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Bioactive compounds, pigment content and antioxidant activity of *Pouzolzia zeylanica* plant collected at different growth stages

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ABSTRACT

The aim of this study was to investigative the effect of different growth stages of Pouzolzia zeylanica plant (young and mature plant) on bioactive compounds content (anthocyanin, flavonoid, polyphenol and tannin); the content of pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids); antioxidant activity through some measurable values such as antioxidant ability index (AAI), ferrous reducing ability power (FRAP) and scavenging capacity 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical; as well as the color parameters of stem and leaves (L^*, a^*, b^* and ΔE). The results showed that the content of anthocyanin, flavonoid, polyphenol, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of young plant was higher than that of mature ones, and had statistically significant difference ($P<0.05$), but the content of tannin was not different between the two groups ($P>0.05$). There was no difference in antioxidant capacity between young and mature plants when performed with scavenging free radical (DPPH) or total reducing power (AAI), but there was significant difference when performed with ferrous reducing ability power (FRAP) method. In addition, there were also statistically significant differences in average values of a^* and b^* between young and mature, stem and leaves, and these two parameters were related to the content of anthocyanin, chlorophyll, carotenoid in Pouzolzia zeylanica plant.

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1 INTRODUCTION

Pouzolzia zeylanica is a medicinal plant in Urticaceae family. It has long been used as one of the components in herbal remedies for treating various diseases. In Vietnam, this plant was 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. It is distributed in tropical and subtropical regions. Nowadays, it is present in many Asian countries such as China including Taiwan, India, Indonesia, Japan, Malaysia, Myanmar, Nepal, Papua New Guinea, Pakistan, the Philipines, Sri Lanka, Thailand, Vietnam, Laos, Singapore, Brunei Darussalam, Bangladesh, Maldives, Polynesia, Yemen (Socotra), El Savodor, and some different places in the world (Adhikari and Babu, 2008). People from many Asian countries have used it to treat various kinds of diseases by traditional method such as poul-

tice to bone fractures, boils and to relieve stomachache, diabetes, cancer, treat eyes injurys; itching, dysentery and loose stools of infant, cure stomach ailments, preventive radiation and confirmed the therapeutic value of polyphenols contained in the leaves (Li, 2006; Yusuf *el al*., 2006; Purkayastha *el al*., 2007; Bhattacharjya and Borah, 2008; Ratnam and Raju, 2008; Mondal *el al*., 2013; Sandhya *el al*., 2013). In modern medicine, *Pouzolzia zeylanica* is also combined with other herbs that could fight cancer cells, against tuberculosis and good for your lungs (Le Thanh Thuy, 2007).

Many recent studies showed that it contains various bioactive comppounds such as alkaloid, polyphenol, flavonoid, tannin, isoflavone, glycoside, phyllanthin, vitexin, friedelin, myricyl palmitate, myricyl alcohol, carotenoid, minerals and their salts etc. and extract has antimicrobial, antifungal, antioxidant and reducing free radical properties (Ghani, 2003; Li *el al*., 2012; Paul and Saha, 2012; Saha and Paul, 2012a and 2012b; Saha *el al.*, 2012; Sarma and Dinda, 2013). Moreover, the product extracted with ethanol from this plant did not show oral toxicity at doses 10 g/kg of body weight for lab mice (Tran Thi My Tien, 2010).

Therefore, *Pouzolzia zeylanica* plants can be considered as raw material for processing precious food products that can support and prevent illness. However, in order to process products from them, the producer must know how to select material sources which have good quality. In addition, the chemical component of this medicinal plant in different growth stages has not been studied yet. Contributing to knowledge of *Pouzolzia zeylanica* uses with the best quality, the study was carried out to survey effect of different growth stages (young and mature plant) on bioactive compounds content (anthocyanin, flavonoid, polyphenol and tannin), the content of 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 this plant.

2 MATERIALS AND METHODS

2.1 Equipment and chemicals

Equipment used 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 consisting of Folin-Cioalteau reagent, Folin-Denis reagent, gallic acid, quercetin, tannic acid, 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferrous sulphate 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

Pouzolzia zeylanica plants were harvested from experimental area of An Giang University, during June, 2016. The young plant sample was harvested at one month of age after planting. The mature plant sample was harvested at three and a half months of age, in flowering stage (Figure 1).

The samples were cleaned, drained preliminarily and cut fine, taking about 5g 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, respectively. 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.

Figure 1: *Pouzolzia zeylanica* plant at young (a) and mature (b) stages

2.3 Analytical methods

Determination of bioactive compounds content

Total monomeric anthocyanin content was determined following different pH method (Lee *el al*., 2005; Ahmed *el al.*, 2013); the result was expressed in milligrams of cyanidin-3-glucoside equivalents (CE) per gram of dry weight (DW). The total flavonoid content was determinated by Aluminum chloride colorimetric method with some modifications (Eswari*el al*., 2013; Mandal *el al.*, 2013); the result was expressed in milligrams of quercetin equivalents (QE) per gram of DW. The total polyphenol content was determined by Folin-Ciocalteu reagent method (Hossain *el al*., 2013); the result was expressed in milligrams of gallic acid equivalents (GAE) per gram of DW. Tannin content was determined by Folin-Denis method (Laitonjam *el al.*, 2013), the result was expressed in milligrams of tannic acid equivalents (TAE) per gram of DW.

Evaluation of antioxidant activity

Antioxidant ability index (AAI) of samples were determined by reducing power method (Nguyen Thi Minh Tu, 2009; Saha *el al*., 2013). Ferrous reducing ability power (FRAP) was performed according to the method of Adedapo *el al.* (2009). Free radical scavenging capacity (DPPH) was estimated using the method of Aluko *el al.* (2014).

Determination of pigments content

The content of chlorophyll and carotenoids of samples were performed according to the method of Singh *el al.* (2014).

Color analysis of Pouzolzia zeylanica plants

Color of stem and leaves was measured in Commission Internationale de l'Eclairage (CIE) the L* a* b* color system using a colorimeter (Konica Minolata, CR-400, Japan). Before the measurement, the colorimeter was calibrated using a white reference tile and a light trap (black tile). In color measurement, CIE the L* a* b* coordinates show the degree of brightness (L), the degree of redness (+a), or greenness (–a), and the degree of yellowness (+b), or blueness (–b), respectively (Tarhan *el al*., 2010). Total color difference (ΔE) indicates the color difference from the standard plate calculated as Rhim *el al*. (1999).

$$\Delta E = \sqrt{(L_0 - L^*)^2 + (a_0 - a^*)^2 + (b_0 - b^*)^2}$$

2.4 Data analysis

All results are presented as the means ± 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; P≤0.05 was considered significantly different.

3 RESULTS AND DISCUSSION

The ability of the synthesis of secondary metabolites depends on the direction of plant growth. Each plant

species enters its specifities into metabolic processes which result in the synthesis of various metabolites. Variations in the concentration of secondary metabolites are the result of both biotic and abiotic factors (Stankovic *el al*., 2010; Stankovic *el al*., 2011). The concentration of bioactive compounds (anthocyanin, flavonoid, polyphenol and tannin) in *Pouzolzia zeylanica* plant at different stages was showed in Table 1.

Table 1: The content of bioactive compounds of whole plant collected at different stages of growth

Growth stage	Anthocyanin (mgCE/g DW)	Flavonoid (mgQE/g DW)	Polyphenol (mgGAE/g DW)	Tannin (mgTAE/g DW)
Young (one month of age)	$3.86^{a} \pm 0.012$	$18.46^{a} \pm 0.471$	$38.68^{a} \pm 1.150$	$30.79^{a} \pm 1.425$
Mature (flowering stage)	$1.96^{b} \pm 0.016$	$14.97^{b} \pm 0.628$	$33.43^{b} \pm 2.389$	$29.70^{a} \pm 2.703$

Notes: Data represent the means (n=3) and ± standard deviation. Values in each column followed by different superscript letters are significantly different at P<0.05

The result showed that young plant contained more bioactive compounds than the mature plant. The content of anthocyanin, flavonoid and polyphenol in young plant was higher than that of mature plant with statistcally significant difference (P<0.05). However, the tannin content was slightly higher than that of mature plant, but this difference was not significant (P>0.05). Nhuan and Hwang (2014) reported that the young leaves contained more polyphenol and total flavonoid than the old leaves. The content of total phenolic compounds was the highest value during April, when the plant is the rosette stage, and then, in stage fruit formation. The highest concentration of flavonoid was just before flowering, and it also increased in fruit formation stage. Total phenolic and flavonoid contents of *Chelidonium majus* ranged from 5.74 to 60.96 mgGAE/g and 7.12 to 291.58 mgRE/g of extract, the changes depended on growth stage of plant and extract solvent (Jakovljevic *el al*., 2013). The concentration of plant metabolites differed from one season to another and between different growth stages (Shuib *el al*., 2011). The study result of Shuib *el al*. (2011) showed that phenolic compounds were one component in plants that were affected by the growth and environmental conditions. The total phenolic content of *Cosmos caudatus* was high and ranged from 19 to 26.04 gGAE/100g. There was a significant difference from the three growth stages and two seasons. The metabolites of the broccoli vegetable also showed significant difference at various growth stages (Vallejo *el al.,* 2003). The content of bioactive compounds (anthocyanin, flavonoid, polyphenol and tannin) of *Pouzolzia zeylanica* were 1.98 to 3.86 mgCE/g; 14.97 to 18.46 mgQE/g; 33.43 to 38.68 mgGAE/g and 29.70 to 30.79 mgTAE/g DW. The result of this study showed that *Pouzolzia zeylanica* at young and mature stages had the different changes in bioactive compounds, the content of anthocyanin, flavonoid, polyphenol, and tannin in young plant were higher than that of mature plant.

The contents of pigment (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) of whole *Pouzolzia zeylanica* plant at different stages of growth are present in Table 2. Results showed that the chlorophyll a, chlorophyll b, total chlorophyll and carotenoids content of young plant were higher than those of mature plant, and had statistically significant difference at P<0.05. In contrast, the research of Nhuan and Hwang (2014) showed that the old aronia leaves contained more chlorophyll than the young leaves, but this difference was not significant. Both young and old leaves contained more chlorophyll a than chlorophyll b. The total carotenoid content of the old leaves was slightly higher than that of the young leaves, but the difference was minimal. The chlorophyll and carotenoid contents of plant leaves vary according to several biotic factor, including species, variety, cultivate, production pratice, maturity, and abiotic factors including light, temperature, and soil properties (Van den Berg *el al.,* 2000; Loranty *el al.,* 2010; Znidarcic *el al*., 2011). Znidarcic *el al*. (2011) reported that total chlorophyll content in some vegetables ranged from 2.00 to 3.59 mg/g DW and the concentration of chlorophyll a (1.42 to 2.61 mg/g DW) was higher than that of chlorophyll b (0.58 to 0.98 mg/g DW). The content of total chlorophyll and carotenoid of aronia leaves ranged from 8.48 to 66.32 mg/g DW and 1.36 to 9.88 mg/g DW, the difference depended on growth stage of plant and extract solvent (Nhuan and Hwang, 2014). The content of total chlorophyll and carotenoid of *Pouzolzia zeylanica* plant ranged 2.09 to 3.01 mg/g and 4.12 to 5.52 mg/g DW. The content of pigments was similar or higher than of that reported by other scientists.

Table 2: The content of pigments of whole plant collected at different stages of growth

Growth stage	Chlorophyll a (mg/g DW)	Chlorophyll b (mg/g DW)	Total chlorophyll (mg/g DW)	Carotenoids (mg/g DW)
Young (one month of age)	$1.947^{a} \pm 0.0347$	$1.063^{a} \pm 0.0618$	$3.009^{a} \pm 0.0278$	$5.519^{a} \pm 0.0693$
Mature (flowering stage)	$1.354^{b} \pm 0.0606$	$0.736^{b} \pm 0.0208$	$2.091^{b} \pm 0.0429$	$4.121^{b} \pm 0.0272$

Notes: Data represent the means (n=3) and ± standard deviation. Values in each column followed by different superscript letters are significantly different at P<0.05

Besides, the study was also analyzed antioxidant activities of ethanol extract of whole *Pouzolzia zeylanica* collected at different stages of growth through AAI, DPPH and FRAP. Results were showed in Table 3.

The result of study showed that FRAP of ethanol extract of mature *Pouzolzia zeylanica* plant was higher than that of young plants, and had significant difference (P<0.05). However, DPPH and AAI of two stages was not significantly different. Antioxidant ability of ethanol extract from this medicinal plant achieved high value, scavenged around 82.53% DPPH free radical and reduced around 659.72 to 673.71 µM $FeSO_4$/g DW, antioxidant index value was from 4.18 to 4.52. The antioxidant potential of the samples was estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) and the reduction of ethanol solution of colored free radical DPPH by free radical scavenger. The scavenging activity was measured as the decrease in absorbance of the samples verus DPPH standard solution. The antioxidant activity (IC_{50}-DPPH) of methanol extract of *Chelidonium majus* obtained the lowest value at rosette stage was 50.72 mg/ml; then initial flowering stage 68.05 mg/ml; flowering stage 196 mg/ml and stage of fruit formation 192.99 mg/ml (Jakovljevic *el al*., 2013). The study indicated that the antioxidant capacity of *Pouzolzia zeylanica* plant could be influenced by growth stage; this finding could be pertinent, as antioxidants may have the potential to prevent a range of chronic, degenerative diseases including cancer, heart disease, and neurological disorders by mitigating oxidative stress in human body.

Table 3: The indexes of antioxidant activity of ethanol extract of whole plant collected at different stages of growth

Growth stage	AAI	DPPH (%)	FRAP (µM $FeSO_4$/g DW)	Moisture (%)
Young (one month of age)	$4.18^{a} \pm 0.068$	$82.53^{a} \pm 0.254$	$659.72^{b} \pm 1.504$	$89.40^{a} \pm 0.0073$
Mature (flowering stage)	$4.52^{a} \pm 0.172$	$82.34^{a} \pm 0.837$	$673.71^{a} \pm 4.286$	$86.24^{b} \pm 0.0037$

Notes: Data represent the means (n=3) and ± standard deviation. Values in each column followed by different superscript letters are significantly different at P<0.05

Analytical result in Table 3 showed that the moisture content of young plant was higher than that of mature plant, and had significant difference (P<0.05). The moisture content of young plant and mature one was 89.40% and 86.24%, respectively.

In additon, the study also performed measuring of color parameters (L^*, a^*, b^* and ΔE) of *Pouzolzia zeylanica* stem and leaves collected at different stages of growth. Results were showed in Table 4.

Table 4: The color parameters of stem, leaves of *Pouzolzia zeylanica* plant collected at different stages of growth

Growth stages	L^*	a^*	b^*	ΔE
Young (Stem)	$32.93^{e} \pm 1.165$	$11.17^{b} \pm 0.879$	$-5.35^{c} \pm 0.596$	$61.64^{a} \pm 1.099$
Mature (Stem)	$35.80^{d} \pm 0.448$	$7.66^{c} \pm 0.179$	$-2.36^{b} \pm 0.292$	$59.55^{a} \pm 0.388$
Young (Leaves – lower face)	$43.38^{bc} \pm 0.558$	$13.55^{a} \pm 0.362$	$-6.68^{c} \pm 0.306$	$51.10^{b} \pm 0.596$
Mature (Leaves – lower face)	$46.48^{a} \pm 0.266$	$12.54^{a} \pm 0.090$	$-6.33^{c} \pm 0.357$	$48.09^{c} \pm 0.307$
Young (Leaves – upper face)	$44.47^{b} \pm 0.607$	$3.25^{d} \pm 0.149$	$0.82^{a} \pm 0.175$	$52.60^{b} \pm 0.539$
Mature (Leaves – upper face)	$41.99^{c} \pm 0.387$	$2.24^{d} \pm 0.961$	$1.22^{a} \pm 1.167$	$55.58^{a} \pm 0.176$
Mean	40.84	8.57	-3.11	54.43
Coefficient of variation (CV, %)	2.0	6.0	21.0	2.0
Significant levels	**	**	**	**
Average value of growth stage				
Young	40.26^{a}	9.32^{a}	-3.74^{b}	55.11^{a}
Mature	41.42^{a}	7.82^{b}	-2.49^{a}	53.74^{a}
Significant levels	ns	**	*	ns
Average value of stem or leaves				
Stem	34.37^{b}	9.41^{b}	-3.86^{b}	60.60^{a}
Leaves – lower face	44.93^{a}	13.05^{a}	-6.50^{c}	49.59^{c}
Leaves – upper face	43.23^{a}	3.25^{c}	1.02^{a}	53.09^{b}
Significant levels	**	**	**	**

Notes: Data represent the means (n=3) and ± standard deviation. Values in each column followed by different super-script letters are significantly different at P<0.05. $^{(ns)}$ not statisticaly significant difference. $^{(*)}$ difference at significant level P<0.05. $^{(**)}$ difference at significant level P<0.01

The L^* parameter was obtained the lowest value (32.93) at stem of young plant and the highest value (46.48) at leaves (lower face) of mature plant. The average value of L^* parameter was different change between young to mature stage, but not significantly different (P>0.05). There was a statistical significantly difference (P<0.01) between stem and leaves (upper face and lower face) of *Pouzolzia zeylanica* plant. Similarly, the a^* parameter was obtained the lowest value (2.24) at leaves (upper face) of maturity and the highest value (13.55) at leaves (lower face) of young stage. There was a statistical significantly difference (P<0.01) between average value of a^* parameter of young and mature stage, stem and leaves.

The b^* parameter was obtained the lowest value (-6.68) at leaves (lower face) of young and the highest value (1.22) at leaves (upper face) of maturity stage. There was a statistical significantly difference (P<0.01) between average value of b^* parameter of young and mature stage, stem and leaves. The ΔE parameter was obtained the lowest value (48.09) at leaves (lower face) of maturity and the highest value (61.64) at stem of young stage. The average value of ΔE parameter changed differently between young and mature stage, but not statistically different (P>0.05). However, there was a statistical significantly difference (P<0.01) between stem and leaves (upper face and lower face) of *Pouzolzia zeylanica* plant. This result showed that there was relationship between a^*, b^* parameters and the content of pigments (chlorophyll and carotenoid) (Table 2) and anthocyanin content (Table 1) in *Pouzolzia zeylanica* plant.

4 CONCLUSION

The content of bioactive compounds, pigments, the antioxidant activity and the color parameters of *Pouzolzia zeylanica* plant were differently present in various stages of growth. The quality characteristics of young plant were higher than those of mature plant. The content of anthocyanin, flavonoid, polyphenol, tannin, chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in young plant were 3.86 mgCE/g DW, 18.46 mgQE/g DW, 38.68 mgGAE/g DW, 30.79 mgTAE/g DW, 1.947 mg/g DW, 1.063 mg/g DW, 3.009 mg/g DW, 5.519 mg/g DW, respectively.

REFERENCES

Adedapo, A.A., Jimoh, F.O., Afolayan, A.J. and Masika, P.J., 2009. Antioxudant Properties of the Methanol Extracts of the Leaves and Stems of *Celtis africana*. Records of Natural Products, 3 (1): 23-31.

Adhikari, B.S. and Babu, M.M., 2008. Floral diversity of Banganga Wetland, Uttarakhand, India. Check list 4 (3): 279-290.

Ahmed, J.K., Salih, H.A.M. and Hadi, A.G., 2013. Anthocyanin in red beet juice act as scavenger for heavy metals ions such as lead and cadmium.

International Jouranl of Science and Technology, 2 (3): 269-273.

Aluko, B.T., Alli, S.Y.R. and Omoyeni, O.A., 2014. Phytochemical analysis and antioxidant activities of ethanolic leaf extract of *Brillantaisia patula*. World Journal of Pharmaceutical Research, 3 (3): 4914-4924.

Bhattacharjya, D.K. and Borah, P.C., 2008. Medicinal weeds of crop fields and role of women in rural health and hygiene in Nalbari district, Assam. Indian Journal of Traditional Knowledge, 7 (3): 501-504.

Eswari, M.L., Bharathi, R.V. and Jayshree, N., 2013. Preliminary phytochemical screening and heavy metal analysis of leaf extracts of *Ziziphusoenoplia* (L) Mill. Gard. International Journal of Pharmaceutical Sciences and Drug Research, 5 (1): 38-40.

Ghani, 2003. Medicinal plants of Bangladesh: Chemical constituents and uses, 2nd Ed. pp. 104, The Asiatic Society of Bangladesh, Dhaka.

Hossain, M.A., Raqmi, K.A.S., Mijizy, Z.H., Weli, A.M. and Riyami, Q., 2013. Study of total phenol, flavonoids contents and phytochemical sreening of various leaves crude extracts of locally grown *Thymus vularis*. Asian Pacific Journal of Tropical Biomedicine 3 (9): 705-710.

Jakovljevic, Z.D., Stankovic, S.M. and Topuzovic, D.M., 2013. Sseasonal variability of *Chelidonium majus* L. secondary metabolites content and antioxidant activity. EXCLI Journal, 12: 260-268

Laitonjam, W.S., Yumnam, R., Asem, S.D. and Wangkheirakpam, S.D., 2013. Evaluative and comparative study of biochemical, trace elements and antioxidant activity of *Phlogacanthus pubinervius* T. Anderson and *Phlocanthus jenkincii* C.B. Clarke leaves. Indian Journal of Natural Products and Resources, 4 (1): 67-72.

Le Thanh Thuy, 2007. Preliminary test of chemical components for *Pouzolzia zeylanica* L. Benn. Chemical Master Thesis, University of Natural Science, HCM city (in Vietnamese).

Lee, J., Durst, R.W. and Wrolstad, R.E., 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study.Journal of AOAC International, 88 (5): 1269-1278.

Li, P., Huo, L., Su, W., *et al.*, 2011. Free radical-scavenging capacity, antioxidant activity and phenolic content of *Pouzolzia zeylanica*. Journal of the Serbian Chemical Society, 76 (5): 709-717.

Li, T.S.C., 2006. Taiwanese Native Medicinal Plants, Phytopharmacology and Therapeutic Values. CRC Taylor & Francis Group, LLC.

Loranty, A., Rembiałkowska, E., Rosa, E.A. and Bennett, R.N., 2010. Identification, quantification and availability of carotenoidsand chlorophylls in fruit, herb and medicinal teas. Journal of Food Composition Analysis, 23 (5): 432-441.

Mandal, S., Patra, A., Samanta, A., *et al.*, 2013. Analysis of phytochemical profile of *Terminalia arjuna bark* extract with antioxidative and antimicrobial properties. Asian Pacific Journal of Tropical Biomedicine 3 (12): 960-966.

Mondal, P., Bhuyan, N., Das, S., Kumar, M., Barah, S. and Mahato, K., 2013. Herbal medicines useful for the treatment of diabetes in North-East India: A review. International Journal of Pharmacy and Biological Sciences, 3 (1): 575-589.

Nguyen Thi Minh Tu, 2009. Study on isolation active compounds from Lingzhi (*Ganoderma lucidium)*. Journal of Science and Technology, Vietnam Academy of Science and Technology, 47 (1): 45-53 (in Vietnamese).

Nhuan, D.T. and Hwang, E.S., 2014. Bioactive Compound Contents and Antioxidant Activity in Aronia (Aronia melanocarpa) Leaves Collected at Different Growth Stages. Prev. Nutr. Food Sci., 19 (3): 204-212

Paul, S. and Saha, D., 2012. In vitro screening of cytotoxic activities of ethanolic extract of *Pouzolzia zeylanica* (L.) Benn. International Journal of Pharmaceutical Innovations (IJPI), 2 (1): 52-55.

Purkayastha, J., Dutta, M. and Nath, S.C., 2007. Ethnomedicinal plant from Dibru-Saikhowa biosphere reserve, Assam. Indian Journal of Traditional Knowledge, 6 (3): 477-480.

Ratnam, K.V. and Raju, R.R., 2008. Traditional medicine used by the adivasis of Eantern Ghats, andhra pradesh – for bone fractures. Ethnobotanical leaflets, 12: 19-22. 2008.

Rhim, J., Wu, Y., Weller, C. and Schnepf, M., 1999. Physical characteristics of a composite film of soy protein isolate and propyleneglycol alginate. Journal of Food Science, 64 (1): 149–152.

Saha, A.K., Rahman, M.R., Shahriar, M., Saha, S.K., Azad, N.S. and Das, S., 2013. Screening of six Ayurvedic Medicinal Plant Extracts for Antioxidant and Cytotoxic Activity. Journal of Pharmacognosy and Phytochemistry, 2 (2): 181-188.

Saha, D. and Paul, S., 2012a. Studies on *Pouzolzia zeylanica* (L.) Benn. (Family: *Urticaceae*). Lap Lambert Academic Publishing, Germany.

Saha, D. and Paul, S., 2012b. Antifungal activity of ethanol extract of *Pouzolzia zeylanica* (L.) Benn. International Journal of Pharmacy Teaching and Pratices, 3 (2): 272-274.

Saha, D., Paul, S. and Chowdhury, S., 2012. Antibacterial activity of ethanol extract of *Pouzolzia zeylanica* (L.) Benn. International Journal of Pharmaceutical Innovations (IJPI), 2 (1): 1-5.

Sandhya, S., Venkata, R.K., Vinod, K.R., Swapna, R. and Asia, B., 2013. Scope of medicinal flora as effective anti ulcer agents. African Journal of Plant Science, 7 (11): 504-512.

Sarma, I.S. and Dinda, B., 2013. A new friedelane triterpene ester from *Pouzolzia indica*. Indian Journal of Chemistry, 52B: 1527-1530.

Shuib, N.H., Shaari, K., Khatib, *et al.*, 2011. Discrimination of young and mature leaves of Melicope ptelefolia using 1H NMR and multivariate data analysis. Food Chem, 126: 640–645

Singh, A., Lawrence, K., Prandit, S. and Lawrence, R.S., 2014. Response of leaves, stems and roots of *Withania somnifera* to copper stress. International Journal of Plant, Animal, and Environmental Sciences, 4 (3): 60-67.

Stankovic, M.S., Niciforovic, N., Topuzovic, M and Solujic, S., 2011. Total phenolic content, flavonoid concentrations and antioxidant activity of the whole plant and plant parts extracts from *Teucrium montanum* L. var. *montnum*, f. *supinum* (L.) Reichenb. Biotechnol Biotechnol Eq, 25: 2222-2227.

Stankovic, M.S., Topuzovic, M., Solujic, S. and Mihailovic, V., 2010. Antioxidant activity and concentration of phenols and flavonoids in the whole plant and plant parts of *Teucrium chamaedrys* L. var. *glanduliferum* Haussk. J Med Plant Res, 4: 2092-2098.

Tarhan, S., Telci, I., Tuncay, M.T. and Polatci, H., 2010. Product quality and energy consumption when drying peppermint by rotary drum dryer. Industrial Crops and Products, 32 (3): 420-427.

Tran Thi My Tien, Nguyen Thi Thu Huong, Duong Thi Mong Ngoc, Tran Cong Luan and La Van Kinh, 2010. Experimental study on some pharmacological effects of *Pouzolzia zeylanica* and *Tinospora crispa*. Medicine Journal of HCM city, special subject Traditional medicine. 14 (2): 116-120 (in Vietnamese).

Vallejo, F., García-Viguera, C. and Tomás-Barberán, F.A., 2003. Changes in Broccoli (*Brassica oleracea* L. Var. Italica) health-promoting compounds with inflorescence development. J Agric Food Chem, 51: 3776-3782.

Van den Berg, H., Faulks, R., Fernando-Granado, H., *et al.*, 2000. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. J Sci Food Agric 80: 880-912.

Yusuf, M., Wahab, M.A., Chowdhury, J.U. and Begum, J., 2006. Ethno-medico biotanical knowledge from kaukhali proper and betbunia of rangamati district. Bangladesh Journal Plant Taxon, 13 (1): 55-61.

Znidarcic, D., Ban, D. and Sircelj, H., 2011. Carotenoid and chlorophyll composition of commonly consumed leafy vegetables in Mediterranean countries. Food Chem., 129: 1164-1168.