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Decreasing Rainfall Increased Secondary Metabolites on Torbangun *(Coleus amboinicus* Lour.)

Penurunan Curah Hujan Meningkatkan Metabolit Sekunder Pada Torbangun (Coleus Amboinicus Lour.)

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ABSTRACT

Torbangun *(Coleus amboinicus* Lour.) is a tropical plant from the Lamiaceae family, with leaves have a distinctive aroma and used more as medicinal plant. Bataknese lactating women in North Sumatra, Indonesia have traditionally consumed Torbangun leaves after birth with beliefs can increase their breast milk production (as lactagogue), anti-fungal and/or anti-bacterial, analgesic, to reduce cholesterol, and to clean the uterus. However, the ecology study of Torbangun is not well documented and scientific evidence is limited, especially about interraction between rainfall with secondary metabolites levels on Torbangun. This research was aimed to provide information of rainfall influence on secondary metabolites levels on Torbangun. This experiment was conducted at Mulyaharja village (6°19'–6°47' S, 106°30'–107°45' E), South Bogor District, Bogor, West Java (Indonesia), ftom January to March. The experiment used three replicates. Observations included components metabolite levels conducted on 10 plant samples, leaf harvested on plant with 5 perfectly opened on the 1st, 3rd, and 5th leaf position in each replication at 3 to 5 months-old. The results showed that decreasing of rainfall increases secondary metabolite levels such as the phenyalanine ammonia lyase (PAL) activity, anthocyanin, total flavonoids and total saponins on Torbangun.

Keywords: abiotic stress, bioactive compounds, drought stress, physiological adaptation, phytochemicals

ABSTRAK

Torbangun (Coleus amboinicus Lour.) adalah tanaman tropis dari keluarga Lamiaceae, dengan daun yang memiliki aroma khas dan lebih sering digunakan sebagai tanaman obat. Wanita menyusui suku Batak di Sumatera Utara, Indonesia, secara tradisional mengonsumsi daun Torbangun setelah melahirkan dengan keyakinan dapat meningkatkan produksi ASI, anti-jamur dan/atau anti-bakteri, analgesik, menurunkan kolesterol, dan membersihkan rahim. Namun, studi ekologi Torbangun belum banyak didokumentasikan dan bukti ilmiahnya terbatas, terutama mengenai interaksi antara curah hujan dengan tingkat metabolit sekunder pada Torbangun. Penelitian ini bertujuan untuk memberikan informasi tentang pengaruh curah hujan terhadap tingkat metabolit sekunder pada Torbangun. Eksperimen ini dilakukan di Desa Mulyaharja (6°19'–6°47' S, 106°30'–107°45' E), Kabupaten Bogor Selatan, Bogor, Jawa Barat (Indonesia), Januari hingga Maret. Penelitian ini menggunakan tiga ulangan. Pengamatan meliputi tingkat metabolit yang dilakukan pada 10 sampel tanaman, daun yang dipanen dari tanaman dengan 5 daun yang terbuka sempurna pada posisi daun ke-1, ke-3, dan ke-5 di setiap ulangan pada usia tanaman 3 hingga 5 bulan. Hasil menunjukkan bahwa penurunan curah hujan meningkatkan tingkat metabolit sekunder seperti aktivitas phenyalanine ammonia lyase (PAL), antosianin, total flavonoid, dan total saponin pada Torbangun.

Kata kunci: adaptasi fisiologis, cekaman kekeringan, fitokimia, senyawa bioaktif, stres abiotik

INTRODUCTION

Torbangun *(Coleus amboinicus* Lour.) is a tropical plant from the Lamiaceae family, with leaves have a distinctive aroma and used more as a medicinal plant (Ekawati, 2013). Bataknese lactating women in North Sumatra, Indonesia, have traditionally consumed Torbangun leaves after birth with beliefs can increase their breast milk production (Damanik et al., 2001; Damanik et al., 2004; Damanik et al., 2006; Damanik, 2009), anti-fungal and/or anti-bacterial (Khattak et al., 2013a; Khattak et al., 2013b), analgesic (Devi et al., 2010; Pramadya, 2010), to reduce cholesterol (Andriani, 2012); and to clean the uterus (Damanik et al., 2001; Damanik et al., 2004; Damanik, 2009). Modern pharmacological studies have confirmed its diverse bioactivities, including lactogenic, antimicrobial, analgesic, hypocholesterolemic, and uterine cleansing properties (Paul et al., 2024). The potential as lactagogue indicated by saponins, flavonoids, polyphenols on the leaves and increase breastfeeding hormones, such as prolactin and oxytocin. Torbangun leaves consumption have a significant effect in increasing some minerals such as K, Ca, Fe, Zn and Mg in breast milk (Damanik et al., 2005; Damanik et al., 2006).

Beyond its ethnomedicinal relevance, *Torbangun* has attracted increasing scientific interest due to its adaptive responses to environmental stressors. Secondary metabolites such as flavonoids, anthocyanins, and saponins are not only central to its pharmacological properties but also play a vital role in plant defense against abiotic stress, including drought and rainfall variability. Recent studies emphasize that fluctuations in rainfall can act as ecological triggers, modulating biosynthetic pathways and enhancing metabolite accumulation in medicinal plants (Złotek et al., 2016). This highlights the importance of integrating ecological parameters into phytochemical research, as rainfall-driven metabolite changes may directly influence both the therapeutic efficacy and cultivation strategies of Torbangun.

Flavonoids is one of phenolics compounds group (Mualim, 2012). Phenolics compounds are produced from the phenylpropanoid pathway with aromatic amino acids as a precursor which is produced from shikimic acid pathway. The biosynthesis of the phenylpropanoid is the deamination of L-phenylalanine to form transcinnamic acid in higher plant which is catalyed by phenyalanine ammonia lyase (PAL) (Mualim, 2012; Reichert et al., 2009). This biosynthetic process is catalyzed by phenylalanine ammonia-lyase (PAL), an enzyme whose activity is influenced by environmental factors such as nutrient availability, light intensity, and temperature (Filipe et al., 2025). The biosynthesis of phenolic compounds has been associated with increased PAL activity. The high PAL activity and followed by increasing of phenolic compound in wet season, promoted synthesis of anthocyanin by shikimic acid pathway such as on waterleaf (Mualim, 2012), corn (Gholizadeh, 2011), and Torbangun (Ekawati, 2013). Similarly, saponins—classified as terpenoid compounds—are synthesized through the mevalonic acid (MVA) and methylerythritol phosphate (MEP) pathways, both originating from acetyl-CoA and glycolytic intermediates. Recent evidence suggests that saponin levels in Torbangun may fluctuate in response to rainfall variability, highlighting the importance of ecological factors in secondary metabolite regulation (Paul et al., 2024). The increasing of saponins also affect the rainfall decreased on Torbangun (Ekawati, 2013).

Despite its ethnomedicinal significance, ecological studies on Torbangun remain scarce, particularly regarding the interaction between rainfall patterns and secondary metabolite accumulation. This research therefore aims to elucidate the influence of rainfall on the biosynthesis of key phytochemicals in Torbangun, thereby contributing to a deeper understanding of its ecological adaptation and pharmacological potential.

MATERIALS AND METHODS

Experimental Site

The research was conducted from January to March at Mulyaharja village (6°19' - 6°47' S, 106°30'-107°45' E), South Bogor District, Bogor, West Java, providing paranet with shade intensity 55%. Analysis of secondary metabolite levels at Plant Analysis and Chromatography, Spectrophotometry, Post-Harvest Laboratory, Department of Agronomy and Horticulture Faculty of Agriculture. Monthly data of rainfall collected from Darmaga Meteorological Stations (BMKG), Bogor, West Java.

Plant materials

The materials used in this research were Torbangun accession RD at 2 months-old, chicken manure 15 tons ha^{-1}. Materials used for chemical analysis were methanol, folin-ciocalteu reagent, sodium carbonate, potassium acetate, chloroform, acetic acid, sulphuric acid, bromocresol green, sodium diphosphate, citric acid, protein buffer, Gallic acid, nitric acid, distilled water and saponins Merck Co. All chemicals used in chemical analysis have pro quality analysis (analytical grade). Tools used were scales, oven, sprayer, camera, laboratory equipment for chemical analysis includes a Shimadzu UV-1800 Spectrophotometer (Japan) associated with UV 2:34 probes for analysis spectrophotometer, Centrifuge Heraeus labofuge-400R, Eyela water bath SB-24 to the incubation mixture solution extracts and other supporting tools.

Treatments

The experiment used three replicates. Observations included components metabolite levels conducted on 10 plant samples, leaf harvested on plant with 5 perfectly opened on the 1st, 3rd, and 5th leaf position in each. Sampling was performed at 3, 4, and 5 months after planting. Analysis of secondary metabolite levels such as phenylalanine ammonialyase enzyme activity (PAL) according to the method Dangcham et al. (2008), anthocyanin (Sims & Gamon, 2002), total flavonoids according to the method of Chang et al. (2002) and total saponins according to the method Fathonah and Sugiyarto (2009), which have been modified. The material for analysis of PAL enzyme activity and anthocyanin using fresh leaves according to the method above. The levels of total flavonoids using dried leaves at an oven temperature of 60 °C were extracted using methanol, while total saponins were extracted using 70% ethanol solution. The data obtained were then analyzed using a student's *t* test. The statistical analysis was performed using SAS Windows 9.1 System software.

RESULTS AND DISCUSSIO

Interraction between Rainfall with PAL activity, Anthocyanin, total Flavonoids and total Saponins

Rainfall data showed that during the experiment were executed on the rainy season (Figure 1, Figure 2, Figure 3 and Figure 4). The highest rainfall occured during the first harvest at 3 months-old was 843 mm per months which led to increased humidity. The cloudy weather conditions often lead to exposure of short duration. Decreasing of rainfall occurs during the second and third harvest at 5 months-old were 527 and 419 mm per months, respectively. The heavy rain occurs during of the second and third harvest. The phenomenon of interraction between rainfall with the levels of secondary metabolites such as PAL activity, anthocyanin, total flavonoids and total saponins.

PAL activity

At 3 months-old, decreasing rainfall affected PAL activity lower than at 4 and 5 months-old (Figure 1). Decreasing rainfall increased PAL activity at 4 and 5 months-old on the 3rd and 5th leaf position were 1.51 and 1.81 mg cinnamic acid equivalents (CAE) g $^{-1}$ protein, respectively.

Anthocyanin

At 3 months-old, decreasing rainfall affected anthocyanin lower than at 4 and 5 months-old (Figure 2). Decreasing rainfall increased anthocyanin at 4 and 5 months-old on the 5th and 3rd leaf position were 0.12 and 0.49 µmol g^{-1} Dry Matter, respectively.

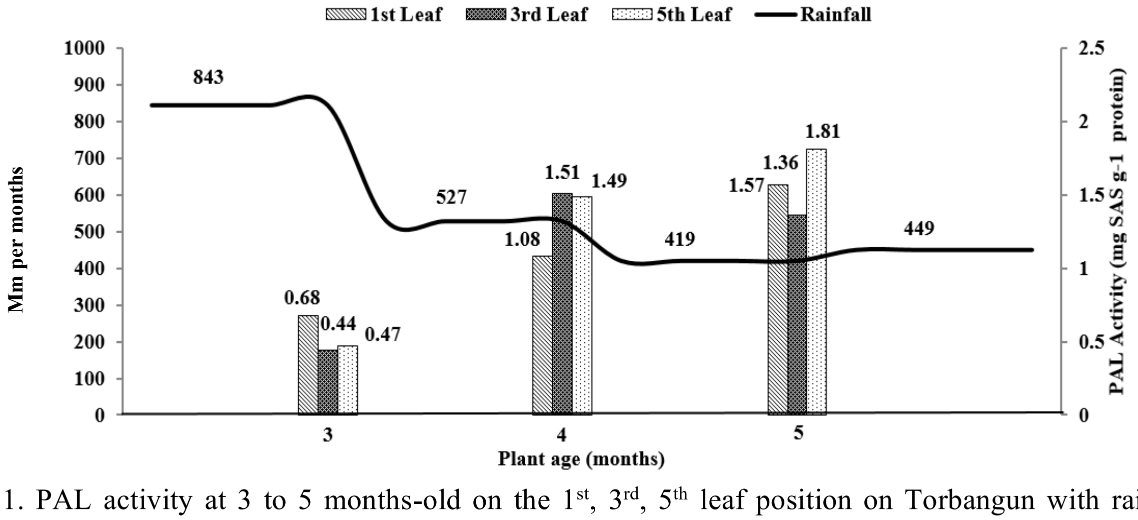

Figure 1. PAL activity at 3 to 5 months-old on the 1st, 3rd, 5th leaf position on Torbangun with rainfall fluctuation. SAS= cinnamic acid equivalents (CAE)

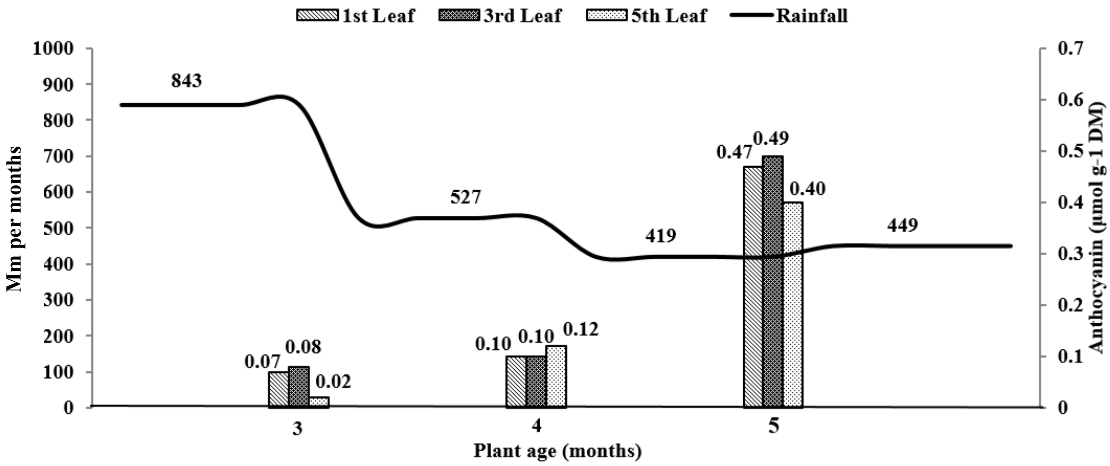

Figure 2. Anthocyanin at 3 to 5 months-old on the 1st, 3rd, 5th leaf position on Torbangun with rainfall fluctuation. DM= Dry Matter

Flavonoids

At 3 months-old, decreasing rainfall affected total flavonoids lower than at 4 and 5 months-old (Figure 3). Decreasing rainfall increased flavonoids at 4 and 5 months-old on the 5th and 3rd leaf position were 19.95 and 21.69 mg Catechin Equivalent (CE) g^{-1} Dry Matter, respectively.

Saponins

At 3 months-old, decreasing rainfall affected total saponins lower than at 4 and 5 months-old (Figure 4). Decreasing rainfall increased saponins at 4 and 5 months-old on the 1st and 5th leaf position were 55.72 and 53.14 mg saponins g^{-1} Dry Matter, respectively.

Discussion

This research principally conducted to provide information of rainfall influence on secondary metabolites levels on Torbangun. Secondary metabolites studied was PAL activity, anthocyanins, flavonoids and saponins. Some bioactive compound studied is an antioxidant that affected by the change or environmental stress. Flavonoids was one of bioactive compound which part of a natural antioxidant compound of phenolic or polyphenolic compounds. Flavonoids have antioxidant activity include flavones, flavonols, isoflavones, catechins, flavanones, and kalkon (Apak et al., 2007). Phytochemical content of waterleaf that affected the antioxidant was determined by environmental factors (such as day length, rainfall, and temperature), genetic (variation within species), and also determined by the input given (Mualim, 2012). The results of this study showed that the differences of rainfall in every age increased secondary metabolites on Torbangun (Figure 1, Figure 2, Figure 3 and Figure 4).

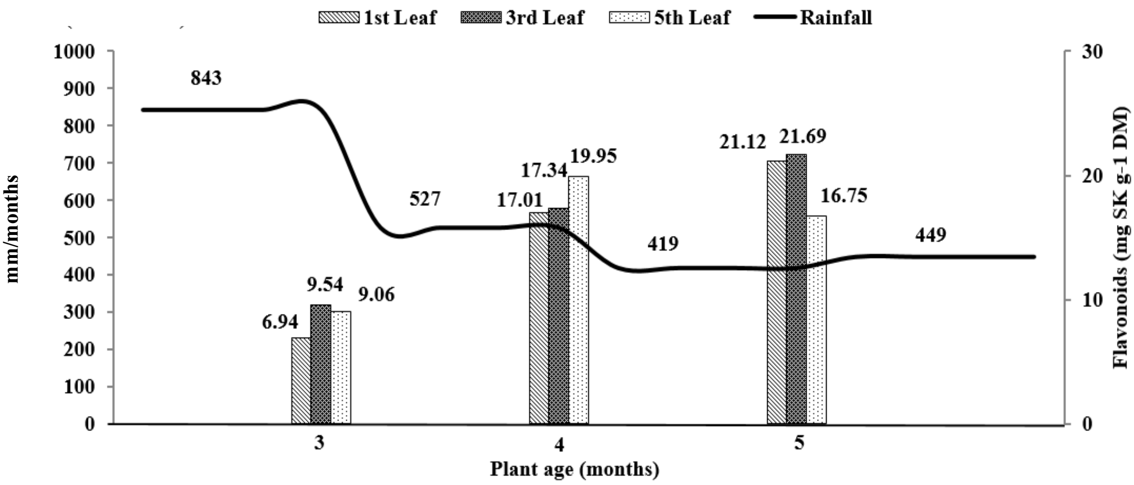

Figure 3. Flavonoids at 3 to 5 months-old on the 1st, 3rd, 5th leaf position on Torbangun with rainfall fluctuation. SK= Catechin Equivalent (CE), DM= Dry Matter

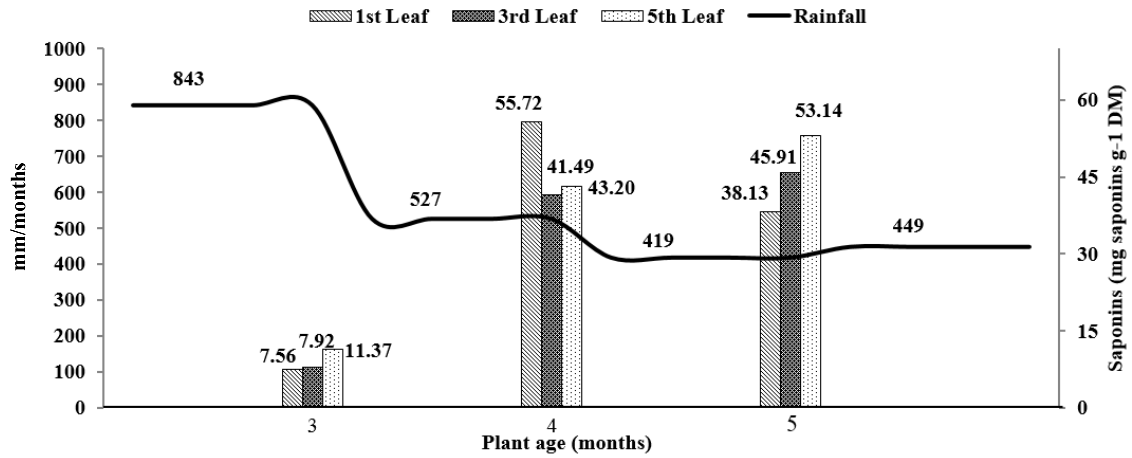

Figure 4. Saponins at 3 to 5 months-old on the 1st, 3rd, 5th leaf position on Torbangun with rainfall fluctuation. DM= Dry Matter

Decreasing rainfall increased PAL enzyme activity at 3 to 5 months-old on the 1st, 3rd and 5th leaf position on Torbangun leaves. Environmental factors such as low nutrient and light can increase the PAL enzyme activity (Tan, 1980). Rainfall during the experiment showed that at 3 months-old, monthly data of rainfall is highly reduced of irradiation conditions. Decreasing rainfall increased the PAL activity at 4 and 5 months-old, although rainfall decreased with the heavy rain condition in a brief time. The PAL activity levels increased along with the age of the plant (Figure 1). Organic fertilizer without or with pruning increases the PAL activity at 8 and 12, at 17 and 20 weeks after treatment (WAT) on torbangun plants along with decreasing of rainfall (Ekawati, 2013).

Decreasing rainfall increased anthocyanin at 3 to 5 months-old on the 1st, 3rd and 5th leaf position on Torbangun leaves. Anthocyanin increased at 3 to 5 months-old, due to there were interraction between anthocyanin biosynthesis with the PAL activity. According Boo et al. (2011) anthocyanin is positively correlated with the PAL activity on lettuce ($r = 0.61$, $p < 0.05$) Furthermore, Ekawati (2013) added that the organic fertilizer increased anthocyanin at 8 and 12 WAT along with decreasing rainfall. It is showed that increasing anthocyanin will be followed by increasing the PAL activity.

Decreasing rainfall increased flavonoids at 3 to 5 months-old on the 1st, 3rd and 5th leaf position on Torbangun leaves. Increasing of total flavonoids at 3 to 5 months-old, due to there were interraction between total flavonoids with the PAL activity. Increasing phenolic compounds (concentration of total flavonoids and total phenolic content) interracted with the PAL activity and more influenced by rainfall. The PAL activity is positively correlated with phenolic compounds on torbangun ($r = 0.62$, $p < 0.05$) (Ekawati, 2013), pistachio *(Pistacia vera* L.) ($r = 0942$, $p < 0.01$) (Nadernejad et al., 2012), and waterleaf (*Talinum triangulare*) (Mualim, 2012). It showed that the precursors of phenolic compounds such as phenylalanine and the formation of phenolic compounds synthesized via the phenylpropanoid pathway with precursors derived from shikimic pathway. Interraction between the PAL activity increased with phenolic compounds formed have been reported, such as *Olea europaea* on (Garcia et al., 2009; Tovar et al., 2002), waterleaf (*Talinum triangulare*) (Mualim et al., 2012), corn (Gholizadeh, 2011), strawberry (Cheng and Breen, 1991), and tomato (Rivero et al., 2001). The simple biosynthesis scheme of primary and secondary metabolites pathway (flavonoids, anthocyanins, saponins) in plants can be seen in Figure 5. The relationship between primary metabolism and secondary metabolite biosynthesis in plants. Carbohydrates produced through photosynthesis enter glycolysis and the pentose phosphate pathway, generating key intermediates such as pyruvate, erythrose-4-phosphate, and acetyl-CoA. These compounds serve as precursors for major biosynthetic pathways, including the shikimic acid pathway, phenylpropanoid pathway, and mevalonic acid pathway. The shikimic acid and phenylpropanoid pathways lead to the formation of phenolic compounds, flavonoids, and anthocyanins, while the mevalonic acid pathway produces terpenoids, including saponins. In addition, amino acids derived from primary metabolism contribute to the synthesis of nitrogen-containing secondary metabolites such as alkaloids. Overall, the figure demonstrates that changes in primary metabolic processes, such as those induced by environmental stresses, can influence the accumulation of secondary metabolites that play important roles in plant adaptation and defense (Figure 5).

Decreasing rainfall increased total saponins at 3 to 5 months-old on the 1st, 3rd and 5th leaf position on Torbangun leaves. Saponins is one of terpene compounds group which the fat synthesized from the primary metabolites of acetyl CoA through of mevalonic acid (MVA) pathway or basic intermediates of glycolysis through of methylerythritol phosphate (MEP) pathway. Three molecules of acetyl CoA merged to form mevalonic acid. Intermediates compounds of 6 carbon occured pyrophosphorilation, carboxylation and dehydration to form isopentenyl pyrophosphate (IPP). IPP is forming compounds (precursors) block 5 C terpenes. IPP can be formed from intermediates of glycolysis or carbon reduction cycle in the photosynthesis process (Taiz & Zeiger, 2002). In this research, there was no correlation between the PAL activity with total saponins.

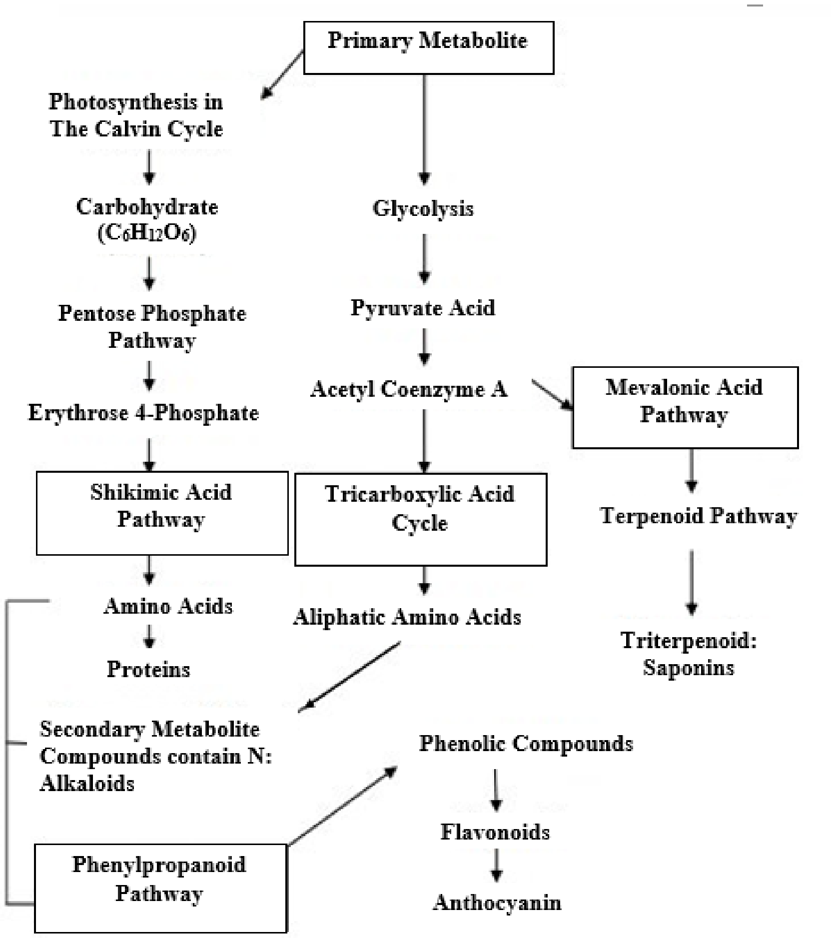

Figure 5. The simple biosynthesis scheme of primary and secondary metabolite pathway in plants. Modification of (Cseke et al., 2006; Ekawati, 2013).

CONCLUSION

This study demonstrated that rainfall variability significantly influences the accumulation of secondary metabolites in Torbangun (*Coleus amboinicus* Lour.). Decreasing rainfall consistently enhanced the biosynthesis of key phytochemicals, including phenylalanine ammonia-lyase (PAL) activity, anthocyanins, total flavonoids, and total saponins across different leaf positions and plant ages. The observed increase in PAL activity under reduced rainfall conditions suggests a strong ecological linkage between environmental stress and activation of the phenylpropanoid pathway, which in turn promotes flavonoid and anthocyanin synthesis. Similarly, saponin levels were elevated during periods of lower rainfall, highlighting the responsiveness of terpenoid biosynthesis to climatic fluctuations.

These findings provide new ecological insights into Torbangun, a plant of high ethnomedicinal and pharmacological relevance, by establishing rainfall as a critical factor regulating its secondary metabolite profile. The results not only strengthen the scientific basis for traditional uses of Torbangun but also underscore the importance of integrating ecological parameters into future cultivation and pharmacological studies. Understanding these interactions may support optimized production strategies for bioactive compounds, contributing to both sustainable agriculture and the development of plant-based therapeutics. However, this study was limited by its focus on rainfall reduction as a single environmental factor, the use of a single location and growing season, and the evaluation of only selected secondary metabolites. Furthermore, physiological and molecular mechanisms underlying metabolite accumulation were not investigated, and potential trade-offs between metabolite enhancement and biomass yield were not comprehensively assessed. Future studies should incorporate multi-environment trials, detailed metabolomic and molecular analyses, genotype comparisons, and evaluations of yield-quality relationships to improve understanding of torbangun responses to water deficit conditions.

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