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Comparative analysis of the bioactive compound, pigment content and antioxidant activity in different parts of *Pouzolzia zeylanica* plant

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ABSTRACT

Plants are a rich source of therapeutically active compounds such as antioxidants, antibiotics, pigments, vitamins, organic acids, glycosides, and other substances of particular importance to human life. The present study was to analyze and compare the content of bioactive compounds (anthocyanin, flavonoid, polyphenol and tannin); pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids); and antioxidant activity in different parts of Pouzolzia zeylanica plant. The antioxidant activities were evaluated using three methods such as antioxidant ability index, ferrous reducing ability power, and scavenging capacity 2,2-diphenyl-1-picrylhydrazyl radical. The results showed that the content of anthocyanin, flavonoid, polyphenol and tannin of young shoots was significantly ($P<0.01$) higher than that of other parts. In constrast, the content of pigments such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of leaves was higher than that of young shoots, whole plants and stems. Besides, the antioxidant capacity of young shoots was also higher than that of leaves, whole plants and stems when performed with three assay methods. It was a correlation between the content of bioactive compounds and antioxidant activities of different parts of Pouzolzia zeylanica plant.

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1 INTRODUCTION

Plants possess various antioxidants which play an important role in the prevention of diseases. It is widely used in many indigenous systems of medicine for therapeutic purposes and increasingly becomes popular in modern society as alternatives to synthetic medicines. Medicinal plant is generally cheaper, accessible or available and are accepted by many people because of the belief that they cause less side effects than some synthetic drugs (Carlson, 2002; Dey and De, 2015).

Pouzolzia zeylanica (L.) Benn. is a perennial herbaceous plant belonging to the *Urticaceae* family, and it is distributed in tropical and subtropical regions. Nowadays, it is present in many Asian countries such as China, India, Indonesia, Japan, Malaysia, Myanmar, Pakistan, Philippines, Sri Lanka, Thailand, Vietnam, Singapore, and some other places in the world (Adhikari and Babu, 2008). It has long been used as one of the components in herbal remedies for treating various diseases by traditional method such as poultices to cure bone fractures, boils and itching; juices or extracts to treat eyes injuries; dysentery and loose stools of infant,

stomach ailments, diabetes, cancer, preventive radiation and confirmed the therapeutic value of polyphenols contained in the leaves (Li, 2006; Yusuf *et al*., 2006; Purkayastha *et al*., 2007; Bhattacharjya and Borah, 2008; Ratnam and Raju, 2008; Mondal *et al*., 2013; Sandhya *et al*., 2013).

In Vietnam, this plant is popularly cultivated in the Mekong Delta; it can be used as fresh or dried plant, decoction drunk to treat cough up phlegm, pulmonary tuberculosis, sore throat, enteritis, dysentery, diuretic, anti-inflammation, urinary infections, galactopoietic, pulmonary disease, etc. (Vo Van Chi, 2012). In modern medicine, *Pouzolzia zeylanica* is also combined with other herbs that could fight cancer cells, tuberculosis and are good for lungs (Le Thanh Thuy, 2007).

The reported studies not only identified the structure and presence of bioactive compounds but also assessed the antimicrobial, antifungal, antioxidant properties of *Pouzolzia zeylanica* plant. However, the chemical components of this medicinal plant in different parts have not been studied yet. The aim of study was to analyze and compare the content of bioactive compounds (anthocyanin, flavonoid, polyphenol and tannin), pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids), and antioxidant activity (AAI – antioxidant ability index, FRAP – ferrous reducing ability power and DPPH – 2,2-diphenyl-1-picrylhydrazyl) of ethanol extract from different parts (shoot, leaf, stem and whole plant) of *Pouzolzia zeylanica*.

2 MATERIALS AND METHODS

2.1 Equipment and chemicals

Equipment used in the study included a spectrophotometer (SPUVS, model SP-1920, Japan), vortex lab (VELP Scientifica, Europe), centrifugal (model EBA 20 Hettich, Germany) and water bath (Menmert, France).

Chemicals that consisted of folin-cioalteau reagent, folin-denis reagent, gallic acid, quercetin, tannic acid, 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), DPPH and ferrous sulfate were supplied by Sigma Chemical Co. (St. Louis, Mo. USA) and Merck (Darmatadt, Germany). Ferric chloride, aluminum chloride, sodium carbonate, sodium acetate, glacial acetic acid, hydrochloric acid and ethanol were supplied by Analytical Reagent (Xilong Chemical Co. Ltd., China) and Himedia (Hemidia Laboratories Pvt. Ltd., India).

2.2 Sample preparation and extraction

Whole plants of *Pouzolzia zeylanica* were collected at the stage of three months of age after being planted from the experimental area of An Giang University, during June, 2016. The height of plants was about 30-35 cm. Then, the shoots, stems and leaves of plants were separated into different parts. Young shoots were taken from the shoot moristems with a length of about 5 cm. The remaining plants were divided into the leaves and stems (Figure 1).

Fig. 1: Whole plants of *Pouzolzia zeylanica* (a), stems (b), shoots (c) and leaves (d)

The samples were cut fine, taking about 5 g of each plant part to extract with extraction conditions including the ethanol concentration of 60% (v/v), ratio of material to solvent of 1/20 (g/mL), extraction time of 60 minutes and temperature of 60^{o}C (Nguyen Trong Diep *et al*., 2013; Nguyen Tien Toan and Nguyen Xuan Duy, 2014). The triangular flask with cover and thermostatic tank were used in this research. The extract was filtered using Buchner funnel with Whatman's No 1 filter paper. The filtrate (crude extract) was diluted in ethanol at an appropriate ratio using for analysis.

2.3 Analytical methods

2.3.1 Determination of anthocyanin content

Total monomeric anthocyanin content was determined following different pH method (Lee *et*

al., 2005; Ahmed *et al.,* 2013); the result was expressed in milligrams of cyanidin-3-glucoside equivalents (CE) per gram of dry weight (DW). Sample absorbance was read against a blank cell containing distilled water. The absorbance (A) of the sample was then calculated according to the following formula:

$$A = (A_{520} - A_{700})\ pH_{1.0} - (A_{520} - A_{700})\ pH_{4.5}$$

Where A_{520} and A_{700} are absorbance of sample in the two pH buffer solutions ($pH_{1.0}$ and $pH_{4.5}$) at the wavelenght λ = 520 and 700 nm.

The total anthocyanin content (TAC) in the original sample was calculated according to the following formula:

$$\text{TAC (mg CE/g DW)} = \frac{[A \times MW \times DF \times 1000] \times V}{(\varepsilon \times 1) \times W}$$

Where MW is cyanidin-3-glycoside molecular weight (449.2 in g/mol); DF is the dilution factor; V is volume of the obtained extracts (L); ε is molar absorptivity (26,900 in L/mol); W is the weight of material sample (g).

2.3.2 Determination of flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination (Eswari *et al*., 2013; Mandal *et al*., 2013). About 1 mL of the crude extracts/standard of different concentration solution was mixed with 3 mL of ethanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 M sodium acetate and 5.8 mL of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with spectrophotometer against blank. The calibration curve was prepared by diluting quercetin in ethanol ($y = 0.0054x + 0.0026$ and $r^2 = 0.9995$). The total flavonoid content (TFC), milligrams of quercetin equivalents (QE) per gram dry weight (DW), was calculated by the following formula:

$$\text{TFC (mg QE/g DW)} = \frac{[A - 0.0026] \times DF \times V}{0.0054 \times W}$$

Where A is the absorbance of the test samples; DF is the dilution factor; V is volume of the obtained extracts (L); W is the weight of material sample (g).

2.3.3 Determination of polyphenol content

Total polyphenol content was determined by folin-ciocalteu reagent method (Hossain *et al*., 2013). Each crude extract (0.2 mL) was taken in a test tube and added 10% Folin-Ciocalteu reagent (1.5 mL). Then all test tubes were kept in a dark place for 5 minutes. Finally, 5% Na_2CO_3 (1.5 mL) was added to solution and mixed well in a vortex. Again, all the test tubes were kept in the dark for 2 hours. The absorbance was measured for all solution by using UV-spectrophotometer at constant wavelength of 750 nm. Total polyphenol concentrations were quantified by calibration curve obtained from measuring the absorbance of a known concentration of gallic acid standard in ethanol ($y = 0.0082x + 0.0595$ and $r^2 = 0.9996$). The total polyphenol content (TPC), milligrams of gallic acid equivalents (GAE) per gram dry weight (DW), was calculated by the following formula:

$$\text{TPC (mg GAE/g DW)} = \frac{[A - 0.0595] \times DF \times V}{0.0082 \times W}$$

Where A is the absorbance of the test samples; DF is the dilution factor; V is volume of the obtained extracts (L); W is the weight of material sample (g).

2.3.4 Determination of tannin content

Tannin content was determined by folin-denis method (Laitonjam *et al.,* 2013). Each crude extract (0.5 mL) and distilled water (0.5 mL) were taken in a test tube. Finally, the samples were treated with 0.5 mL of freshly prepared folin-denis reagent, and 20% sodium carbonate (2 mL) was added, shaken well, warmed on boiling water-bath for 1 minutes and cooled to room temperature. Absorbance of the colored complex was measured at 700 nm. Tannin concentration was quantified basing on the calibration curve of tannic acid in ethanol ($y = 0.0098x + 0.0478$ and $r^2 = 0.9996$). The tannin content (TC), milligrams of tannic acid equivalents (TAE) per gram dry weight (DW), was calculated by the following formula:

$$\text{TC (mg TAE/g DW)} = \frac{[A - 0.0478] \times DF \times V}{0.0098 \times W}$$

Where A is the absorbance of the test samples; DF is the dilution factor; V is volume of the obtained extracts (L); W is the weight of material sample (g).

2.3.5 Determination of AAI

AAI of samples were determined by reducing power method (Nguyen Thi Minh Tu, 2009; Saha *et al*., 2013). Two ml of plant extract was mixed with 2.5 ml phosphate buffer (pH 7.4) and 2.5 ml of 1% aqueous postassium ferriccyanide solution. This mixture was kept at 50°C in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloroacetic acid was added and centifuged at 3,000 rpm for 5 minutes. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of 0.1% freshly prepared ferric chloric solution. Then the absorbance of solution was measured at 700 nm using a spectrophotometer against blank. AAI calculated by the following formula:

AAI = Abs sample/Abs blank

Where Abs sample is the absorbance of extract; Abs blank is the absorbance of distilled water

2.3.6 Determination of FRAP

FRAP assessment was performed according to the method of Adedapo *et al.* (2009). The stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the solution was raised to 37°C before use. Plant extracts (150 µL) were allowed to react with 2,850 µl of the FRAP solution
for 30 minutes in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve of $FeSO_4$ was established (y = 0.5177x + 0.0855 and r^2 = 0.9981). Results were expressed in µM $FeSO_4$/g dry weight (DW).

$$\text{FRAP (µM } FeSO_4\text{/g DW)} = \frac{[\text{Abs} - 0.0855] \text{ x V x } 1000}{0.5177 \text{ x W}}$$

Where Abs is the absorbance of sample; V is volume of the obtained extracts (L); W is the weight of material sample (g).

2.3.7 Determination of DPPH radical scavenging capacity

The scavenging ability of extract against DPPH radical was determinaed using the method of Aluko *et al.* (2014). One millilitre of 0.135 mM of DPPH in ethanol was mixed with 1 ml of test solution. The mixture was kept in a dark cupboard for 30 minutes. The absorbance of the resulting solution was measured spectrophotometerically at 517 nm and the scavenging ability of the extract was calculated as:

DPPH radical scavenging activity (%) = [(Abs control – Abs sample)/Abs control] x 100

Where Abs control is the absorbance of DPPH radicals + ethanol; Abs sample is the absorbance of DPPH radical + extract

2.3.8 Determination of pigments content

The content of chlorophyll and carotenoids of samples were performed according to the method of Singh *et al.* (2014). Sample extracts were measured at 663, 645 and 480 nm wavelengths, with 60% ethanol as the blank. The chlorophyll content was calculated by the following formula:

Chlorophyll a (mg/g DW) = [(12.7x A_{663} – 2.69 x A_{645})/(1000 x W)] x V

Chlorophyll b (mg/g DW) = [(22.9 x A_{645} – 4.68 x A_{663})/(1000 x W)] x V

Total chlorophyll (mg/g DW) = [(20.2 x A_{645} – 8.02 x A_{663})/(1000 x W)] x V

Carotenoids (mg/g DW) = A_{480} + (0.114 x A_{663}) – (0.638 x A_{645})

Where A is the absorbance of the extract at respective wavelengths, V is the volume of extract (ml), and W is the weight of the sample (g)

2.4 Data analysis

All results were presented as means and standard deviation. A statistical analysis system (Statgraphic software package, version 16.0) was used to perform all statistical analyses. Data were compared by one-way analysis of variance; the analysis of LSD was considered significantly different at P≤0.05.

3 RESULTS AND DISCUSSION

Almost all of the parts of the plants namely leaf, flower, fruit, stem and root have their own bioactive compounds which can be used for therapeutic purpose. Typically, medicinal plants ensure an extensive supply of antibiotic, antifungal, antiseptic, analgesic compounds etc. (Pandurangan *et al.,* 2018). Several studies reported that the aerial parts of the plants, such as stems and leaves, are normally used for the extraction of active phytochemicals. According to previous findings of medicinal herbs researches, there are some determining factors of the amount and types of phytochemicals content. Other researchers claimed that growth stage of plants contributes to the level of phytochemical content (Raya *et al.,* 2015). *Pouzolzia zeylanica* has been known as medicinal plant which contains various bioactive comppounds such as polyphenol, flavonoid, tannin, isoflavone, glycoside, phyllanthin, vitexin, carotenoids, etc. (Ghani, 2003; Le Thanh Thuy, 2007; Saha and Paul, 2012). The result of the present study showed that the content of bioactive compounds in different parts of *Pouzolzia zeylanica* plant was different (Table 1).

Table 1: The content of bioactive compounds in different parts of *Pouzolzia zeylanica*

Different parts	Anthocyanin (mgCE/g DW)	Flavonoid (mgQE/g DW)	Polyphenol (mgGAE/g DW)	Tannin (mgTAE/g DW)
Young shoots	3.12 ± 0.132^{a}	18.72 ± 0.487^{a}	39.32 ± 1.526^{a}	29.54 ± 0.568^{a}
Leaves	2.65 ± 0.059^{b}	17.39 ± 0.165^{b}	32.47 ± 0.926^{b}	26.87 ± 0.508^{b}
Stems	0.89 ± 0.039^{d}	6.68 ± 0.497^{d}	20.06 ± 0.975^{c}	20.75 ± 0.941^{c}
Whole plants	2.06 ± 0.082^{c}	14.88 ± 0.166^{c}	30.53 ± 1.031^{b}	26.18 ± 0.722^{b}

Note: Data represent the means (n=3) and ± standard deviation. Values in each column followed by the same super-script letters are not significantly different by LSD at P≤0.05.

Phenolic compounds are secondary metabolites and naturally present in plants. They have great importance for the food and drink products derived from plants, since these compounds are responsible for their organoleptic properties (Dvořáková *et al*., 2007). Anthocyanins are responsible for attractive colors of flowers, fruits and vegetables as well as their products (Mazza and Brouillard, 1990). In addition, anthocyanin also have multiple biological roles, e.g. antioxidant activity, anti-inflammatory action, inhibition of blood platelet aggregation and antimicrobial activity, treatment of diabetic retinopathy and prevention of cholesterol-induced atherosclerosis (Mazza and Miniati, 1993; Wang *et al*., 1997; Cliford, 2000; Espin *et al.,* 2000). Flavonoids can have a wide range of biological activities, the protective role of flavonoids in living systems was mostly due to their antioxidant potential, which is related to transfer of reactive oxygen species, chelation of metal catalysts, activation of antioxidants enzymes and inhibition of certain type of oxidases and colon cancer (Heim *et al*., 2002; Chidambara Murthy *et al*., 2012). Flavonoids also have the potency to stimulate the immune system, induce protective enzymes in the liver or block damage to genetics materials (Zarina and Tan, 2013). Polypenols are present in various plants and have been shown to be good antioxidant in both in vitro and in vivo studies. It helps reduce the risk for various life style-related diseases including cancer and cardiovascular diseases, which have been linked to the formation of active oxygen species (Yoshida *et al.,* 2000). Tannin is present in varying concentrations in plants, and plays important roles in modulating cardiac action potential repolarization and tumor cell biology (Chu *et al*., 2015).

The results in Table 1 showed that the content of anthocyanin and flavonoid in whole *Pouzolzia zeylanica* plant was 2.06±0.082 mg CE/g DW and 14.88±0.166 mg QE/g DW, respectively, and there was statistically significant difference between parts of plants such as young shoots, leaves, stems and whole plants with P≤0.01. In particular, young shoots contained the highest anthocyanin and flavonoid content, with 3.12±0.132 mg CE/g DW and 18.72±0.487 mg QE/g DW, followed by leaves, whole plants and stems. Similarly, the highest content of polyphenol and tannin were recorded in young shoots, with 39.32±1.526 mg GAE/g DW and 29.54±0.568 mg TAE/g DW, followed by leaves and whole plants, and there was no statistically significant difference between leaves and whole plants (P≤0.01). The lowest content of these compounds was observed in stems. The result of Raya *et al.* (2015)'s study also showed that the content of total phenolic and flavonoid in *Clinacanthus nutans* were significantly influenced by plant parts. The content of these compounds was higher in leaves than that in stems. Quantification of secondary metabolites in the root, stem and foliar tissues of *Centella asiatica* revealed the presence of various bioactive compounds at varying concentrations. The concentrations of phenols, tannin and flavonoid was higher in the leaves than that in stems and roots (Vaddadi *et al.,* 2017). The phenolics content of *Moringa oleifera* plant was higher in leaf than that in stems and stalks (Shih *et al.,* 2011). Each plant part has different content of chemical substances, for example, total phenolic content and antioxidant composition of *Urtica dioica* L. vary with plant parts (Khare *et al.*, 2012).

Phenolic compounds of the extracts are probably involved in their antiradical activity. Phenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity because of their scavenging ability due to their hydroxyl groups (Shih *et al.,* 2011). A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, DPPH. DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of plant extracts. One important mechanism of antioxidation involves the scavenging of hydrogen radicals. DPPH has a hydrogen free radical and shows a characteristic absorption at 517 nm. After encountering the proton-radical scavengers, the purple color of the DPPH solution fades rapidly (Deighton *et al*., 2000). The method of AAI assay showed that antioxidants can donate an electron to free radicals,

which leads to the neutralization of the radical. Reducing power was measured by direct electron donation in the reduction of $Fe^{3+}(CN-)_6$–$Fe^{2+}(CN-)_6$. The extract was visualized by forming the intense Prussian blue color complex and then measured at λ 700 nm (Yen and Chen, 1995). In addition, FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine [Fe^{3+}-TPTZ] complex and producing a coloured ferrous tripyridyltriazine [Fe^{2+}-TPTZ] (Benzie and Strain, 1996). Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom (Duh *et al.,* 1999). FRAP assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction (Guo *et al.,* 2003). The ethanol extracts of different parts of *Pouzolzia zeylanica* plant were able to reduce the unstable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The results of the evaluation of the antioxidant activity of various plant parts were presented in Table 2.

Table 2: Antioxidant activity and moisture in different parts of *Pouzolzia zeylanica*

Different parts	AAI	DPPH (%)	FRAP (µM FeSO4/g DW)	Moisture (%)
Young shoots	5.52 ± 0.172^a	88.29 ± 0.942^a	578.10 ± 8.371^a	83.23 ± 0.589^c
Leaves	4.84 ± 0.077^b	85.14 ± 1.184^b	529.08 ± 10.101^b	82.67 ± 0.406^c
Stems	3.93 ± 0.111^c	58.56 ± 0.799^d	501.20 ± 6.843^c	86.97 ± 0.155^a
Whole plants	4.71 ± 0.060^b	78.11 ± 1.264^c	546.11 ± 5.171^b	85.28 ± 0.094^b

Note: Data represent the means (n=3) and ± standard deviation. Values in each column followed by the same superscript letters are not significantly different by LSD at P≤0.05.

Table 2 showed that ethanol extract of young shoots had the highest antioxidant activity among the three tested methods, followed by leaves, whole plants and stems (AAI method), and followed by whole plants, leaves and stems (FRAP method), and there was no statistically significant difference between leaves and whole plants. While there was statistically significant difference (P≤0.01) in various parts such as young shoots > leaves > whole plants > stems (DPPH method). The lowest antioxidant value was found in stems. For example, the young shoots extract had AAI of 5.52; scavenging 88.29% free radical of DPPH and 578.10 µM $FeSO_4$/g DW. The study result of Raya *et al.* (2015) showed that antioxidant power was higher in young plant than that in old plant irrespective of plant parts. The highest DPPH was observed in young leaves followed by young stems. The lowest DPPH was recorded with matured stems. Ethanol extracts of *Centella asiatica* root, stem and leaf were tested for their scavenging activities. Result showed that leaf extracts have shown high DPPH scavenging activities compared with those of root and stem extracts (Vaddadi *et al.,* 2017). The methanolic extract of Moringa showed strong scavenging effect of DPPH radicals and reducing power. The trend of antioxidative activity as a function of the part of *Moringa oleiferwas*: leaf > stem > stalk for samples investigated (Shih *et al*., 2011).

The analysis of the moisture content of different parts of *Pouzolzia zeylanica* plant showed that the highest moisture content was observed in stems, followed by whole plants, young shoots and leaves. There was statistically significant difference (P≤0.01) between these parts of plant. The moisrure content ranged from 82.67 to 86.97% (Table 2).

Chlorophyll is a specifically pigment of green plants, which plays a key role in photosynthesis. In plants there are several types of chlorophyll, denoted by letters of a, b, c, d. Chlorophyll has effects on the human body. External acts as deodorant and skin tonic, internally, stimulates respiration, helps in cleansing waste and helps combat anemia (Dumbrava *et al*., 2012). The major cholorophylls in plants include choloropyll a and cholorophyll b, which are usually present at a ratio of 3 (Chen and Chen, 1993). Chlorophyll a is recognized as the main pigments which convert light energy into chemical energy. Chlorophyll b as accessory pigments acts indirectly in photosynthesis by transferring the light that it absorbs to chlorophyll a. The chlorophyll molecule has Mg^{2+} at its center which makes it ionic and hydrophilic, and a ring that is hydrophobic in nature with a carbonyl group at its tail which makes it polar. It is held in place in the plant cell within a water-soluble chlorophyll-binding protein. Chlorophyll-b differs from chlorophyll-a only in one functional group (i.e -CHO) bounded to the porphyrin ring, and is more soluble than chlorophyll-a in polar solvents because of its carbonyl group (Costache *et al.,* 2012; Sumanta *et al.,* 2014). Carotenoids are located in chromoplast, contribution color to vegetables/fruits, and also in chlorophylls, where together with chlorophylls involved in the two photosystems. Carotenoids group and their derivatives consist of about 70 compounds that are present in most vegetables and fruits. The carotene pigments were the most important photosynthetic

pigments, and they prevented chlorophyll and thylakoid membrane from the damage of absorbed energy by peroxidation (Costache *et al.,* 2012; Sumanta *et al.,* 2014). Analytical result in this study showed that *Pouzolzia zeylanica* plant was also present chlorophylls and carotenoids pigments (Table 3).

Table 3: The content of pigments in different parts of *Pouzolzia zeylanica*

Different parts	Chlorophyll a (mg/g DW)	Chlorophyll b (mg/g DW)	Total chlorophyll (mg/g DW)	Carotenoids (mg/g DW)
Young shoots	2.203 ± 0.073^{a}	1.601 ± 0.066^{b}	3.802 ± 0.138^{b}	7.725 ± 0.096^{b}
Leaves	2.292 ± 0.068^{a}	2.164 ± 0.104^{a}	4.455 ± 0.038^{a}	8.152 ± 0.020^{a}
Stems	0.681 ± 0.015^{c}	0.690 ± 0.029^{d}	1.371 ± 0.043^{d}	3.171 ± 0.089^{d}
Whole plants	1.375 ± 0.062^{b}	1.056 ± 0.048^{c}	2.430 ± 0.110^{c}	5.128 ± 0.167^{c}

Note: Data represent the means (n=3) and ± standard deviation. Values in each column followed by the same superscript letters are not significantly different by LSD at P≤0.05.

Table 3 showed that the highest content of chlorophyll a was observed in leaves, with 2.292±0.068 mg/g DW, followed by young shoots, whole plants and stems, and there was statistically significant difference between leaves, whole plants and stems, but there was no statistically significant difference between leaves and young shoots. The highest content chlorophyll b, total chlorophyll and carotenoids were also recorded in leaves, with 2.164±0.104 mg/g DW, 4.455±0.038 mg/g DW, 8.152±0.020 mg/g DW, respectively, followed by young shoots, whole plants and stems, there was statistically significant difference between these different parts (P≤0.01). In the tested samples a ratio between chlorophyll a and chlorophyll ranged from 0.99 to 1.38, meaning that chlorophyll a was the main form of chlorophyll in young shoots, and chlorophyll b was the main form of chlorophyll in stems. Other scientists also reported that changes in the color and the content of chlorophylls were related to the genotype but not to the growing conditions (Bekhradi *et al*., 2015). The result of the present study was in line with the reported result of Straumite *et al.* (2015), in the stems chlorophyll content was significantly lower than in leaves. The highest chlorophyll content was observed in young leaves which contained 72% higher chlorophyll than matured leaves. The lowest chlorophyll content was found in matured stems (Raya *et al.*, 2015). The basic pigments of green plants are chlorophylls, always accompanied by carotenoids. In part of samples, significantly higher concentration of carotenoids in stems was observed (*Mentha suaveolens*) and significantly higher content of carotenoids in leaves only in *Mentha piperita* was determined. For other samples, differences between the leaves and the stems were not significant (Straumite *et al.,* 2015).

4 CONCLUSIONS

The content of bioactive compounds, pigments and the antioxidant activity of *Pouzolzia zeylanica* plant were differently present in various parts of plant. The quality characteristics of young shoots were higher than those of leaves, whole plants and stems. The content of anthocyanin, flavonoid, polyphenol, tannin, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in young shoots was 3.12 mg CE/g DW, 18.72 mg QE/g DW, 39.32 mg GAE/g DW, 29.54 mg TAE/g DW, 2.203 mg/g DW, 1.601 mg/g DW, 3.802 mg/g DW, 7.725 mg/g DW, respectively. This result showed that young shoots of *Pouzolzia zeylanica* plants can be used to process tea. It can be considered as good sources of natural products that may be employed in the treatment of the different diseases associated to the oxidative stress.

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