UDC 631

# HABITAT STUDY AND CONTRIBUTION OF MICRO CLIMATE AND LUJA (PERISTROPHE BIVALVIS MERRILL) LEAF EXTRACTION AS NATURAL DYES FOR TEXTILE IN NORTH MALUKU

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## ABSTRACT
Luja is categorized as fiber dye plant and can potentially be used as plant-based dyes for textile. Plant-based dyes exploration begins with studying plant habitat and which part of plant that produces color, for example its leaf. The objective of the study was to describe contribution of micro climate towards growth and color quality, and total phenol based on position of leaf. The methodology was survey and laboratory testing. Coefficient of determination and SAS 9.3 program were used to analyze the contribution of micro climate and growing media towards plant growth while t-test was used to compare leaf position. The finding showed that luja was grown in Balisoang village, West Halmahera and Bido village in Morotai Island. Temperature, intensity of light and pH of the soil were elements of the micro climate that contributed to growth of luja in Balisoang while intensity of light and pH of the soil were the micro climate elements contributing to the plant growth in Bido. Position of light influenced quality of color and total phenol in both locations. The blades tend to produce brighter color and the shoots produced red color while the leafstalk produced yellow color. In Balisoang, the shoots contained higher phenol than the leafstalk, but the same amount of phenol as the blade. In Bido, all parts of luja leaf had the same amount of phenol.

## KEY WORDS
Luja, habitat, micro climate, color.

There is an increasing demand for textile with plant-based dye, for example batik and woven fabric with ethnic pattern and coloring, from both domestic and international customers. Synthetic dyes damage the environment. Crafters in North Maluku, Indonesia use plants from *Acanthaceae* family for dyes. Besides North Maluku, plants from *Acanthaceae* family are also grown in Java (Girmansyah, 2014) but further analysis is needed to identify its habitat and advantages. One of the plants that can be used for natural dyes is luja leaf (*Peristrophe bivalvis* Merrill). Until recently, the plant has been growing in the wild (Melati, 2016). An effort to introduce luja as natural dyes is to analyze contribution of micro climate towards luja in its natural habitat (Tesitel, et al*.,* 2015) and to identify bioactive chemicals in the leaf as plant-based textile dyes.

Luja leaf is a source of pigment producing red and yellow color due to its phenol content. Researchers should identify types of color different parts of luja leaf produces. The shoots and leafstalk contain different substances. Leaf position is one of the factors that influences bioactive content, in which younger leaf of herbs have higher nitrogen content compared to the older leaf and the nitrogen content tends to be stable (Wiedemuth, et.al*.,* 2005). Therefore, it is important to describe type of colors luja leaf extract produces. Another indicator to determine types of color leaf extract produces is yellow, and brownish red substance such as phenol and flavonoid (Alfian and Susanti, 2012 ; Aksara, et al., 2013) while quality of color can be determined by analyzing color intensity that consists of brightness, reddish and yellowish level (Aryanti, et.al., 2016). Leaf extract characterizes type of substance the leaf contains, for instance phenol that produces brown to brownish red color. Other indicators of color quality are brightness (L), reddish (a) and yellowish (b).

## MATERIALS AND METHODS OF RESEARCH

The survey was conducted between May and September 2016 in Balisoang village, West Halmahera and Bido village, Morotai Islands, North Maluku. The color quality and phenol content testing was conducted in the Laboratory of Agricultural Technology and Biochemistry of Brawijaya University.

The methods implemented in the study were survey, observation and laboratory testing. The sampling technique was purposive sampling, in which the criteria were individuals familiar with natural habitat of luja and used the plant frequently (Fachrul, 2008). Snowballing sampling technique was used to decide the setting of the study (Sugiono, 2007). Information about natural habitat of luja was obtained from the respondents. The researchers observed some physical components of the environment where the plant grew, for example altitude (m asl) using altimeter, location using GPS, topography and other plants associated to luja in the area, and acidity, humidity and temperature of soil using soil pH and moisture meter. Environment meter was the instrument used to identify components of micro climate such as temperature ($^{o}$C), intensity of light (lux), and humidity (%). The researchers also observed growth of the plant using some indicators, namely height (cm), diameter (cm), number of branch and leaf, and length of roots. Fresh luja leaves was picked compositely from three sample plants from three areas in each location. The leaves were categorized based on their position on the plant. Based on their position, the samples were categorized into shoots, blade and leafstalk. Shoots were leaves of which position was between the tip of the plants and the third segment of the shoots. Blade was green leaves of which position was between the fourth segment and leafstalk. Leafstalk was greenish yellow leaves on the leafstalk. The researchers observed lightness, redness, yellowness and total phenol of the leaves.

*Color Intensity Analysis.* Color intensity was analyzed using Color Reader based on the Hunter's Lab Colorimetric System. The symbolic or notation system used three types of scoring, namely lightness (L), redness (a), and yellowness (b). The score for white color (lightness) = 100 and black color = 0. The "a" consisted of +a = red and –a = green, while "b" consisted of +b = yellow and –b = blue. The sample, in the form of liquid, was extracted with methanol previously. 5 grams of fresh luja leaf was extracted with 10 milliliters of methanol and then rested for 30 minutes. 5 milliliters of the extract was poured into a cuvette. The liquid-filled cuvette was covered using black carbon and placed near the sensor (Razak, et.al, 2011; Caliskan *et al.,* 2016). The color reader worked after pressing the "on" button. The result appeared on the monitor was recorded. This experiment was conducted three times (3x).

*Phenol Testing Method.* Two grams of fresh leaf was dissolved in 5 milliliters of 96% ethanol and rested for 30 minutes. The extract was detected using 2 drops of 1% $FeCl_3$. When the extract turned into yellow-orange-brownish color, it positively contained phenol. Total phenol was determined using Folin-Ciocalteu reagent calorimeter, which Wojdylo, et.al (2007) adopted previously. 0.2 milliliters of Folin–Ciocalteu and 1 milliliter of 20& sodium carbonate were added to 100 millilters of luja extract. The solution was rested for 30 minutes. Absorbance score was observed using 361 wavelengths (λ) with Spectrofotometer Genesys. The observation was conducted 3 (three) times.

*Data Analysis.* Coefficient of determination testing was used to analyze contribution of micro climate and growing media towards growth of the plant. The independent variable (X) was micro climate which consisted of temperature, intencity of light, humidity, soil temperature and soil pH, while the dependent variables (Y) were plant height, diameter, number of leaves, number of branches and twigs and length of roots. SAS 9.3 was the program used for the data analysis. T-test was used to compare color quality and phenol content in different parts of the leaf.

## RESULTS AND DISCUSSION

Luja (*Peristrhope bivalvis* Merrill) grew in North Maluku, particularly Balisoang village in the west part of Halmahera Island and Bido village in the north part of Morotai Island. The villages were located 180 and 20 m asl consecutively. Type of soil in Balisoang was sandy loam while that in Bido was sandy clay loam. The plant grew in plantation area under the tree. The topography of the areas was flat. Table 1 described the physical component of the plant natural habitat during the study.

Table 1 – Physical Component of Growing Area and Micro Climate in the Two Locations as the Natural Habitat of Luja

| Growing Area | Balisoang | Bido |
|---|---|---|
| Physical Component | | |
| Altitude (m asl) | 180 | 20 |
| Position | NL 01$^o$ 10' 17,145" and SL 27$^o$ 27' 14,98" | NL 02$^o$ 16' 46,565" and SL 108$^o$ 38' 08,727" |
| Topography | Flat | Flat |
| Associated Plants | Nutmeg (*Myristica fragrans*), coconut (*Cocos nucifera*), durian (*Durio zibethinus* Murr.), mangosteen (*Garcinia mangostana* L.), banana (*Musa paradisiaca*), ferns (*Lycopodinae*), willow (*Justicia gendarus*), *keladi* (*Caladium*), *pegagan* (*Centella asiatica*), "sambang getih" (*Hemigraphis alternata*) | Sagu (*Metroxylon sagu Rottb*), coconut (*Cocos nucifera),* screwpine (*Pandanus amaryllifolius*), ginger (*Zingiberaceae*), willow (*Justicia gendaru)*, kidney tea plants (*Orthosiphon aristatus*), ferns (*Lycopodinae)* |
| Growing Area | Balisoang | Bido |
| Micro Climate and Growing Media | | |
| Temperature ($^O$C) | 31.2 – 31.5 | 32.8 – 33.5 |
| Intensity of Light between canopy (lux) | 146 - 611 | 365 - 461 |
| Humidity (%) | 77.2 - 82 | 71 – 84.3 |
| Soil Temperature ($^O$C) | 25 - 27 | 26.7 – 29.3 |
| pH of Soil | 5.6 – 5.7 | 7.0 – 7.1 |
| Type of Soil | Sandy Loam | Sandy-Clay Loam |
| Location | Plantation | Plantation (in the river stream) |

Luja which belongs to the Achantaceae family can grow in semi-dry to humid area with dry and watery soil such as Balisoang and Bido villages. Distribution of luja is similar to that of *Justicia spicigera* that grows in some areas in Mexico, is used as medicinal plants and fiber dyes and can grow up to 3000 m asl (Baqueiro-Pena and Guerrero-Beltran, 2014).

Temperature in Balisoang was between 31.2 and 31.5$^o$C and that in Bido was higher, between 32.8 and 33.5 $^o$C. Intensity of light in Balisoang was between 146 and 611 lux while that in Bido was between 365 and 461 lux. Humidity in both villages was 77.2 – 82% and 71 – 84.3% consecutively. Soil temperature in Balisoang was between 25 and 27 $^o$C while that in Bido was between 26.7 and 29.3 $^o$C. pH of the soil in Balisoang (5.6 – 5.7) was lower than that in Bido (7.0 – 7.1). In Balisoang, luja grew in dry yet humid soil and the plant grew along the river stream in Bido. The area where luja is grown in Bido is similar to the area where the plant grew in India (Tanavade, et.al, 2014).

Finding of the study showed that microclimate and soil contributed to growth of the plant. The temperature in Balisoang (31.2 - 31.5$^o$C) had 72.25% contribution to crown diameter, 75.69% contribution to the number of branches and 82.81% to the plant's longest roots. The light intensity (146 - 611 lux) had 54.76% contribution towards the diameter, 88.36% contribution towards the number of branches, 84.64% contribution towards the number of leaves and 67.24% contribution towards the root length. The temperature and light intensity did not have any significant contribution towards the plant height. The humidity in Balisoang did not have significant contribution towards the agronomic characters of the plant because it had negative effect towards the number of leaves (Table 2).

Negative influence was seen during high humidity (77.2% - 82%) resulting in 49.00% decrease in the number of branch and 56.25% decrease in the number of leaves. Humidity had 46.24% contribution to the plant height, 8.41% contribution to the crown diameter and 3.24% contribution to the length of roots. The soil temperature had similar contribution towards the growth character as humidity, negative influence with major contribution. In other words, 25-27$^oC$ was not an ideal temperature for luja to grow because the soil temperature only had 22.09% positive contribution towards the plant height.

The microclimate component in Bido had both positive and negative correlations (Table 2). Higher temperature resulted in a decrease in the plant growth. The temperature had negative contribution towards the plant height (75.69%), number of branch (92.16%) and crown diameter (20.25%). The temperature had positive influence towards the length of roots (1.69%), but the percentage was very low making the temperature had zero effect on the plant growth. The intensity of light (365- 461 lux) had significant influence towards the plant height, crown diameter, number of leaf and length of roots but it did not have any influence towards the number of branch. The intensity of light had 60.84% contribution towards the plant height, 98.01% contribution towards the crown diameter and 75.69% contribution towards the length of roots. Therefore, the intensity of light in the plant natural habitat was a part of the microclimate that had significant role towards the growth of Luja in Bido village, while the average humidity, soil temperature and soil pH did not have any contribution towards the growth. Different micro climate in both villages shows that luja depends heavily on intensity of light and temperature to grow.

Table 2 – Coefficient of Correlation and Determination (%) between Microclimate and Growth Component in Two Different Locations

| Microclimate | Height | Crown Diameter | Number of Branch | Number of Leaf | Length of Roots |
|---|---|---|---|---|---|
| Balisoang | | | | | |
| Temperature (31.2 – 31.5 $^oC$) | 0.53<br>28.09 | 0.85*<br>72.25* | 0.87*<br>75.69* | 0.83*<br>68.89* | 0.91*<br>82.81* |
| Intensity of Light (146 – 611 lux) | 0.37<br>13.69 | 0.74*<br>54.76* | 0.94*<br>88.36* | 0.92*<br>84.64* | 0.82*<br>67.24* |
| Humidity (77.2 – 82 %) | 0.68<br>46.24 | 0.29<br>8.41 | -0.70<br>49.00 | -0.75*<br>56.25* | 0.18<br>3.24 |
| Soil Temperature (25 - 27$^oC$) | 0.47<br>22.09 | 0.03<br>0.09 | -0.87*<br>75.69* | -0.90*<br>81.00* | -0.09<br>0.81 |
| Soil pH (5.6 – 5.7) | -0.47<br>22.09 | -0.03<br>0.09 | 0.87*<br>75.69* | 0.90*<br>81.00* | 0.09<br>0.81 |
| Bido | | | | | |
| Temperature (32.8 – 33.5$^oC$) | -0.87*<br>75.69* | -0.45<br>20.25 | -0.96*<br>92.16* | -0.5<br>25 | 0.13<br>1.69 |
| Intensity of Light (365 – 461 lux) | 0.78*<br>60.84* | 0.99*<br>98.01* | 0.11<br>1.21 | 0.99*<br>98.01* | 0.87*<br>75.69* |
| Humidity (71 – 84.3%) | -0.46<br>21.16 | -0.88*<br>77.44* | 0.30<br>9.00 | -0.85*<br>72.25* | -0.99*<br>98.01* |
| Soil Temperature (26.7 – 29.3$^oC$) | -0.59<br>34.81 | -0.03<br>0.09 | -0.99*<br>98.01* | -0.09<br>0.81 | 0.54<br>29.16 |
| Soil pH (7.0 – 7.1) | -0.46<br>21.16 | -0.02<br>0.04 | 0.86*<br>73.96* | 0.89*<br>79.21* | 0.08<br>0.64 |

*Note: Number followed by asterisk (*) means significant difference at 95% level of confidence.*
*Number on top = correlation score (r) and number on the bottom = coefficient of determination ($r^2$).*

Luja is a type of plant that can adapt to low light. Luja requires fertile soil (sandy loam and sandy clay loam), high humidity, low temperature, low intensity of light and normal pH to grow (Dang et al., 2014). However, the plant can still live when its growing media are filled with water. It is in line with several previous studies that habitat and climates will have impact towards morphology, physiology, and chemical content of a species (Tomlinson, et. al, 2013). Light has pivotal role to determine composition of plants in a community, which is indicated by correlation between light and growth of herbaceous and cover crop plants (Tinya, et.al, 2009).

The analysis showed that position of leaf influenced quality of color the leaf extract produced. It may be seen in terms of lightness (L) and redness (a) of the extract taken from Balisoang (Table 3). The blade (47.30) had higher lightness compared to the shoots (32.07) and the leafstalk (37.30) that meant the blade tended to fade more. The shoots had the highest reddish level (4.03) followed by the blade (2.73). These two were different from the leafstalk, but there was no different between the shoots and the blade.

Table 3 – Average Color Intensity of Different Parts of Luja Leaf in Balisoang

| Color Intensity | Shoots: Blade | | Shoots: Leafstalk | | Blade: Leafstalk | |
|---|---|---|---|---|---|---|
| Lightness (L) | 32.07 | 47.30* | 32.07 | 37.30 | 47.30* | 37.30 |
| Redness (+a, -a) | 4.03 | 2.73 | 4.03* | 1.40 | 2.73* | 1.40 |
| Yellowness (+b, -b) | -0.16 | -0.60 | -0.17 | -0.70 | -0.60 | -0.70 |

*Description: * = different; +a = reddish; -a = greenish; +b yellowish; -b = bluish.*

Luja grew in Bido had different color intensity (lightness, redness and yellowness) (Tabel 4). The blade (37.63) had the highest lightness, followed by the leafstalk (34.87) and the shoots (31.03). The blade (8.10) also had the highest redness (8.10) followed closely by the shoots (7.67). The leafstalk (3.97) had the lowest redness level. Furthermore, all parts of the leaf had the same level of yellowness.

Table 4 – Average Color Intensity of Different Parts of Luja Leaf in Bido

| Color Intensity | Shoots: Blade | | Shoots: Leafstalk | | Blade: Leafstalk | |
|---|---|---|---|---|---|---|
| Lightness (L) | 31.03 | 37.63* | 31.03 | 34.87 | 37.63 | 34.87 |
| Redness (+a, -a) | 7.67 | 8.10 | 7.67* | 3.97 | 8.10* | 3.97 |
| Yellowness (+b, -b) | 1.27 | 0.27 | 1.27 | 2.00 | 0.27 | 2.00* |

*Description: * = different; +a = reddish; -a = greenish; +b yellowish; -b = bluish.*

Different parts of the leaf did not have different yellowness level. They produced bluish color (-b). The blade extract was brighter because it has more dominant green pigment compared to the shoots and leafstalk extracted. Luja can produce more than one colors such as yellow, orange, brown, red, green and blue, while luja found and used by the indigenous community in North Vietnam produces red, yellow and purple color (Thuy, et.al, 2012; Luu-dam, et.al, 2016). Besides that, luja color extract may degrade due to condition of its growing media. Dang, et.al. (2014) showed that red turned to orange and yellowish green when pH of the media was higher than 12 making the red less stable. Color of the extract relied upon substances it contained. Color will change when acidity of growing media changes, high acidity of soil changes red to yellow and so does increasing temperature (Reshmi, et.al*.,* 2012). Chlorophyl and other substances such as phenol characterized by brownish yellow color are sources of pigment on leaves (Arum, et.al, 2012). Plants categorized as Achanthaceae family can be used as both natural dyes and medicine (Sengupta and Paul, 2016).

Table 5 – Average Phenol Content in Different Parts of Leaf from Two Locations

| Location | Shoots: Blade | | Shoots: Leafstalk | | Blade: Leafstalk | |
|---|---|---|---|---|---|---|
| Balisoang | 0.20 | 0.19 | 0.20* | 0.18 | 0.19 | 0.18 |
| Bido | 0.11 | 0.11 | 0.11 | 0.10 | 0.11 | 0.10 |

**Truly different.*

The results showed that the location of the leaves affect the total content of phenols. Phenol differences are seen between leaf shoots and leafstalk leaves, whereas there is no difference between leaf shoots with blades and blades with leafstalk leaves in Balisoang village. Different things are seen in luja from Bido village where, it has the same phenol content in each leaf location (Table 5). Leaf shoots contain more phenols because the leaf shoots are the leaves that actively perform photosynthesis and receive enough light compared to the leaves that are at the very bottom. Sufficient light will be a stimulus in producing phenol. The difference of this compound also occurs in the leaves of Ribes nigrum L, where the shoots have a high phenol content because it is influenced by phenological factors (ontogeny), soil nutrients, the environment grows like the light and heat units received by the plant (Vagiri et al., 2015) . The content of phenol is also related to the degree of leaf aging (Ghasemzadeh et al., 2010).

The finding also showed that different parts of the plant leaf had different phenol content. From the samples taken from Balisoang, the shoots and the leafstalk had different phenol content but the shoots and the blade as well as the blade and the leafstalk had similar phenol content. On the other hand, all parts of the samples taken from Bido had the same phenol content in all parts of the luja leaf (Table 5). The shoots had the higher phenol content because it receives more light compared to other lower parts of the leaf and because of photosynthesis. Adequate light was the stimulus for phenol production. Similar phenomenon occurs for *Ribes nigrum L* leaves, in which its shoots have higher phenol due to phenologic (ontogeny) factors, soil nutrition, and the environment (light and heat the plant gets) (Vagiri, et.al*.,* 2015). Phenol content is also related to maturity level of leaf (Ghasemzadeh, et.al*.,* 2010).

## REFERENCES


1. Aksara R., Musa, W.J.A., Alio, L. 2013. Identifikasi Senyawa Alkaloid dari Ekstrak Metanol Kulit Batang Mangga (Mangifera indica L), Jurnal Entropi 8 (1): 514-519.
2. Alfian R & Susanti, H. 2012. Penetapan Kadar Fenolik Total Ekstrak Metanol Kelopak Bunga Rosella Merah (Hibiscus sabdariffa Linn) dengan Variasi Tempat Tumbuh secara Spektrofotometri. Jurnal Ilmiah Kefarmasian, 2 (1): 73 – 82.
3. Arum Y.P., Supartono, & Sudarmin. 2012. Isolasi and Uji Daya Antimikroba Ekstrak Daun Kersen (Muntingia calabura), Jurnal MIPA 35 (2): 165-174.
4. Baqueiro-Pena. I & Guerrero-Beltran. J.A. , 2014. Uses of Justicia spicigera in Medicine And as a Source of Pigments (Review), Functional Foods in Health and Disease, 4 (9): 401-414.
5. Dang B. K., Dam, S.M., Pham, M.T., Dang, T.B.O., & Le, T.H.V. 2014. Peristrophe roxburghiana - A Review, Annals. Food Science and Technology, 15 (1): 1-9.
6. Fachrul, M.F. 2008. Metode Sampling Bioekologi. Jakarta: Bumi Aksara.
7. Ghasemzadeh, A., Jaafar, H. Z. E., Rahmat, A., Edaroyati, P., Wahab, M., Abdulhalim, M.R. 2010. Effect of Different Light Intensities on Total Phenolics and Flavonoids Synthesis and Anti-oxidant Activities in Young Ginger Varieties (Zingiber officinale Roscoe), Int. J. Mol. Sci., 11: 3885-3897.
8. Girmansyah D. 2014. Validasi, Distribusi and Pemanfaatan Acanthaceae Di Jawa (Komunikasi Pendek), Berita Biologi 13 (1): 107-113.
9. Melati, R. 2016. Keragaman Sumber Pewarna Nabati yang Digunakan oleh Masyarakat Maluku Utara. Proceeding, National Seminar of Agriculture in "Optimalisasi Sumber Daya Lokal Berbasis Riset". Faculty of Agriculture University of Ternate, First Publication, p. 65-70.
10. Luu-dam N.A., Ban K.N., & Yoshinori, S. 2016. Ethnobotany of Colorant Plants in Ethnic Communities in NorthernVietnam. Anthropol 3 (2): 2-6.
11. Razak, A.N., Tumin, S.M., & Tajuddin, R. 2011. Effect of Temperature on the Color of Natural Dyes Extracted Using Pressurized Hot Water Extraction Method. American Journal of Applied Sciences 8 (1): 45-49.
12. Reshmi, S.K., Aravindhan, K.M., & Devi, P.S. 2012. The Effect of Light, Temperature, pH on Stability of Betacyanin Pigments in Basella Alba Fruit, Asian J. Pharm. Clin. Res., 5 (4): 107-110.
13. Sengupta, S.D. & Paul, R. 2016. Exploration of Knowledge on New Ethno-Botanical Value of Justicia spicigera Schltdl. Global J Res. Med. Plants & Indigen. Med. 5 (10): 261–266.
14. Sugiyono. 2007. Statistika untuk Pertanian. Bandung: Alfabeta.
15. Tanavade, S.S., Naikwade, N.S., & Chougule, D.D. 2012. Antimicrobial Activity of Ethanolic Extracts of Leaves and Stems of Peristrophe bivalvis Merrill, International Journal of Biomedical Research, 3 (2): 106-108.
16. Tesitell, J., Fibichl, P., De Bello, F., Chytry, M., & Lepsl, J. 2015. Habitats and Ecological Niches of Root-Hemiparasitic Plants: An Assessment Based on a Large Database of Vegetation Plot. Preslia (87): 87–108.

17. Thuy, T. T., Huong, N.T.T., Nhung, L.T.H., Thi Ninh, P., Delfino, D.V., & Van Sung, T. 2012. Natural phenoxazine alkaloids from Peristrophe bivalvis (L.) Merr, Biochemical Systematics and Ecology, 44: 205–207.
18. Tinya, F., Marialigeti, S., Kiraly, I., Nemeth, B., & Odor, P. 2009. The effect of light conditions on herbs, bryophytes and seedlings of temperate mixed forests in Orseg Western Hungary. Plant Ecol (204): 69–81.
19. Tomlinson K. W., L. Poorter, F. J. Sterck, F. Borghetti, D. Ward, S. De Bie1 and F. Van Langevelde, 2013. Leaf Adaptations of Evergreen and Deciduous Trees of Semi-Arid and Humid Savannas on Three Continents, Journal of Ecology (101): 430–440.
20. Vagiri, M., S. Conner, D. Stewart, S. C. Andersson, S. Verrall, E. Johansson & K. Rumpunen, 2015. Phenolic Compounds in Blackcurrant (Ribes nigrum L.) Leaves Relative to Leaf Position and Harvest Date, Food Chemistry (172): 135–142.
21. Wiedemuth, K., J. Muller, A. Kahlau, S. Amme, H. P, Mock, A. Grzam, R. Hell, K. Egle, H. Beschow & K. Humbeck, 2005. Successive maturation and senescence of individual leaves during barley whole plant ontogeny reveals temporal and spatial regulation of photosynthetic function in conjunction with C and N metabolism. Journal of Plant Physiology, 162: 1226–1236.
22. Wojdylo A., J. Oszmian'ski, & R. Czemerys, 2007. Antioxidant activity and phenolic compounds in 32 selected herb, Food Chemistry, 105: 940–949.