

Evaluation of bioactive compounds and minerals from leaves, stems and roots of burdock (*Arctium lappa* L.)

Sergiu Pădureț^{1*}

¹¹Faculty of Food Engineering, Stefan cel Mare University of Suceava, Suceava, Romania

Abstract

This study aims to characterize the burdock leaves, stems and roots as a novel food product and for this purpose the physicochemical parameters (the concentration of soluble substances, pH, total acidity and CIEL*a*b* color parameters), the antioxidant activity, total chlorophylls, carotenoids, tannins, total polyphenol and mineral content were investigated. Burdock samples were divided in three categories: roots, leaves and stems, each sample contained parts of several plants. One - factor analysis of variance – ANOVA strengthened that analyzed burdock samples differ significantly in terms of bioactive compounds ($p < 0.001$). The highest antioxidant capacity was recorded by stems and the lowest by burdock roots; one way ANOVA analysis highlighted this difference at a level of $p < 0.001$. Another important aspect is the minerals content; the burdock samples had a significant level of calcium, magnesium, sodium, zinc.

Keywords: burdock; color; minerals; tannins; total chlorophylls

1. Introduction

Plants have been used by humans as food sources, forages, embellishment or for medicinal purposes since ancient times. Due to the geographic position and climatic conditions; Romania has rich vegetation, being the meeting place of the Mediterranean and Eurasian flora. In Romanian plant heritage there are more than 3600 species of plants, of which approximately 700 have medicinal uses. The first more precise data about potentially medical plants in today's area of Romania country were stated by the Greek doctor Pedanius Dioscurides Anazarbeus in the five-volume thesis on plants "De Materia Medica" [1].

Arctium lappa, (of the *Asteraceae* family), normally known as burdock is a perennial herb, common throughout Romania, well-known since antiquity by Geto-Dacian population, it was one of the most valuable and the oldest plant-medicament practiced by our folk medicine. Burdock has progressively achieved international appreciation for its culinary use as a root vegetable due to its nutritional importance and health benefits, being promoted as a healthy and nutritious food product, consumed directly or in the form of tea [1, 2].

Burdock's roots, leaves, stems and seeds have been used for therapeutic purpose by Americans, Asians and also Europeans for hundreds of years; traditionally it is used to treat diseases like throat inflammation, diabetes, inflammatory diseases, skin eruption and various skin problems [3, 4].

The major active compounds identified in this plant are: tannins, polyphenols such as caffeic and chlorogenic acid, beta-eudesmol, dietary fiber such as inulin and lignan, (e.g., arctigenin, arctiin,) and sterols (e.g., sitosterol, stigmasterol), diartigenin, essential oils, numerous vitamins (B vitamins, C, K and E) and minerals (like Na, Ca, Fe, Cu, Mg, P, K, and Zn), [5]. In many studies it is suggested that burdock has antioxidative, anti-inflammatory, anti-cancer activities [6], antitumor, antibacterial (against *Bacillus subtilis*, *Lactobacillus acidophilus*, *Candida albicans*, *Escherichia coli* and *Micrococcus luteus*) and antiviral (against herpes virus HSV1 and HSV2, adenovirus ADV3 and ADV11) activities too [5].

Burdock is an edible plant used in folk medicine, its root being frequently consumed as food by Asians due to their low content of starch, high

concentration of oligosaccharides and have recently achieved interest because they have fructans that are resistant to human digestive enzymes, being considered fibers with prebiotic potential, (hypoglycemic effect) [7, 8].

In the literature, there are more frequent studies of various compounds present in burdock root and fewer of the aerial parts of the plant (leaves, stems, seeds). The present research aims to characterize the whole burdock plant in terms of bioactive compounds, antioxidant activities and mineral content.

2. Materials and methods

Materials. The burdock samples (*Arctium lappa L.*) were collected in spring time from Suceava area (Romania) and divided into three categories: roots, leaves and stems, each sample contained parts of several plants. All plant samples were washed and dried prior analysis.

Physicochemical parameters. The color of burdock samples was measured with a chromameter CR400 (Konica Minolta, Japan); hue angle/tone (h^0) was calculated using Equation 1 and chroma/color intensity (C^*) was calculated using Equation 2 [9]:

$$h^0 = \tan^{-1}(b^*/a^*) \quad (1)$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

The concentration of soluble substances, pH [10] and total acidity [11] were also determined.

The determination of minerals content was performed by ICP-MS (atomic emission in plasma coupled with mass spectrometry), Agilent Technologies 7500cs Series device, following the procedure described by Amariei 2018, [12], which involves calcining (650 °C) an exact quantity of the sample to be analyzed and then acidification of the ash with HNO₃ [13]. For solutions double deionized water (18 MΩ cm⁻¹) was used.

Total content of chlorophyll and carotenoids was determined spectrophotometrically (3600UV-VIS-NIR Shimadzu Spectrophotometer), using 1g of each sample homogenized with 10 mL of acetone (80% v/v).

Total chlorophyll (Equations 3, 5 and 6) and carotenoids (Equation 4) was calculated using the equations of Lichtentaler and Wellburn [14, 15]:

$$\text{Total chlorophylls} = 7.15 \cdot A_{664} + 18.71 \cdot A_{647} \quad (3)$$

$$\text{Total carotenoids} = (1000 \cdot A_{460} - 1.82 \cdot C_a - 85.2 \cdot C_b) / 198 \quad (4)$$

$$\text{Chlorophyll a (C}_a\text{)} = 12.25 \cdot A_{664} - 2.79 \cdot A_{647} \quad (5)$$

$$\text{Chlorophyll b (C}_b\text{)} = 21.50 \cdot A_{647} - 5.10 \cdot A_{664} \quad (6)$$

The antioxidant activity of burdock samples was performed by DPPH method and the scavenging effect of the radical was calculated with equation 7:

$$\text{DPPH}_{\text{scavenging effect}}(\%) = 100 \cdot (A_C - A_{P5\text{min}}) / A_C \quad (7)$$

Where: A_C was the absorbance of the control and $A_{P5\text{min}}$ was the absorbance in the presence of the sample measured after 5 minutes.

The extraction process consisted of mixing one part of burdock sample with nine parts of methanol following the protocol described by Oroian 2017 [16], the absorbance of methanolic extract (0.5 mL) and DPPH solution (2.5 mL of 6·10⁻⁵ M) mixture were measured at 517 nm after 5 minutes [15, 17].

The total tannins of burdock samples were evaluated according to Diaconeasa 2015 [18]; 1 mL of sample was diluted with 49 mL distilled water and from this final volume 2 mL was mixed with 1 mL distilled water and 3 mL of HCl (12M). This mixture was divided in two, one part was heated (30 min and then cooled on bath ice (A)), while the second part was kept at room temperature (B). Finally, to each sample, 0.5 mL of 95% ethanol was added and the absorbance was read at 550 nm. Total tannins (g/Kg) were calculated as follows:

$$\text{Total Tannins} = 19.33 \cdot (AbsA_{(550)} - AbsB_{(550)}) \quad (8)$$

The total polyphenol content was measured by Folin-Ciocalteu assay [19]. The obtained extract (0.1mL) was mixed with (0.9 mL) Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and after stirring it was added 1 mL of Na₂CO₃ (7.5 %). The absorbance was measured at 765 nm after 30 min in the dark at room temperature using a 3600UV-VIS-NIR Shimadzu Spectrophotometer [20]. The total polyphenol were expressed as mg Gallic acid equivalent/100g fresh weight sample. All reagents were purchased from Sigma-Aldrich (Germany).

Statistical analysis. To describe the relationship between burdock samples and measured parameters Principal Component Analysis was performed using Unscrambler 9.7 software (CAMO Process AS, Oslo, Norway) and one - factor analysis of variance -ANOVA by STATGRAPHICS CENTURION XVI (Trial Version).

3. Results and Discussion

In this research the burdock importance and usefulness was investigated as a valuable food ingredient; for this purpose the color parameters, the concentration of soluble substances expressed as °Brix, pH, total acidity and the content of bioactive compounds such as tannins, polyphenol substances, chlorophylls and carotenoids were conducted. In Table 1 are presented the physicochemical results of burdock's leaves, stems and roots.

Color is an important parameter of raw materials and food product that influence the consumer's choice and preferences, being the first quality parameter evaluated by consumers [21]. Burdock brightness (L^*) ranges between 50.19 and 34.13, leaf samples had the lowest brightness while root samples had the highest brightness.

The a^* color parameter represents the red–green axis of CIE $L^*a^*b^*$ color space and its value was in the negative region (green) for leaf and stem samples while the value for root samples was in the positive region. In case of b^* color component (yellow–blue axis) the ANOVA factorial analysis showed an insignificant difference ($p > 0.05$) between analyzed samples. Of the calculated color parameters, color intensity (C^*) of leaves presented the highest value (27.37) whereas the highest color tone value (h^0) was recorded by burdock roots ($p < 0.001$). The concentration of soluble substances ranges between 3.03 to 6.86 °Brix; the burdock roots being characterized by the highest content of soluble substances compared to other burdock samples.

The pH values of burdock were close to neutral (6.38-6.71) and the acidity ranges between 0.0088-0.0113 cm^3 NaOH/100g fresh weight.

The ANOVA results of analyzed burdock samples are shown in Table 2 and we can observe that burdock is an exceptional plant with a wide variety of valuable compounds. The antioxidant activity of burdock samples measured by DPPH assay range between 5.63% and 90.26%, the highest capacity being recorded by stems and the lowest by burdock roots; one way ANOVA analysis highlighted this difference at a level of $p < 0.001$. The chlorophyll a, chlorophyll b and total carotenoids are the primary pigments conferring color quality to burdock samples. Burdock leaves have a high content of total chlorophylls (68.76 mg/100 g) and total carotenoids (23.06 mg/100 g) compared to the stem samples in which the total chlorophylls level was 7.48 mg/100 g and total carotenoids was 0.56 mg/100 g; in root samples these compounds were not detected. Of total chlorophylls content the chlorophyll b presented higher values than chlorophyll a, in leaves the difference was 57.56% and in stems the difference was 25.96%.

Tannins are water-soluble polyphenolic compounds, have antioxidant properties (free radical scavenging and metal chelating activities) and are found in a variety of plants used for human consumption including fruits, nuts, vegetables, certain grains, cocoa and beverages like red wine, coffee or tea; leading to an estimated daily intake of between 1 and 3 grams polyphenols per day and that of total tannins to be from tens to several hundred milligrams per day [22, 23].

Table 1. ANOVA physicochemical parameters of burdock samples

Parameter		Burdock samples - mean (SD)			F - ratio
		Leaves	Stems	Roots	
Color	L^*	34.13 (2.22)c	42.06 (1.88)b	50.19 (2.02)a	45.95***
	a^*	-15.34 (1.67)c	-6.86 (1.66)b	0.38 (0.40)a	96.98***
	b^*	22.66 (2.88)a	18.24 (0.73)b	18.74 (1.30)b	4.99 ^{ns}
	h^0	-0.97 (0.01)b	-1.21 (0.08)c	1.54 (0.02)a	2411.66***
	C^*	27.37 (3.29) a	19.53 (0.62)b	18.75 (1.29)b	15.79**
°Brix		3.03 (0.15)c	4.97 (0.28)b	6.86 (0.25)a	194.49***
pH		6.71 (0.01)a	6.39 (0.05)b	6.38 (0.01)b	838.91***
Acidity (cm^3 NaOH/100g)		0.0069 (0.0009)b	0.0113 (0.0018)a	0.0088 (0.0018)b	12.37**

Note: NS – not significant ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Different lowercase letters (a–c) in a row show significant differences between the groups ($p < 0.05$).

Table 2. ANOVA of burdock bioactive compounds

Bioactive compounds		Burdock samples - mean (SD)			F - ratio
		Leaves	Stems	Roots	
Antioxidant activity	[%]	79.97 (1.51)b	90.26 (0.87)a	5.63 (1.40)c	3854.94***
Total chlorophylls	mg/100 g	68.76 (0.24)a	7.48 (0.13)b	-	166164.42***
Total carotenoids	mg/100 g	23.06 (0.06)a	0.56 (0.09)b	-	121541.21***
Chlorophyll a	mg/100 g	25.12 (0.18)a	1.54 (0.12)b	-	37446.46***
Chlorophyll b	mg/100 g	43.64 (0.20)a	5.93 (0.13)b	-	84980.03***
Total tannins	mg/100 g	8.96 (0.30)b	6.97 (0.45)c	9.95 (0.33)a	139.31**
Total polyphenol	g/100 g	17.81 (0.03)a	7.77 (0.28)c	10.92 (0.10)b	4871.24***

Note: NS – not significant ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Different lowercase letters (a-c) in a row show significant differences between the groups ($p < 0.05$).

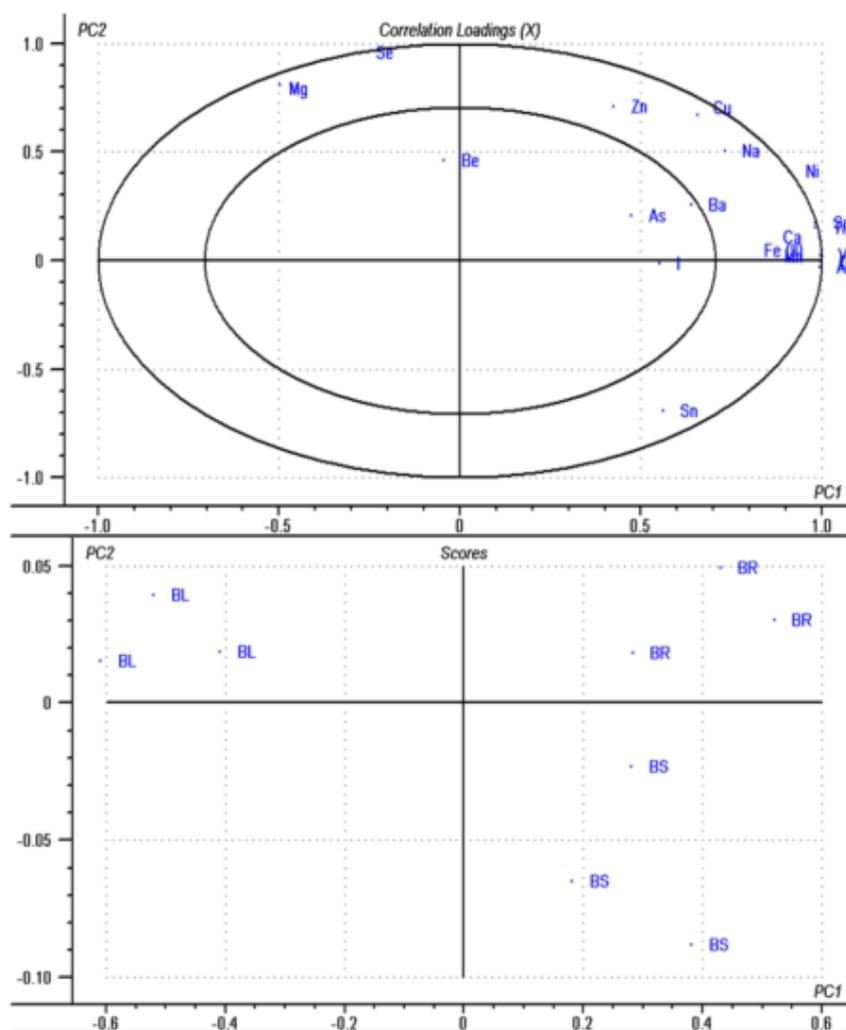


Figure 1. PCA loadings and scores of burdock samples minerals. BL-burdock leaves, BS-burdock stems, BR-burdock roots.

The burdock roots present a higher concentration of total tannins (9.95 mg/100 g) followed by leaf samples (8.96 mg/100 g), the difference between these two samples being relatively small. In case of total tannins measurement one - factor analysis of variance - ANOVA showed a smaller difference $p < 0.01$ between analyzed samples. Compared to berries, (Diaconeasa et al., 2015) [18], burdock samples have lower total tannin content.

The total polyphenol content in burdock samples, expressed as Gallic acid, range from 17.81 to 7.77 g/100 g fresh weight. The highest total polyphenol levels were detected in leaves extracts (17.81 g/100 g) and the lowest in stems extracts (7.77 g/100 g), the roots extracts presented a level of 9.92 g/100 g. The total polyphenol content of leaves was in the same range with those reported by Ferracane 2010, [24].

The results of burdock bioactive compounds strengthen that burdock can be a valuable food ingredient or food flavoring, its consumption bringing great benefits to human health. As food, burdock can be consumed dry (food flavoring), fresh (like a vegetable), cooked or as beverage (tea, juice).

Burdock roots had significant content of calcium (39 mg/100g), magnesium (24 mg/100g), sodium (15 mg/100g), zinc (0.15 mg/100g) while burdock leaves and stems presented high level of magnesium (58 mg/100g-leaves, 49 mg/100g stems) and almost a double content of sodium (26 mg/100g-leaves, 29 mg/100g stems). The total polyphenol, chlorophylls, carotenoids and tannins contents along with minerals like selenium zinc and copper protect the body against oxidative stress, infections and free radicals.

The Principal Component Analysis (PCA) was performed on minerals content of burdock samples (leaves, stems and roots), the two principal components (PCs) explain all the data variation, first one (PC1) explains 76% of data variance while second one (PC2) explains 24% of the variance in the minerals results. PCA is a factorial analysis method whose main objective is to take out the important information from the measured data, to decrease the initial number of variables, to show it as a series of new orthogonal variables named main components and to presents the similarity of the observations and of the variables as points in graph [25].

In Figure 1 are represented the PCA correlations loadings and based on those the burdock samples are divided in different quadrants; the mineral elements in center of the ellipse (iodine, barium) have an insignificant influence in burdock samples differentiation, whereas the mineral elements from outside of ellipse (calcium, magnesium, sodium, zinc) present a strong influence in samples differentiation. From Correlation Loadings (Figure 1), it can be observed that burdock leaves samples projection was significantly influenced by magnesium and selenium while the burdock roots samples projection was influenced by sodium, zinc, copper.

4. Conclusion

The study carried out on different parts of burdock (leaves, stems and roots) highlighted that this plant can be used successfully as a valuable food ingredient or food flavoring, representing an important and accessible source of natural antioxidant substances; high level of total chlorophylls, total carotenoids and total polyphenol being recorded on leaves samples. The roots samples were characterized by high content of total tannins, mineral elements and a lower level of total polyphenol. All analyzed samples have a low content of soluble substances, low acidity and a pH close to neutral which makes this plant fit with the alkaline diet. Further research will aim to investigate the burdock seeds and to integrate this seeds into different food products.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

References

1. Fierascu, R. C., Fierascu, I., Ortan, A., Avramescu, S. M., Dinu-Pirvu, C. E., & Ionescu, D., Romanian Aromatic and Medicinal Plants: From Tradition to Science. *Aromatic and Medicinal Plants: Back to Nature*, **2017**, 149.
2. Lee, D., & Kim, C. Y., Influence of Roasting Treatment on the Antioxidant Activities and Color of Burdock Root Tea. *Preventive nutrition and food science*, **2017**, 22(1), 21.
3. Chan, Y. S., Cheng, L. N., Wu, J. H., Chan, E., Kwan, Y. W., Lee, S. M. Y., Chan, S. W., A review of the pharmacological effects of *Arctium lappa* (burdock). *Inflammopharmacology*, **2011**, 19(5), 245-254.

4. Predes, F. S., Ruiz, A. L., Carvalho, J. E., Foglio, M. A., & Dolder, H., Antioxidative and in vitro antiproliferative activity of *Arctium lappa* root extracts. *BMC complementary and alternative medicine*, **2011**, 11(1), 25.
5. Pirvu, L., Nicorescu, I., Hlevca, C., Albu, B., & Nicorescu, V., Burdock (*Arctium lappa*) Leaf Extracts Increase the In Vitro Antimicrobial Efficacy of Common Antibiotics on Gram-positive and Gram - negative Bacteria, *Open Chemistry*, **2017**, 15(1), 92-102.
6. Chen, W. C., Hsu, Y. J., Lee, M. C., Li, H. S., Ho, C. S., Huang, C. C., & Chen, F. A., Effect of burdock extract on physical performance and physiological fatigue in mice. *Journal of Veterinary Medical Science*, **2017**, 17, 0345.
7. Cao, J., Li, C., Zhang, P., Cao, X., Huang, T., Bai, Y., & Chen, K., Antidiabetic effect of burdock (*Arctium lappa* L.) root ethanolic extract on streptozotocin-induced diabetic rats. *African Journal of Biotechnology*, **2012**, 11(37), 9079-9085.
8. Moro, T. M. A., Celegatti, C. M., Pereira, A. P. A., Lopes, A. S., Barbin, D. F., Pastore, G. M., & Clerici, M. T. P. S., Use of burdock root flour as a prebiotic ingredient in cookies. *LWT*, **2018**, 90, 540-546.
9. Pădureț, S., Oroian, M., Gutt, G., & Amariei, S. (2017). Evaluation of strawberry texture in close relation with their anisotropy. *International Journal of Food Properties*, **2017**, 20(2), 247-259.
10. ISO 1842, *Fruit and vegetable products - Determination of pH*, **1991**
11. ISO 750: *Fruit and vegetable products determination of titratable acidity*, **1998**
12. Amariei, S., Norocel, L., Pădureț, S., & Gutt, G., Effect of grape seed flour on the quality of summer salami. *Journal of food processing and preservation*, **2018**, 42(5), e13601
13. Fernandez-Turiel, J. L., Llorens, J. F., López-Vera, F., Gómez-Artola, C., Morell, I., & Gimeno, D., Strategy for water analysis using ICP-MS. *Fresenius J Anal Chem.*, **2000**, 368(6), 601-606.
14. Morna, A., Chlorophyll and carotenoid content in lettuce (*Lactuca Sativa* L.) and nettle leaves (*Urtica Dioica* L.). *Analele Universității din Oradea, Fascicula: Ecotoxicologie, Zootehnie și Tehnologii de Industrie Alimentară*, **2015**, XIV/A,
15. Pădureț, S., Amariei, S., Gutt, G., & Piscuc, B., The evaluation of dandelion (*Taraxacum officinale*) properties as a valuable food ingredient. *Rom Biotechnol Lett*, **2016**, 21(11) 569-75.
16. Oroian, M., Leahu, A., Dutuc, A., & Dabija, A., Optimization of Total Monomeric Anthocyanin (TMA) and Total Phenolic Content (TPC) Extractions from Red Cabbage (*Brassica oleracea* var. capitata f. rubra): Response Surface Methodology versus Artificial Neural Network. *International Journal of Food Engineering*, **2017**, 13(3).
17. Ulewicz-Magulska, B., & Wesolowski, M., Total Phenolic Contents and Antioxidant Potential of Herbs Used for Medical and Culinary Purposes. *Plant Foods Hum Nutr.*, **2018**, 1-7
18. Diaconeasa, Z., Ranga, F., Leopold, L., Oana, P. O. P., Vodnar, D., Cuiabus, L., & Socaciu, C., Phenolic content and their antioxidant activity in various berries cultivated in Romania. *Bulletin UASVM Food Science and Technology*, **2015**, 72, 1.
19. Horng, C. T., Wu, H. C., Chiang, N. N., Lee, C. F., Huang, Y. S., Wang, H. Y., & Chen, F. A., Inhibitory effect of burdock leaves on elastase and tyrosinase activity. *Experimental and therapeutic medicine*, **2017**, 14(4), 3247-3252.
20. Yamaguchi, T., Katsuda, M., Oda, Y., Terao, J., Kanazawa, K., Oshima, S., Matoba, T., Influence of polyphenol and ascorbate oxidases during cooking process on the radical-scavenging activity of vegetables. *Food Science and Technology Research*, **2003**, 9(1), 79-83.
21. Pathare, P. B., Opara, U. L., & Al-Said, F. A. J. (2013). Colour measurement and analysis in fresh and processed foods: a review. *Food and bioprocess technology*, **2013**, 6(1), 36-60.
22. Combs, C. A. (Ed.). (2016). *Tannins: Biochemistry, Food Sources and Nutritional Properties*. Nova Science Publishers.
23. Montes Ávila, J., López Angulo, G., & Delgado Vargas, F., Tannins in fruits and vegetables: Chemistry and biological functions. *Fruit and Vegetable Phytochemicals: Chemistry and Human Health. 2nd ed. Chichester: John Wiley & Sons, Ltd*, **2017**, 221-268.
24. Ferracane, R., Graziani, G., Gallo, M., Fogliano, V., & Ritieni, A., Metabolic profile of the bioactive compounds of burdock (*Arctium lappa*) seeds, roots and leaves. *Journal of pharmaceutical and biomedical analysis*, **2010**, 51(2), 399-404.
25. Abdi, H., & Williams, L. J. (2010). Principal component analysis. *Wiley Interdiscip Rev Comput Stat.*, **2010**, 2(4), 433-459.