

Morphological characteristics and flavonoid accumulation of *Echinacea purpurea* cultivated at various salinity

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Abstract. Choirunnisa JP, Widiyastuti Y, Sakya AT, Yunus A. 2021. Morphological characteristics and flavonoid accumulation of *Echinacea purpurea* cultivated at various salinity. *Biodiversitas* 22: 3716-3721. Purple coneflower (*Echinacea purpurea* (L.) Moench) is an introduced medicinal plant from North America. *E. purpurea* has high morphological characteristics on stems, leaves and flowers. This plant has not much cultivated as a raw material for traditional medicine in Indonesia due to not much information about flavonoid accumulation of *E. purpurea* in this country. The purpose of this research was to study morphological characteristics from three accessions of *E. purpurea* cultivated with various salinity and to select *E. purpurea* accessions that have high flavonoid accumulation. This study design using a factorial Completely Randomized Design (CRD). The first factor is 3 accessions of *E. purpurea* (E1; E2; E3). The second factor is 4 levels of CaCl₂ (0 ppm; 2500 ppm; 5000 ppm; 10000 ppm). The study was conducted by observing the morphological characteristics of stems, leaves flowers, and herb extract and flavonoid accumulation were analyzed using SPSS. The results demonstrated that morphological characteristics are easy to observed on leaf shape and flower color. The highest herb extract with 10.043% and flavonoid accumulation with 0.510% were in accession 2 with the addition of CaCl₂ concentration of 10000 ppm. This study concludes that there are morphological characteristics of *E. purpurea* cultivated at various salinity and the highest CaCl₂ concentration can increase with significance to herb extract and flavonoid accumulation.

Keywords: Accession, CaCl₂, characteristics, *Echinacea purpurea*, flavonoid

INTRODUCTION

Echinacea purpurea (L.) Moench or purple coneflower is a medicinal plant from North America introduced in Indonesia. *E. purpurea* began to be studied in Indonesia in 1998, the results showed that *E. purpurea* is well grown to altitude range 450-1100 meters above sea level (m asl) in the tropics (Rahardjo 2000). *E. purpurea* cultivated in Indonesia has many morphological changes due to different climate conditions from North America. Indonesia has tropical climate characterized by high and longer rainfall, and frequent high temperatures (Center for Research and Development 2010). North America has subtropical climate characterized by full sun exposure and low temperatures (Mangini and Areta 2018). Kindscher (2016) stated that *E. purpurea* cultivated in Canada has risen red and dark purple flower colors, while Sidhiq et al. (2020) obtained flower color variations including pale pink, dark pink, and dark purple on *E. purpurea* in Karangpandan, Central Java, Indonesia. Morphological differences are influenced by environmental factors, genetics, and interaction between two factors (Benito et al. 2016). Morphological characteristics are currently used in plant classification and classified as the fastest method for preparation of plant diversity. Morphological characters are

more easily recognized and simple to observe than other plant characters (Landey et al 2013).

Traditional medicine production from 2018 to 2019 increased by 18.58% (Central Bureau of Statistics 2020). The increasing health costs and many disease outbreaks attack the human body, causing medicinal plants as raw materials for traditional medicines to increase (Sofowora et al. 2019). *E. purpurea* is useful as an antioxidant, anti-inflammatory, and immunomodulator, and has no hypersensitivity in clinical trials (Sidhiq et al. 2020). Flavonoids are secondary metabolites from phenolic compound derivatives are the dominant compounds in *E. purpurea* (Kurkin et al. 2011). Flavonoid compounds indicate antioxidant and immunomodulatory effects by boosting the immune system (Khazdair et al. 2018). The adaptation of *E. purpurea* cultivated in Indonesia has a promising opportunity to develop as a raw material for traditional medicine. Nonetheless, *E. purpurea* as an introduced plant has not much research in Indonesia about the diversity of *E. purpurea* with maximizing flavonoid accumulation.

The flavonoid accumulation can be increased by abiotic stress such as stress salinity by CaCl₂. Khorasaninejad et al. (2020) stated that stress salinity can increase the flavonoid accumulation by 90% in *E. purpurea*. Research results in

other Asteraceae species by Madany and Khalil (2017), sunflower plants with 5 mM CaCl₂ treatment can increase the flavonoid accumulation with 0.1 mg This study aims to determine the morphological variations and flavonoid accumulation in three accessions of *E. purpurea* and to determine plants having good potential to be developed based on the flavonoid accumulation.

MATERIALS AND METHODS

Experimental design

This study used a factorial Completely Randomized Design (CRD) method. The first factor was accession (E) consisted of 3 levels (E1; E2; E3). The second factor was CaCl₂ concentration consisted of 4 levels, namely 0 ppm (C1), 2500 ppm (C2), 5000 ppm (C3), and 10000 ppm (C4), so it had 12 treatment combinations. *E. purpurea* was planted at the Jumantono Experimental Field, Sebelas Maret University, Karanganyar, Central Java, Indonesia (293 m asl) in December 2020 to June 2021.

Field experimental procedure

Accession selection

Three accessions of *E. purpurea* were obtained from mass selection from the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT), Tawangmangu, Indonesia. *E. purpurea* accessions were selected from identifying morphological differences by covering leaf shape, flower shape, flower color and stem color.

Application of CaCl₂

CaCl₂ solution was applied 4 times in the plant vegetative phase, namely on plants aged 4 WAP (Week After Planting), 5 WAP, 6 WAP, and 7 WAP with watering intervals of once a week. Watering volume according to treatment concentration determined by field capacity.

Morphological identification

Identification of morphological characters in *E. purpurea* accession used descriptive non-experimental method and observed directly. The observed variables included morphological characters of stem color, stem surface texture, leaf shape, leaf margin, leaf tip, upper leaf surface texture, lower leaf surface texture, leaf color, flower shape, flower edge shape, flower petals tip shape, flower edge density, flower color, and flower bud color.

Analysis of herb extract

Extract of *E. purpurea* taken from all plant parts (stems, leaves, flowers, and roots) that have been harvested and dried for 7 days at room temperature then dried again with oven at 50°C temperature. The dried herbs were ground into powder and weighed 5 g as the initial sample weight. The 5 g powder sample was put in a bottle by adding 50 ml of 70% ethanol and macerated for 3 days, then filtered and placed in a cup that had previously weighed as initial weight of the cup then dried. The dried extract was weighed as a final weight of the cup and calculated weight

of the herbal extract by subtracting the cup's initial weight from the cup's final weight (Center for Research and Development of Medicinal Plants and Traditional Medicines 2018). The herb extract of *E. purpurea* was calculated using the formula:

$$r (\%) = \frac{x}{y} \times 100$$

Where:

r : Herb extract content (%)

x : Herbal extract weight (g)

y : Initial sample weigh (g)

Analysis of flavonoid

Analysis of the flavonoid accumulation was performed using the Aluminum chloride colorimetric method from Chang et al. (2002) with slight modification. The thick extract was weighed 100 mg and dissolved with 70% ethanol to a volume of 10 ml, then sonicated for 15 minutes and deposited overnight. The extract was taken 4 ml and put in oven at 50°C temperature, then dissolved with 8 ml methanol, after that sonicated for 15 minutes and deposited overnight. Make 2 solutions for flavonoid testing by adding 0.2 ml extract solution and 4.8 ml aquabidest as the first sample solution, make second sample solution by adding 0.2 ml extract solution, 1 ml AlCl₃ and 3.8 ml aquabidest, then each sample solution was incubated for 15 minutes. The first sample solution was read at the maximum wavelength using *UV-VIS Spectrophotometer* at 370-450 nm wavelength, determine operating time with reading the most stable absorbance value at 415 nm wavelength then make a standard curve by pipetting 2 ml second sample solution. After that reading absorbance value at the maximum wavelength and operating time obtained from the first sample solution. The quercetin concentration was calculated by the curve equation of $y = 0.0383x + 0.0911$, y value is absorbance of curve standard and x value is concentration of quercetin (mg/L). Flavonoid accumulation in the extract was calculated using the formula:

$$F = \frac{C \times V \times FP \times 10^{-3}}{M} \times 100$$

Where:

F : Flavonoid accumulation (%)

C : Quercetin concentration (mg/L)

V : Extract volume (L)

FP : Dilution factor

M : Sample weight (mg)

Data analysis

Qualitative data of morphological characteristics were analyzed descriptively and quantitative data of herb extract and flavonoid accumulation were analyzed by Analysis of Variance (ANOVA), simple correlation analysis, and Duncan Multiple Range Test (DMRT) level of 5% using SPSS (Statistical Product and Service Solution) 21.0.

RESULTS AND DISCUSSION

Morphological variations in stems, leaves, and flowers occur in 60 *E. purpurea* plants cultivated at various salinity levels. Almost 70% variation occurs in the morphology of leaves and flowers. In accordance with Subositi and Fauzi (2012), there are 8 accessions of *E. purpurea* that have morphological variants, especially in leaf and flower morphology. Salinity stress by CaCl_2 can increase herb extract by more than 24% and flavonoid accumulation by more than 29%. Sarker and Oba (2018) stated that salinity stress can increase the production of secondary metabolites such as phenolic compounds and flavonoids in plants.

Morphological characters of *E. purpurea*

Stem

Morphological variations occur almost 30% in stems of *E. purpurea*. Morphological variations in stems can be easily observed based on stem color and stem surface texture. Accession 1 has a light green stem color with dark green and white spots. Accession 2 has a yellowish-green stem color with purple spots. Accession 3 has a dark green stem color with brownish-red spots (Figure 1). The stem surface texture of accession 1 has a slightly rough, while accession 2 and accession 3 had a rough stem surface texture. Banga and Ardelean (2008) stated that 7 accessions of *E. purpurea* collected in Cluj Napoca Romania had morphological diversity in stems. The accessions in the study were different from their previous parents. This was thought to be propagated using seeds so as to produce new variants that were different from the parent. Subositi and Fauzi (2016) reported that new morphological variants are caused by mass selection using seeds, resulting in some offspring having morphological differences with their parents. This is in accordance with the statement of Baum et al. (1999) that genetic variability in *E. purpurea* causes of generative propagation. Mass selection using seeds causing differences of morphological from 4 species *E. purpurea* (Yavari et al. 2017).

Leaf

Leaf morphology of 3 accessions *E. purpurea* grown at various concentrations of CaCl_2 had almost 60% variation in leaf shape (Figure 2). Accession 1 has an oblong leaf shape that is elongated with a wavy tapered leaf tip. Accession 2 has an oval leaf shape and extending towards the base (lanceolate) with a tapered leaf tip, while leaf shape of accession 3 is widened to the center (ellipse) with a blunt leaf tip. Accession 1 and accession 2 had the same leaf margin, which is jagged grooves, while the leaf margin of accession 3 is flat and serrated. Accession 2 has the smoothest upper leaf surface texture and the lower leaf surface texture is sparse and rough-haired. Accession 1 and accession 3 had the same leaf surface texture of upper and lower which is rough. Accession 2 has a green leaf color as the brightest leaf color. The color leaf of accession 1 and accession 3 is dark green. Pop (2010) stated that 10 accessions of *E. purpurea* produced morphological variations in leaves, such as leaf shape, leaf length, and leaf width. The different leaf morphology of *E. purpurea* from

its parents is caused by environmental factors. Humidity, temperature, and stress are environmental factors that can affect the metabolism of plant and causing morphology differences in plant (Sidhiq et al. 2020), such as the change of leaf shape. According to Lin-na (2013), the differences in morphological characters of 14 accessions *E. purpurea* were easily affected by growth environmental factors such as temperature and sunlight. Color changes in plant morphology are caused by lack of nutrients, water, or sunlight due to environmental stress (Pratiwi et al. 2019). According to Varban et al. (2010), plants have different adaptation levels for environmental changes that synergize with genetic factors. *E. purpurea* has a different leaf morphology in leaf shape and color shape caused the different tolerance levels between accessions of *E. purpurea* to changes in environmental conditions.

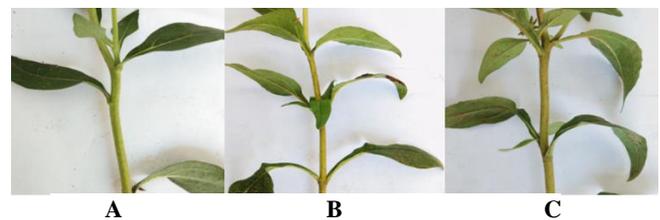


Figure 1. Stem morphology of three *E. purpurea*: A. Accession 1, B. Accession 2, C. Accession 3

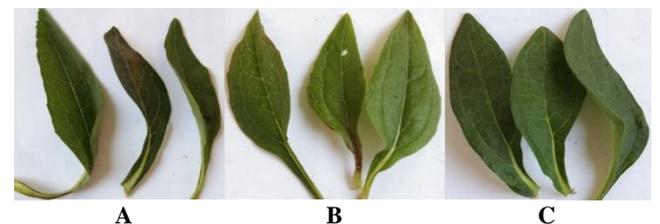


Figure 2. Leaf morphology of three *E. purpurea*: A. Accession 1, B. Accession 2, C. Accession 3



Figure 3. Flower morphology of three *E. purpurea*: A. Accession 1, B. Accession 2, C. Accession 3

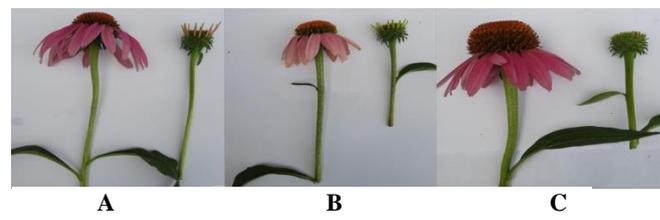


Figure 4. The morphology of color leaf and color shoots in three *E. purpurea*: A. Accession 1, B. Accession 2, C. Accession 3

Flower

Flowers have the most striking morphological differences and are easily visually observed. The diversity of flower morphology can easily be observed in the flower shape (Figure 3), accession 1 has a flat flower shape, while accession 2 and accession 3 have a downward curving flower shape. Accession 1 and accession 3 have the same flower edge shape which is elongated oval, while flower edge shape of accession 2 is lanceolate. Accession 3 has the flower petals tip is shaped like the letter V with 1 indentation. The flower petals tip of accession 1 and accession 2 are shaped like the letter W with 2 indentations. Accession 2 has the densest flower edge density and flower edges each other support and accession 3 has flower edge density is tight but not mutually supportive between flower edges, widely accession 1 has flower edge density that rarely or not overly tight. The diversity of flower color is also easy to observe, accession 2 has the most different flower color with accession 1 and accession 3. Accession 2 has a pale pink flower color with yellowish-green flower bud color. Accession 1 has a purplish-pink flower color with the flower bud color is pink green and accession 3 has dark-pink flower colors with light green flower bud color (Figure 4). Almost 70% of flower shapes and colors differ from their parents due to self-incompatibility in *E. purpurea*. The diversity of agronomic and morphological in *E. purpurea* species is caused by cross-pollination with generating *E. purpurea* seeds with different morphology from the parent (Sidhiq et al. 2020). *E. purpurea* is a plant with self-incompatibility that can cause a high diversity in plant morphology (Subositi and Widyastuti 2013). According to Kang et al. (2015), the cross of hibiscus (*Hibiscus rosasinensis*) flower using intraspecific hybridization method can produce the difference of flower shape, flower size, and flower color.

Herb extract

The results of the herb extract analysis are presented in Figure 5. Based on data analysis, there was no interaction between accession and addition of CaCl₂ concentration to herb extract. The highest herb extract was obtained by accession 2 in the concentration of 10000 ppm CaCl₂ with a value of 10.043%, significantly different from accessions 1 and 3. The concentration of 10000 ppm CaCl₂ can increase the herb extract of accession 1 with a value of 30.88%, then in the herb extract of accession 2 with a value of 23.80%, and the herb extract of accession 3 with a value of 27.62%.

The higher CaCl₂ concentration has resulted in the highest extract of *E. purpurea* herb (Figure 5), due to can cause plant stress and can affect the production of active compounds in herb extract. According to research Amallia et al. (2020), the highest extract of *Centella asiatica* herb with a value of 13.80% was found in salt content of 3000 ppm, due to increased production of active compounds in the herb extract as a response of plants to stress conditions. The high herb extract indicates many active compounds in plant extracts, according to statement of Zohra and Fawzia (2014) that active compounds content in plants is related to value of herb extract. The higher of herb extract, the more active compounds are attracted to the solvent (Quispe et al. 2017).

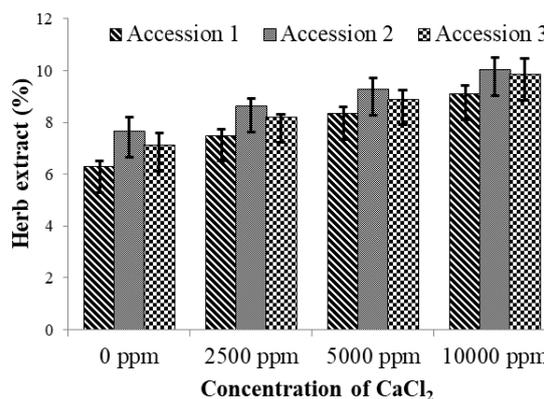


Figure 5. Herb extract of three *E. purpurea* accessions at various CaCl₂ concentrations

Table 1. Flavonoid accumulation of three *E. purpurea* accessions that resulted at various CaCl₂ concentrations

Accessions	CaCl ₂ concentrations			
	0 ppm	2500 ppm	5000 ppm	10000 ppm
1	0.267 a	0.313 ab	0.367 b	0.428 d
2	0.356 b	0.408 c	0.459 e	0.510 g
3	0.308 ab	0.361 b	0.420 d	0.469 f

Note: Numbers followed by different lowercase letters in each column indicate a significant difference in DMRT level of 5%

Table 2. Correlation analysis between variables

Variables	Flavonoid accumulation
Accessions	1.000**
CaCl ₂ concentrations	0.484**
Herb extract	0.982**

Note: The sign ** indicates very significant difference in simple correlation analysis level of 5%

Accumulation of flavonoid

Flavonoids are secondary metabolites colored yellow from a group of phenolic compound. Flavonoids have three physiological functions for plants, including as color pigments (anthocyanins), protection to high UV radiation (flavonols and flavones), and acting as plant defense signals in stress conditions (isoflavones) (Pambudi et al. 2014). Flavonoid pigments are also useful in humans as immunomodulators and antioxidants (Heldt and Piechulla 2011). The results of analysis of flavonoid accumulation are presented in Table 1. Based on data analysis, there was an interaction between accession and addition of CaCl₂ concentration to flavonoid accumulation. Accession 2 with the treatment of 10000 ppm CaCl₂ concentration had the highest flavonoid accumulation with a value of 0.510%, while accession 1 without the addition of CaCl₂ resulted in the lowest flavonoid accumulation with a value of 0.267%. The accumulation of flavonoids in accession 2 was significantly different at various concentrations of CaCl₂. The response of each accession in the confront of salt stress

showed different results in the accumulation of flavonoids, accession 2 showed a better response than accessions 1 and 3. In addition to environmental factors, variations in morphology and content of secondary metabolites in plants are caused by genetic factors in each accession (Yang et al. 2016).

The higher CaCl_2 concentration can increase the flavonoid accumulation in all accessions of *E. purpurea* (Table 1). This is caused by the high concentration of CaCl_2 causing plant stress, so that plants will respond in the form of osmoregulation which involves osmotic regulation in the confronts of salt stress. Kaouther et al. (2013) stated that osmoregulation in saline-stressed plants would involve synthesizing organic acid compounds and amino acids to increase turgor pressure and decrease cell osmotic potential. Synthesis of amino acid compounds can increase the activity of the PAL (Phenylalanine Ammonia-Lyase) enzyme as an important compound in flavonoid biosynthesis through the pathway of phenylpropanoid. According to Valifard et al. (2015) salinity stress can synthesize the amino acid L-phenylalanine to produce PAL enzymes. The biosynthesis of phenolic compound groups of flavonoids requires a PAL enzyme as a precursor in the phenylpropanoid pathway (Zhang et al. 2021). Wang et al. (2021) reported that 5 mmol-L-1 CaCl_2 increased 25% PAL enzyme activity and 18% flavonoid accumulation in dwarf saltwort (*Salicornia bigelovii* Torr) plants.

Based on Table 2, we showed that the flavonoid content is positively correlated with accession 2, CaCl_2 concentration and herb extract. The higher concentration of CaCl_2 in *E. purpurea* can produce bioactive compounds in the herb extract, affecting the higher accumulation of flavonoids. Aghdam et al. (2013) stated that salt stress using CaCl_2 can be used to increase the production of secondary metabolites in the form of flavonoids. The results of Chakraborty et al. (2015) stated that the CaCl_2 concentration of 5 mM can increase 40% of total flavonoids in tomato plants. Khorasaninejad et al. (2020) stated that 50 mM salinity increased 72% of the flavonoid content in *E. purpurea*.

The high salt content causes an increase in the number of bioactive compounds (Kamiab et al. 2012). Herb extract value is related to the number of bioactive compounds, the higher the number of bioactive compounds (Altemimi et al. 2017). Syafitri et al. (2014) stated that the herb extract of Harendong ripe fruit was 50.89% indicating the highest total flavonoid content (105.93 mg/g), while the lowest total flavonoid content was found in the slight amount of extract (42.08%).

The results of this study concluded that the morphological variations of three *E. purpurea* accessions cultivated under various salinity stresses could be distinguished based on the morphology of stems, leaves and flowers descriptively. Morphological variations were found in stem color, flower color, leaf shape and flower shape. CaCl_2 concentration of 10000 ppm increased 24-31% herb extract and 29-38% flavonoid accumulation in three accessions of *E. purpurea*. Accession 2 had the highest herb extract and the highest accumulation of

flavonoids, so that it showed characteristics of more tolerance to salinity stress than accession 1 and accession 3.

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