number, the time of quartenary branches appearance, fruits number, leaf wet and dried weight (at the beginning and the end of the experiment), leaf area index (LAI, at the beginning and the end of the experiment), leaf area plant⁻¹ (=leaf weight plant⁻¹/leaf weight 50 leaf⁻¹ x leaf area 50⁻¹leaf⁻¹; leaf area 50⁻¹leaf⁻¹ = length x width of the 50 leaf sample), flavonoid production (= flavonoid concentration in the leaf x leaf dry weight) at the beginning and the end of the experiment.

RESULTS

Overall Condition. Pretreatment to make the plants uniform were given in the middle of March 2006, with rain intensity 138.30 mm month⁻¹ and 84% humidity. The first leaf harvesting was at the end of May 2006, with high rain intensity of 324 mm month⁻¹. Average humidity was almost the same from the condition at the beginning of the uniforming the plants. Rain intensities fluctuated up to the end of the experiment (at beginning of November 2006), but for overall throughout the experiment, the months were dry months (Figure 1). Overall condition of the leaf, flower and fruit growth can be seen at Table 2.

Growth phases significantly affected the fruit number at 1-13 WO. Harvesting significantly affected the leaf number 1-17 WO, the tertiary branches number 10-22 WO, the quartenary branches number 6-22 WO, the time of quartenary branches appearance and leaf wet weight at the end of the experiment (Data not showed). Treatments interaction significantly affected flavonoid production at the end of the experiment.

Leaf Increase. The two growth phases had almost similar leaf increase, but at 2-10 WO, the vegetative phase plants treatment had more increase than generative phase; at 11-17 WO the generative phase plants had more leaf increase, and at 18-22 WO the vegetative phase plants had more leaf increase (Data not showed). Table 3 showed

Table 1. Leaf harvesting treatment of red guava

Initial harvest of tertiary branches at 0 WO) (the beginning of the observation)	Combination of leaf harvesting treatment	
	The end of the experiment at 22 WO	Quaternary branches of 75% of the plants with the same treatment had pentanary branches with 3 pairs of leaf and etc
0 (without)	100	100
25	25	25
50	50	50
100	100	100

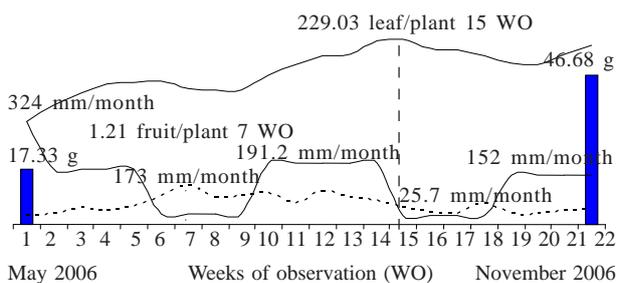


Figure 1. Leaf number increase, fruit number and flavonoid production at the beginning and the end of the experiment. —: Flavonoid production, —: Leaf number increase, - - -: Fruit number, —: Rainfall intensity.

Table 2. Overall condition of the leaf, flower, and fruit growth

Harvesting	Sites of new leaf growth, flower and fruit growth
Tip of the branches	-New lateral branches at the tip of the branches -In the middle of the branches that has no leaf -From the base of the leaves stalk
100% tertiary branches with more than 6 pairs of leaf	-Quarternary branches formed -Restricted flowers growth or aborted
100% tertiary branches with less than 6 pairs of leaf	-Almost no quaternary branches formed, leaf bud not harvested still intact and grow -Triggered more new tertiary branches -Restricted flowers growth or aborted
50 and 100% tertiary branches	-Abnormal fruit development from small to normal size -Fruit abortion at 3-5 WAP
25% tertiary branches	Normal flower and fruit development

Table 3. Leaf increase affected by leaf harvesting

Weeks after pretreatment	Leaf harvesting (%)			
	0	25	50	100
2	114.00a	41.25b	-41.25c	38.13b
3	156.13a	81.00b	3.13b	63.50b
4	185.00a	113.00ab	26.00c	77.38bc
5	155.63a	143.75a	16.25b	94.50ab
6	142.75a	165.50a	18.63b	106.75ab
7	103.38a	137.25a	4.38b	98.00a
8	79.50ab	120.75a	11.13b	105.88ab
20	-48.30b	270.40a	158.40ab	213.50ab
21	-25.80b	312.80a	197.50ab	237.80ab
22	-3.50b	354.80a	227.10ab	261.40ab

Figures followed by different letters in the same row significantly different at 5% DMRT.

Table 4. The number of tertiary and quaternary branches

Treatment	The number of tertiary branches.plant ⁻¹		The number of quaternary branches.plant ⁻¹		The time of first quaternary branches appearances (WO)
	1 WO	22 WO	1 WO	22 WO	
<i>Growth Phases</i>					
Vegetative	1.44	17.38	1.13	21.75	3.31
Generative	0.88	15.88	0.94	20.06	3.25
<i>Leaf Harvesting (%)</i>					
0	1.63	11.13b	0.75	11.50b	5.50a
25	1.13	20.63a	1.25	31.25a	2.00c
50	0.63	12.88b	1.00	28.00a	2.00c
100	1.25	21.88a	1.13	12.88b	3.63b
Interaction	-	-	-	-	-

Figures followed by different letters in the same row significantly different at 1% DMRT test, log x for leaf number and $(x+1.5)^{1/2}$ for the number of tertiary and quaternary branches.

Table 5. Wet and dried leaf weight at the beginning and the end of the experiment

Treatment	The beginning of the experiment		The end of the experiment	
	Leaf wet weight	Leaf dried weight	Leaf wet weight	Leaf dried weight
g/plant				
<i>Growth Phase</i>				
Vegetative	200.19b	93.89b	193.09	99.82
Generative	343.31a	157.92a	206.94	115.45
<i>Leaf Harvesting (%)</i>				
0	277.96	135.90	150.38ab	78.50ab
25	179.60	83.36	301.31a	156.94a
50	297.45	131.61	273.31a	135.45ab
100	332.00	152.74	75.06b	59.65b
Interaction	-	-	75.06b	59.65b

Figures followed by different letters in the same column significantly different at 1% DMRT test, with $(x+1.5)^{1/2}$ transformation.

that the highest leaf number increase occurred at 25% leaf harvesting (354.8 leaf plant⁻¹) at the end of the experiment.

The Number of Tertiary and Quarternary Branches. Uniform growth on the tertiary branches showed at the beginning of the observation after the first leaf harvesting. Table 4 showed that leaf harvesting 25 and 50% produced more quaternary branches than tertiary branches, whereas 100% leaf harvesting produced more tertiary branches. The data showed that some of the leaf that were still intact, produced assimilate on tertiary branches of 25 and 50% leaf harvesting for the growth of lateral branches more than 100% leaf harvesting treatment.

The uniform growth on the quaternary branches at the beginning of the treatment had the same pattern as the growth of tertiary branches. Significantly different growth on the tertiary branches showed at 10 WO and for quaternary branches at 5 WO.

The time of quaternary branches appearances affected significantly by leaf harvesting. Delayed emergence of the first quaternary branches to 5.5 WO was found on trees without leaves harvesting treatment compared to 2.0 WO for 25 and 50% leaf harvesting treatments.

Generative Growth. The highest fruit number occurred at 2 WO (3.44 fruit plant⁻¹) on generative phase treatment. Fruit abscission started at 3 WO up to the end of the experiment. Almost all the fruit were produced at the rainy season and decreased at the time when the experiment was in the drought season. Small number of flower and fruit were still produced by the plants with the vegetative phase treatment at the lateral branches, despite all the ± 1 cm fruit in diameter were picked. Several plants with the generative phase treatment has medium size fruits, and became matured, but plants with smaller size fruits became hampered or abscised. Only fruit number on 50% leaf harvest (0.75 plant⁻¹) was significantly higher than the other leaf harvest treatments at 20 WO.

Leaf Wet, Dried Weight LAI, and Leaf Flavonoid Production. At the beginning of the experiment (Table 5), generative growth phase gave significantly higher leaf wet and dried weight than vegetative phase, 71.40 and 68.20%, respectively. LAI (Table 6) at the end of the experiment of leaf harvesting 25% treatment was significantly higher 291.67% (LAI 0.47), than 100% leaf harvesting treatment (0.12). The significantly lowest flavonoid production (Table 7) was found on 100% leaf harvesting in vegetative phase (7.82 g) and the highest

on 50% leaf harvesting in generative phase (89.90 g), which is 1149% higher.

Interaction of Growth Phase and Leaf Flavonoid Production with Time. Growth phase, leaf harvesting and

Table 6. Leaf area index

Treatment	LAI of the experiment	
	at the beginning	at the end
<i>Growth Phases</i>		
Vegetative	0.46	0.33
Generative	0.43	0.31
<i>Leaf Harvesting (%)</i>		
0	0.59	0.27ab
25	0.41	0.47a
50	0.42	0.42ab
100	0.37	0.12b

Figures followed by different letters in the same column significantly different at 1% DMRT test, with $(x+1.5)^{1/2}$ transformation.

Table 7. Treatments interaction on leaf flavonoid production at the end of the experiment

Growth phases	Harvesting (%)				Average
	0	25	50	100	
Vegetative	32.53ab	69.06ab	69.64ab	7.82b	44.77A
Generative	16.87ab	35.45ab	89.90a	52.10ab	48.58B
Average	24.70B	52.27AB	79.77A	29.96B	

Figures followed by different small letters in the same rows and column significantly different at 1% DMRT test, with $(x+1.5)^{1/2}$ transformation. Figures followed by different capital letters in the same rows or column significantly different at 1% DMRT test, with $(x+1.5)^{1/2}$ transformation.

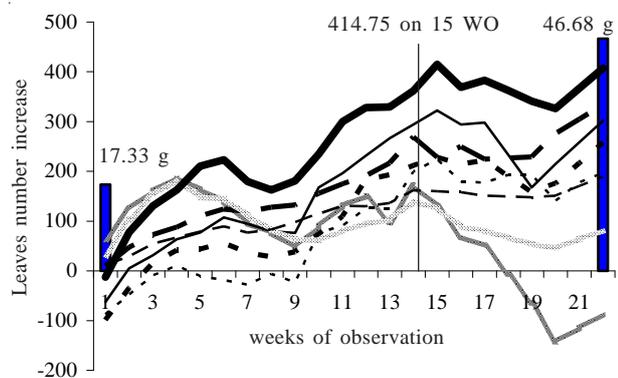


Figure 2. Interaction between growth phases, leaf harvesting and time of observation at the beginning and the end of the experiment on vegetative phase (ovp). ■: Flavonoid production, —: Without leaf harvesting ovp, - - -: 25% leaf harvesting ovp, . . . : 50% leaf harvesting ovp, - . - : 100% leaf harvesting ovp, —: Without leaf harvesting ovp, - - -: 25% leaf harvesting ovp, . . . : 50% leaf harvesting ovp, - . - : 100% leaf harvesting ovp.

time interaction affected leaf increase, fruit number and flavonoid concentration and production in the leaf. The highest leaf increase was at 15 WO (229.03 leaf plant⁻¹), the highest fruit number at 7 WO (1.21 plant⁻¹), and the highest flavonoid at the end of the experiment (22 WO) was 46.68 g plant⁻¹ (Figure 1). Although leaf wet weight-plant⁻¹, leaf dried weight-plant⁻¹ and LAI (Table 8) in the beginning of the experiment were higher than at the end of the experiment, but flavonoid production-plant⁻¹ and leaf flavonoid concentration were higher at the end of the experiment.

Growth phase interaction with leaf harvesting and time of observation significantly affected leaf increase. Plants harvested 50% of its leaf in generative phase at 4-22 WO had the highest leaf number increase than other treatment and also produced the highest flavonoid production. No leaf harvesting at 15-22 WO decreased the leaf number increase on vegetative phase plants, as the lowest leaf number increase (Figure 2).

DISCUSSION

Guédon *et al.* (2007) stated that observed growth, as given, for instance, by the length of successive annual shoots along the main axis of a plant, is mainly the result of two components: an ontogenetic component and an environmental component. In this experiment, LAI used as one of relative growth rate (RGR) variable based on leaf weight, whereas Sulistijorini *et al.* (2008) used plant height. The result of the harvesting treatment showed that no leaf harvesting and 100% leaf harvesting significantly had the lowest leaf flavonoid production than 50% leaf harvesting, whereas 25% leaf harvesting had the same leaf flavonoid production with the other three treatments. Trees with no leaf harvesting treatment experience no disturbance because of the leaf harvesting at the beginning of observation, such as that the tree grow at the normal growth throughout the experiment. The flower and fruit still intact on trees on generative phase gave the needed sink, caused the partitioning of the photosynthate for the growth and the generative organ, and made the leaf flavonoid production lower (16.87g tree⁻¹) than on vegetative phase (32.53 g tree⁻¹). No leaf harvesting treatment had the highest leaf increase at 2 to 4 WO, the highest leaf abscission 1 at 20 WO (November 2006), the slowest quaternary branches (5.5 WO) emergence, LAI at the beginning (0.59) = 2 at the end of the experiment (0.27), the lowest number of tertiary (11.13) and quaternary branches at 22 WO (11.50). This treatment experience no induction of pruning effect, whereas on vegetative phase, the flower and fruit picking

Table 8. Leaf production at the beginning and the end of the experiment

Time of observation	Leaf wet weight.plant ⁻¹	Leaf dried weight.plant ⁻¹	LAI	Flavonoid production.plant ⁻¹	Leaf flavonoid concentration (ppm)
Beginning	271.75	132.28	0.45	17.33b	138.08b
End	194.48	109.98	0.32	46.68a	437.94a

Figures followed by different small letters in the same column significantly different at 1% DMRT test, with $(x+1.5)^{1/2}$ transformation.

had negative effect on the trees, and caused the trees to have no sink and the highest leaf abscission.

Trees with 100% leaf harvesting treatment had the most severe treatment from leaf harvesting and forced condition of vegetative phase treatment, so that made the trees had the lowest flavonoid production (7.82 g tree^{-1}) than all the other treatments. Moderate leaf number increase, the lowest LAI at the beginning (0.37) and the end (12.88) of the experiment, leaf abscission occurred especially on vegetative phase, with smaller leaf size and the lowest leaf water content in the end of the experiment (20.53%). High stress caused the trees with 100% leaf harvesting on vegetative phase produced smaller leaf size in lower quantity on the lowest number of leaf on tertiary branches (21.88) and quaternary branches (12.88) at 22 WO.

Trees with 25% leaf harvesting treatment had more leaf increase on vegetative phase than generative phase, that made the similar trend in leaf flavonoid production. The effect of pruning was the second highest on this treatment, causing the highest number of leaf increase (354.8 tree^{-1}), the highest number of tertiary (20.63 tree^{-1}) and quaternary branches (31.25 tree^{-1}), the lowest wet weight at the beginning (179.60 g tree^{-1}), but the highest wet weight at the end of the experiment (301.31 g tree^{-1}), leaf water content 47.91% at 22 WO, and almost the same LAI value (0.41 at the beginning, and 0.47 at the end), almost the same number of leaves found on tertiary and quaternary branches. The data suggested that 25% leaf harvesting showed the stabilized growth of the guava.

Trees with 50% leaf harvesting treatment had the most severe negative effect on leaf number increase (-41.25 tree^{-1}) from first harvest that caused leaf abscission, but leaf number increase was moderate at 22 WO which was similar with 100% leaf harvesting treatment. The first harvest also caused the lowest number of tertiary branches (0.63 tree^{-1}) at 1 WO and lowest tertiary branches (12.88 tree^{-1}) at 22 WO, but the highest quaternary branches (28 tree^{-1}) at 22 WO, the same LAI value (0.42), and the second highest wet weight at the beginning and the end of the experiment. Treatment of 50% leaf harvesting promoted more quaternary branches with most of leaves on these branches, this data also suggested a stabilized growth. Treatment of 50% leaf harvesting had the similar highest leaf number increase at 15 WO/September and 22 WO/November (414.75 g tree^{-1}).

From 11 WO of the experiment, the tree with vegetative phase treatment almost had no fruit left to the end of the experiment. Whereas the generative still had 1 fruit tree^{-1} up to 10 WO, and declining to the end of the experiment. Vegetative phase had lower LAI (99.82 tree^{-1}), and higher water leaf content (51.32%) than generative phase (115.45 tree^{-1} and 43.45%). The data suggested that leaves on generative phase weight more than on vegetative phase. On the other hand flavonoid content of the leaf was also higher at the end (437.94 ppm) than at the beginning of the experiment (138.08 ppm).

Maintenance respiration costs, which are calculated on the basis of the Q_{10} concept, have first priority. Vegetative and reproductive growth are given second and

third priority. Daily carbon demands for the vegetative and reproductive organs are based on an analytical formulation of the potential growth rate at any time (Lescourret *et al.* 1998).

Fruit number on 50% leaf harvest (0.75 plant^{-1}) was significantly higher than the other leaf harvest treatments at 20 WO, was playing an important role for the sink. Lescourret *et al.* (1998) also stated that in a simulation model of daily C assimilation and allocation in an isolated shoot-bearing fruit, the pool of C assimilates available daily for distribution is the daily assimilation of C, plus that mobilized from reserves if the demand of sink organs exceeds the product of photosynthesis. Lopez *et al.* (2008). Carbohydrate partitioning was related to carbon allocation, such as organ growth, carbohydrate assimilation, reserve dynamics, and respiration maintenance.

Rain intensities fluctuated up to the end of the experiment (at beginning of November 2006), but for overall throughout the experiment, the months were dry months. Drought has dramatic negative effects on plants' growth and crop productivity. Although some of the responses and underlying mechanisms are still poorly understood, there is increasing evidence that drought may have a negative effect on photosynthetic capacity (Damour *et al.* 2008). This condition made the red guava trees forced on the generative state, not on flush state. Chaubert-Pereira *et al.* (2010), stated that tree growth is assumed to be mainly the result of three components: (i) an endogenous component assumed to be structured as a succession of roughly stationary phases separated by marked change points that are asynchronous between individuals; (ii) a time-varying environmental component assumed to take the form of synchronous fluctuations between individuals; (iii) an individual component corresponding mainly to the local environment of each tree. The result suggested that harvesting 50% leaf on tertiary branches in generative phase can be used to produce the highest flavonoid ($89.90 \text{ g tree}^{-1}$) on red guava leaf.

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