Phytochemical Analysis of Purple Sweet Potatoes (*Ipomoea batatas*) Roots Extract From Lawang and Kawi Mountain Cultivar, East Java, Indonesia

Fajar Mustika Alam[1,4], Nia Kurnianingsih[2,4*], Fatchiyah Fatchiyah[3,4]

[1]Bachelor Program of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, Indonesia
[2]Department of Physiology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, Indonesia
[4]Research Center of Smart Molecules of Natural Genetic Resources, Universitas Brawijaya, Malang, Indonesia

Abstract

Indonesia has wide cultivation of purple sweet potatoes (PSP) commodities, particularly in East Java province. However, the difference of phytochemical profiles in PSP among geographical regions has not been fully explored. This study aimed to analyze the phytochemicals, anthocyanin, and antioxidant activity profiles from two different cultivars of PSP from Lawang and Kawi Mountain region, East Java, Indonesia. The acidified methanol extract was identified for a phytochemical compound using standard methods. Antioxidant activity was analyzed using a ferric reducing assay. Anthocyanins were screened using ultraviolet-visible spectroscopy and total calculation. Both extracts have positive values in their alkaloid, phenolic, flavonoid, glycoside, and tannin content. Antioxidant activity was high with IC_{50} value 2.5 and 2.3 µg. mL^{-1} for Lawang and Kawi Mountain, respectively. Each cultivar has a similar peak at 521 nm at pH 1 and 530 nm at pH 4.5. Total anthocyanin calculation was showed that Lawang has higher anthocyanin content than Kawi Mountain cultivar. We concluded that PSP from Lawang has better anthocyanin content than the Kawi Mountain cultivar. We proposed that PSP from the Lawang cultivar has the potential to be explored in further research and health-related product development.

Keywords: anthocyanin, antioxidant, geographical, purple sweet potatoes.

INTRODUCTION

Sweet potatoes (*Ipomoea batatas* (L) Lam) is an important annual perennial plant that contributed as global food security [1]. Indonesia is one of the major supplier of sweet potatoes commodity worldwide. The biodiversity of sweet potatoes in Indonesia is addressed into natural resources that should be taken into consideration, especially purple sweet potatoes varieties [2,3]. Purple sweet potatoes (PSP) cultivation is dominant in East Java Province, particularly in Malang District at Lawang and Kawi Mountain region [4]. The attractive color pigment and higher anthocyanin compound are more favorable than another phenotypes. Purple sweet potatoes are the main source of energy, amino acid, macronutrient, and anthocyanin. Anthocyanin in PSP previously reported has beneficial biological activity on inflammation, cancer, mutation, oxidative stress, liver protection, etc. [5,6].

Purple sweet potatoes from the Kawi Mountain region displayed potential health benefits in inhibiting the brain cell apoptosis in an animal model of diabetes as well as enhancing the antioxidant enzyme in atherogenic rats [7,8]. However, research on the nutritional content of PSP from Kawi Mountain cultivar is limited to amino acid characterization [9]. Meanwhile, nutritional identification of PSP cultivated in Lawang is limited to a major proximate composition, for instance, protein, carbohydrates, fat, water content, and ash [10].

The growth environment is strongly correlated with the synthesize of plant bioactive compounds [11]. The validation of plant growth conditions is important in the functional food development process to ensure product quality and safety [12]. Thus, the profiling of biochemical compounds of PSP from Lawang and Kawi Mountain is necessary to support the potential of both cultivars to be developed into functional food. This research was evaluated the differences of phytochemicals, anthocyanins, and antioxidant activity of PSP from those different geographical. A recent study provides the recommendation of the utilization of both cultivars according to their biochemical compounds.

MATERIAL AND METHOD

Plants extraction

Tuber roots of purple sweet potatoes (PSP) cultivar of Lawang and Kawi Mountain were obtained from the local traditional farmer and immediately transfer to the laboratory. Solid extraction was performed according to a previous protocol with minor modification. In brief, the procedure was performed by grinding the fresh tuber roots (50 g) followed by

[1] Correspondence Address:
Nia Kurnianingsih
Email : nia_fkub@ub.ac.id
Address : Faculty of Medicine, Universitas Brawijaya, Veteran Malang, 65145.

maceration in 100 mL acidified methanol 1% (CAS No. 67-56-1, Merck) for 24 hours at 25°C. The homogenates were filtrated using Whatman paper 0.45 µm. Repeated maceration and filtration were conducted until colorless extract was obtained. The filtrates were evaporated at 50°C using a rotary evaporator RE-25C 1L series. The filtrates were then stored at a temperature of 4°C until used [13].

Qualitative phytochemical screening

A standard method as previously described was performed to evaluate the alkaloid, phenolics, flavonoid, glycosides, and tannin The absorbance of alkaloid, phenol, flavonoid, glycoside, and tannin was measured at 470, 280, 430, 210, and 700 nm wavelength, respectively [13].

Ultraviolet-visible spectroscopy

Anthocyanin preliminary structure determination was conducted by measuring the absorbance with UV-1700-spectrophotometer (Shimadzu, Japan) at pH 1 and pH 4.5 conditions. The maximum absorbance in acidic solution was used for indicating the basic structure of anthocyanins. A ratio of 1:100 (v/v) PSP extract and buffer solution was used for absorbance measurement. A total 1 mL PSP extract was dissolved with 0.25 M HCl-KCl (CAS No. 7447-40-7 , Merck) buffer (pH 1) or 0.5 M sodium acetate buffer (pH 4.5) [6,13,14].

Anthocyanin calculation

Total anthocyanin content (TAC) was expressed as cyanidin-3-glucoside per 100 g ($C3Gmg.100g^{-1}$). The calculation of TAC was conducted using the following formula [15]:

$$TAC = \left(\frac{A}{\varepsilon \times 1}\right) \times MW \times DF \times \left(\frac{v}{Wt}\right) \times 100$$

Description:

TAC = Total Anthocyanin Content
A = Absorbance
MW = molecular weight (449.2 $gmol^{-1}$)
DF = dilution factor
ε = molar extinction coefficient (26.900 $molcm^{-1}$)
L = cuvette diameter
V = final volume (L)
Wt = extract weight (g)

Antioxidant activity

The reducing power assay method was performed to measure the antioxidant activity of both extracts with minor modification. A series of extract concentration of 0, 2, 4, 6, 8, and 10 $\mu g.mL^{-1}$ was made by dilution of 2.5 mL, pH 6.6 pH phospate buffer and 2.5 mL, 1% potassium ferrcyanide. The mixture was incubated in a water bath at 50°C for 30 minutes with a slightly shaking movement. Subsequently, a total of 2.5 mL 10% trichloroacetic acid was added to the mixture. The mixture (5 mL) was dissolved with aquadest (5 mL) and 0.1% $FeCl_3$ (1 mL). The absorbance of the reaction mixtures was measured at 700 nm. Ascorbic acid was used as a positive control [16]. The IC_{50} value was calculated as previous report [17].

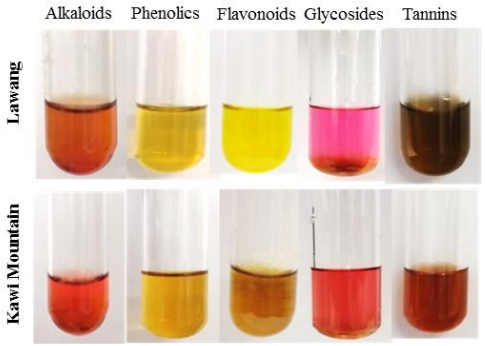
Statistical analysis

The differences of total anthocyanin purple sweet potatoes Lawang and Kawi mountain were analyzed using an independent-sample of t-test by SPSS software. Meanwhile, the IC_{50} difference between ascorbic acid of Lawang and Kawi Mountain cultivar was analyzed using one-way ANOVA. Data were shown as mean ± SD from three replication. The level of significance was set as $p<0.05$.

RESULT AND DISCUSSION

Phytochemical Profiles of Purple Sweet Potatoes From Lawang and Kawi Mountain.

Qualitative phytochemical screening was revealed the presence of alkaloid, phenolic, flavonoid, glycoside, and tannin in both extracts of PSP. However, the cultivar of Lawang was a relatively low color intensity for alkaloid and higher color intensity for flavonoid (Fig. 1).



	Alkaloids	Phenolics	Flavonoids	Glycosides	Tannins
Lawang	+	++++	+++	++++	+
Kawi Mountain	++	++++	++	++++	+

Figure 1. Qualitative phytochemical profies from extracts of purple sweet potatoes Lawang and Kawi Mountain cultivars. The presence of tested compounds was visualized as + low, ++ medium, +++ high and ++++ very high intensity color

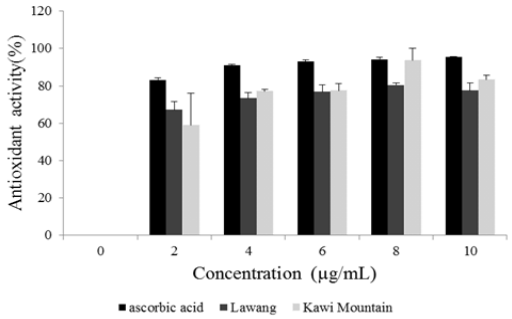
The present study was revealed that both cultivars have similar phytochemical content. Phytochemical content in sweet potatoes can be affected by several factors, for instance, the

ISSN. 2087-2852
E-ISSN. 2338-1655

genetic color of tuber roots. The purple and red color of tuber flesh were reported higher flavonoid and phenolic content than other white flesh [18]. The application of plant growth regulators has increased the quantitative phytochemical matters in sweet potatoes [19]. A previous study was suggested that phytochemical compounds such as alkaloids, glycosides, tannins, flavonoids, and phenolics in PSP are related to antioxidant activity [20].

Antioxidant activity of Purple Sweet Potatoes From Lawang and Kawi Mountain.

Antioxidant activity and IC_{50} value are negative correlations. Both PSP cultivars were shown antioxidant activity was directly proportional to the increase in concentration. PSP Lawang cultivar was not significantly different from PSP Kawi Mountain cultivar at the IC_{50} value, i.e 2.5, and 2.3 $\mu g.mL^{-1}$ for Lawang and Kawi Mountain, respectively (Fig. 2). Thereupon, the antioxidant activity of the Lawang cultivar was relatively similar to Kawi Mountain cultivar.



	Ascorbic acid	Lawang	Kawi Mountain
IC50 (µg/mL)	1.7[a]	2.5[a]	2.3[a]

Figure 2. Similar antioxidant activity is observed from the value of IC50. The same notation is reflected no significant value (p=0.076). Data were shown as mean value ±SEM.

The higher antioxidant activity is also influenced by the presence of phenolics and flavonoids in purple sweet potatoes [21]. In addition, purple sweet potatoes have higher levels of anthocyanin as a powerful antioxidant [22].

Anthocyanin in Purple Sweet Potatoes From Lawang and Kawi Mountain.

The UV-visible spectroscopy analysis was demonstrated that both extracts were absorbed UV light at a range of 200-324 nm and visible light at a range of 469-572 nm under pH 1 condition. Both cultivars have the same peak of absorbance at 521 nm in pH 1 and 530 nm in pH 4.5 (Fig. 3A and 3B). The range of peak between 520-530 nm is characterized as anthocyanin absorption wavelength. A similar study by Li *et al.* reported maximum absorption from purple sweet potato extracts at 520-530 nm using Uv-vis spectrophotometer analysis [23].

The similar range of hump and peak on UV-spectroscopy indicated that both cultivars have a similar basic structure of anthocyanin. The peak of absorbance under the UV-light at 521 nm and the acid condition was correlated to anthocyanin pigment content prediction in plants. Basic structures of anthocyanin in both cultivars are predicted as acetylated anthocyanin as previously reviewed. Acetylated anthocyanin is formed by sugar attachment on anthocyanidin moiety [6,24].

Even though both cultivars have similar wavelength hump peaks of absorbance, the 521 nm absorbance was higher in Lawang cultivar than the Kawi Mountain cultivar. It correlates with the higher total anthocyanin content in Lawang cultivar than Kawi Mountain cultivar, i.e., 10 $mg.100g^{-1}$ and 5 $mg.100g^{-1}$ respectively, with p=0.000 (Fig. 3C). The flesh color of various potatoes previously was correlated with different total anthocyanin content. It is supported by Teow *et al.*, which displayed a not detected level of anthocyanin in white and orange sweet potatoes. Despite the different anthocyanin content among cultivars, the antioxidant activity was similar (p=0.076). Phytochemical composition affects the antioxidant activity in plants, such as phenol, phenols, ortho-diphenols, flavonoids, and tannins [25].

Environmental factors such as the difference in growth altitude, soil pH, daily temperature, and light intensity were contributed to the content of phytochemical compounds in plants [26]. The range of daily temperature has affected the accumulation of anthocyanin biosynthesis. The increase of night temperature from 12-22°C and day temperature to 25°C promotes the increase of anthocyanin as well as radical absorbance capacity [27]. Soil characteristics were contributed to anthocyanin synthesis in plants [28,29]. Lawang and Kawi Mountain region has distinct environmental characteristics such as temperature and soil pH. The Lawang region has a daily temperature of 4°C lower than the Kawi Mountain region. The soil pH of the Kawi Mountain region is more acid than Lawang i.e., 4.71 and 5.25, respectively.

The previous review was reported that the accumulation of anthocyanin is affected by water drought and salt stress through cytoplasm dehydration [11]. However, the potatoes plant

ISSN. 2087-2852
E-ISSN. 2338-1655

was shown distinct responses towards drought stress. Drought has resulted in the decrease of tuber yield without a significant difference in anthocyanin and antioxidant measurement. Nevertheless, simultaneous application of drought and wound to purple potatoes significantly reduce the Trolox equivalent. It suggested that purple potatoes are more sensitive to drought, especially during tuber bulking, with a less pronounced effect on anthocyanin production [30].

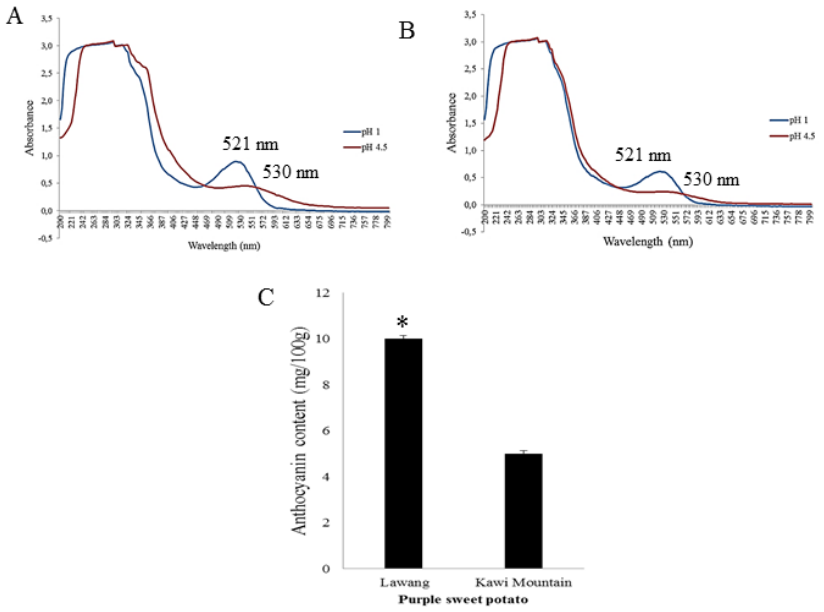


Figure 3. Anthocyanin contents from two cultivars of purple sweet potatoes. A) The aborbance of UV-Vis spectroscopy profilesof Lawang cultivar and B) Kawi Mountain cultivar. Despite both cultivasr have similar peak at 521 nm wavelength under pH 1 (blue line) and shifted to 530 nm in pH 4.5 (red line), the absorbance level of Lawang Cultivar at 521 nm is higher than Kawi Mountain cultivar C). The total anthocyanin of Lawang cultivar significantly higher than Kawi Mountain cultivar with p=0.000. The (*) notation identified significantly different. The data were shown mean value ±SEM.

CONCLUSION

Purple sweet potatoes from Lawang and Kawi Mountain region of East Java Indonesia have similar qualitative phytochemical profiles and antioxidant activity. However, anthocyanin content in Lawang cultivar was higher than Kawi Mountain cultivar. We proposed the necessary study related to environmental characteristic profile in both regions to support the growth requirements enrichment to enhance the anthocyanin as well as other secondary metabolites in both cultivars.

Acknowledgements

This research was partially funded by Research and Community Services Agencies, Faculty of Medicine, Universitas Brawijaya, no. 214.26/SK/UN10.F08.06/PN/2020. We thank all members of Smart Molecule of Natural Genetics Resource Research Centre Universitas Brawijaya.

REFERENCES

[1] Chandrasekara, A., T.J. Kumar. 2016. Roots and tuber crops as functional foods: A review on phytochemical constituents and their potential health benefits. *Int. J. Food Sci.*. 2016. Article ID 3631647. DOI: 10.1155/2016/3631647.

[2] Suryani, R. 2016. Outlook komoditas pertanian sub sektor tanaman pangan ubi jalar. Data Center and Information Syste of Agriculture. Indonesian Ministry of Agriculture. Jakarta.

[3] Dewi, R., S.D. Utomo, M. Kamal, P.B. Timotiwu, S. Nurdjanah. 2019. Genetic and phenotypic diversity, heritability, and correlation between the quantitative characters on 30 sweet potato germplasms in Lampung, Indonesia. *Biodiversitas*. 20(2). 380–386.

[4] Ariadi, B.Y. 2006. Comparison among various sweet potato varieties focused from farm income and marketing at Malang Regency. *Jurnal Pembangunan Pedesaan.* 6(2). 104-113.

[5] Kurnianingsih, N., R. Ratnawati, T. Nazwar, M. Ali, F. Fatchiyah. 2021. Purple sweet

potatoes from East Java of Indonesia revealed the macronutrient, anthocyanin compound and antidepressant activity candidate. *Med. Arch.* 75(2). 94-100.

[6] Li, A., R. Xiao, S. He, X. An. Y. He, C. Wang, *et al.* 2019. Research advances of purple sweet potato anthocyanins: Extraction, identification, stability, bioactivity, application, and biotransformation. *Molecules*. 24(21). 3816. DOI: 10.3390/ molecules24213816.

[7] Darwatik, R. Ratnawati, S. Rianawati, I. Sarwono. 2018. Effect of purple sweet potato anthocyanin towards TNF-α, apoptosis, and spatial memory in hippocampus of rat model of Diabetes Mellitus. *Jurnal Kedokteran Brawijaya*. 30(1). 12–18.

[8] Maharani, T., D. Sargowo, A. Tjokropranowo, R. Ratnawati. 2014. Effect of extrac purple *Ipomoe batatas* cultivar Kawi mountain chronic inflammation in Wistar Rats with atherogenic diet. 3(1). 1–7.

[9] Kurnianingsih, N., R. Ratnawati, F Fatchiyah, W. Barlianto, M.M. Ali, A. Safitri, E. Suyanto. 2019. The difference of amino acid profiling from two morphological purple sweet potatoes from Kawi Mountain cultivars, East Java, Indonesia. *J. Phys. Conf. Ser.* 1374(1). 012017. DOI: 10.1088/1742-6596/1374/1/ 012017.

[10] Yaningsih, H., B. Admati H., S. Mulyani. 2016. Studi karakteristik gizi ubi jalar ungu (*Ipomoea batatas* var Gunung Kawi) pada beberapa umur panen. *Jurnal Rekayasa dan Manajemen Agroindustri.* 1(1). 21–30.

[11] Ramakrishna, A., G.A. Ravishankar. 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal. Behav.* 6(11). 1720–1731.

[12] Mukherjee, P.K., R.K. Harwansh, S. Bahadur, V. Duraipandiyan, N.A. Al-Dhabi. 2017. Factors to consider in development of nutraceutical and dietary supplements. Elsevier Inc.

[13] Fatchiyah, F., D. Ratih, T. Sari, A. Safitri, J.R.K. Cairns. 2020. Phytochemical compound and nutritional value in black rice from Java Island, Indonesia. *Sys. Rev. Pharm*. 11(7). 414–421.

[14] Amelia, F., G.N. Afnani, A. Musfiroh, A.N. Fikriyani, S. Ucche. 2013. Extraction and stability test of anthocyanin from buni fruits (*Antidesma bunius* L.) as an alternative natural and safe food colorants. *J. Food Pharm. Sci*. 1(2). 49–53.

[15] Chen, C.C., C. Lin, M.H. Chen, P.Y. Chiang. 2019. Stability and quality of anthocyanin in purple sweet potato extracts. *Foods*. 8(9). 1–13.

[16] Ahmed, D., M.M. Khan, R. Saeed. 2015. Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from Adiantum caudatum leaves. *Antioxidants*. 4(2). 394–409.

[17] Nugroho, D.W., D.A. Daratika, M. Kamila, L. Togatorop, M.A. Rifada. 2020. Effect of mechanical milling on the total phenolic content and antioxidant activity of *Garcinia mangostana* pericarp. *Makara J. Sci.*, 24(2). 65-71. DOI: 10.7454/mss.v24i2. 1190.

[18] Brown, C.R. 2005. Antioxidants in potato. *Am. J. Potato Res.* 82(2). 163–172.

[19] Ghasemzadeh, A., D. Talei, H.Z.E. Jaafar, A.S. Juraimi, M.T.M. Mohamed, A. Puteh, M.R.A. Halim. 2016. Plant-growth regulators alter phytochemical constituents and pharmaceutical quality in sweet potato (*Ipomoea batatas* L.). *BMC Complement. Altern. Med.* 16(1). 1–13. DOI: 10.1186/s12906-016-1113-1.

[20] Sendangratri, R. Handayani, B. Elya. 2019. Inhibitory effects of different varieties of sweet potato (*Ipomoea batatas* L.) tubers extracts on lipoxygenase activity. *Pharmacogn. J.* 11(6). 1195–1198.

[21] Hue, S.M., A.N. Boyce, C. Somasundram. 2012. Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (*Ipomoea batatas*). *Aust. J. Crop Sci.* 6(3). 375–380.

[22] Amir, H.M.S., N. Nurun, N. Iqbal, F.R. Nur, L.H. Lee, F.M.N. Mariam, *et al.* 2016. Effect of heat treatment on the antioxidant activities of two cultivars of sweet potatoes. *Jurnal Teknologi.* 78(6–12). 111–115.

[23] Li, J., L. Zhang, Y. Liu. 2013. Optimization of extraction of natural pigment from purple sweet potato by response surface methodology and its stability. *J. Chem.* Article ID 590512. 1–6.

[24] Saha, S., J. Singh, A. Paul, R. Sarkar, Z. Khan, K. Banerjee. 2021. Anthocyanin

profiling using UV-vis spectroscopy and liquid chromatography mass spectrometry. *J. AOAC Int.* 103(1). 23–39.

[25] Teow, C.C., V. Den Truong, R.F. McFeeters, R.L. Thompson, K.V. Pecota, G.C. Yencho. 2007. Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. *Food Chem.* 103(3). 829–838.

[26] Setyati, D., H. Sulistiyowati, M.P. Erizcy, T. Ratnasari. 2020. The Flavonoid and alkaloid content of *Cyclosorus parasiticus* (Linn.) farwell ferns at the plantation areas of Jember Regency. *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan)*. 7(1). 23–37. DOI:10.31289/biolink.v7i1.3026.

[27] Wang, S.Y., W. Zheng. 2001. Effect of plant growth temperature on antioxidant capacity in strawberry. *J. Agric. Food Chem.* 49(10). 4977–4982.

[28] Khusnia, A. 2017. Keanekaragaman serangga tanah di perkebunan teh PTPN XII Wonosari Lawang. Bachelor Thesis. Department of Biology, UIN Maulana Malik Ibrahim Malang.

[29] Putra, M.J.N.F.I.A., S. Soemarno, R. Suntari. 2016. Humification degree and its relationship with some soil physical characteristics on robusta coffee (Coffea canephora) plantation. *J. Degrad. Min. Lands Manag.* 3(4). 649–658. DOI: 10.15243/jdmlm.2016.034.649.

[30] Wegener, C.B., G. Jansen. 2013. Antioxidants in different potato genotypes: Effect of drought and wounding stress. *Agriculture.* 3(1). 131–146.

ISSN. 2087-2852
E-ISSN. 2338-1655