Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2021.61.66

Research Article
Comparative Analysis of Bioactive Compounds, Antioxidant and Anti-inflammatory Activities of Apple Varieties

[1]Abeer H. Elhakem, [2,3]Manal M. Almatrafi, [4]Nada Benajiba, [5]Marwa Y. Koko and [2]Rokayya Sami

[1]Department of Biology, College of Sciences and Humanities, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia
[2]Department of Food Science and Nutrition, Taif University, Al-Huwayah, Taif, 888, Saudi Arabia
[3]Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA
[4]Department of Basic Health Sciences, Deanship of Preparatory Year, Princess Nourah Bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia
[5]National Food Research Centre, P.O. Box 213, Shambat, Khartoum North, Sudan

Abstract

Background and Objective: Apples play an essential role in human nutrition, as they are a well-documented source of natural antioxidants as vitamins, carotenoids and phenolic compounds. This research was conducted to study some bioactive compounds and activities of five apple varieties (Black, Apricot, Jester, Big Ariane and Medium Ariane). **Materials and Methods:** *In vitro* antioxidant and anti-inflammatory activities were determined and analyzed by using a spectrophotometer. Replications of all apple varieties were applied to ANOVA test using SPSS (20.00). Significant differences between means (±SD) were significant at ($p<0.05$) by using Duncan's New Multiple Range Test. **Results:** Medium Ariane apples had the highest total antioxidant value followed by Big Ariane, Apricot, Black and Jester apples. Apricot had the highest antioxidant activities, (93.67, 7.71 and 85.98 µmol TE g^{-1} fw) for DPPH, ABTS and FRAP, respectively. Total phenol values were 21.65-35.11 mM and the total flavonoids were 36.86-55.52 mg Qe g^{-1}. The extracts showed high anti-inflammatory activity values (0.32-5.46 µg mL^{-1}). Medium Ariane, Jester and Apricot had highly anti-inflammatory activity followed by Big Ariane and Black extracts, respectively. **Conclusion:** The results presented that apples, especially Apricot variety had the highest bioactive compounds, antioxidant and anti-inflammatory activities.

Key words: Comparative analysis, antioxidant, anti-inflammatory, bioactive compounds, apple, varieties

Citation: Elhakem, A.H., M.M. Almatrafi, N. Benajiba, M.Y. Koko and R. Sami, 2021. Comparative analysis of bioactive compounds, antioxidant and anti-inflammatory activities of apple varieties. Asian J. Plant Sci., 20: 61-66.

Corresponding Author: Rokayya Sami, Department of Food Science and Nutrition, Taif University, Al-huwayah, Taif, 888, Saudi Arabia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

An increasing amount of research *in vitro* and *in vivo* is being done nowadays on apple genotypes due to their potential high antioxidant, anti-inflammatory activities for the use of value-added food processing[1]. Apple fruit has excellent antioxidant potential against free radicals scavenging. Antioxidants created by apples in response to the environmental stresses and infections caused by micro-organisms or even mechanical damage[2]. The antioxidant activities were evaluated in several reports by various assays[3-5]. Chen *et al.*[6] studied the intracellular signaling pathways by dietary flavonoids against inflammation. Besides, Chu *et al.*[7] reported the flavonoids contents and antioxidant activities of apple fruits. Magalhaes *et al.*[8] mentioned some evaluations of apple antioxidant properties *in vitro*. While, Anna *et al.*[9] studied the impact of some processing factors on health-promoting phytochemicals in apples and some fruits. The FRAP, DPPH and ABTS are the common assays used for measuring the antioxidant activities[10].

Oxidative stress of apples is linked to inflammation against the pathogenesis of cardiovascular diseases, cancers, asthma and diabetes. Raw apples and their extracts have been established to decrease cholesterol, lipid oxidation and inhibit cancer cell[11,12]. Epidemiological *in vivo* studies have confirmed that antioxidant and anti-inflammatory dietary intakes are essential for lowering inflammations and oxidative stresses[13-15].

This research aimed to evaluate bioactive compounds (phenol, flavonoids), antioxidant activities (FRAP, ABTS, DPPH) and anti-inflammatory activities of different varieties of apple fruit.

MATERIALS AND METHODS

Study area: The present study was conducted during January-June, 2020 at the Department of Biology Prince Sattam Bin Abdulaziz University, Saudi Arabia

Chemicals and cells: RAW 264.7 cells, (MTT), (DMEM), Abnova Kit (KA 1342), Trolox, Quercetin, DPPH, ABTS, FRAP, Folin reagents and ascorbic acid were from Sigma, Co., USA.

Sample preparation: Five apple varieties as; Black variety; apples are medium in size and red, despite its name, it (often) does not have a black hue. Apricot and Jester apples are small in size approximately 31.45-39.64 g in weight. Ariane apples are (Medium and Big) approximately 137.49-188.34 g in weight, respectively. Apples were obtained from a public market in Taif City, Saudi Arabia. Apple varieties were cleaned, cut, freeze-dried, milled and stored until use at (-80°C).

***In vivo* antioxidant extraction:** Apple samples were mixed with an equal volume of (80%) methanol, centrifuged at (15 min, 6900 g) with chilling and the supernatants were kept for further study at 4°C.

Bioactive compounds determination: Total antioxidant capacity was evaluated by ascorbic acid as a standard and results were considered as ascorbic acid equivalent (AAE). The total phenol content was evaluated by the Folin-Ciocalteu method and considered as mM (TEAC)[13]. Flavonoids were detected 415 nm and expressed as Quercetin (QE)[16].

Antioxidant assays determinations (ABTS), (DPPH) and (FRAP) assay: ABTS assay was evaluated according to a protocol to stabilize the ABTS+cation-radical[17]. DPPH assay was to determine the capability of the antioxidants for the radical DPPH (in ethanol). The FRAP reagent included the antioxidant potential through the ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}) reductions in apple extracts[18]. Results were considered at µmol TE g^{-1} fw.

***In vitro* anti-inflammatory extraction for cell cultures:** The dried apple varieties (5 g) were mixed and immersed in 20 mL of (80%) distilled methanol and centrifuged. The filtrates were collected and evaporated until dryness. The apple residues were dissolved in (DMSO) (20 mg mL^{-1}) as final concentrations.

MTT assay: MTT assay is responsible for assessing cytotoxicity or cell viability. This assay evaluates the principally of cell viability through mitochondrial function determination by the mitochondrial enzyme activity as succinate dehydrogenase. Briefly, cells were incubated with 5 mg mL^{-1} MTT for 4 hrs after solubilizing in DMSO 150 µL per well and measured at 490 nm[19].

NO production assay measurement: For measuring NO production generated; the amount of nitrate in the media supernatant was extracted by using Abnova Kit (KA 1342). Briefly, (200, 100 and 100 µL) of assay buffer, standard solution and apple extracts respectively beside the addition of (50 µL for R1) and (50 µL for R2). Cells were incubated for (10 min) and measured at 540 nm[20].

Statistical analysis: Replications of all apple varieties were applied to ANOVA test using SPSS (20.00). A difference between means (±SD) was significant at ($p<0.05$) by using Duncan's new multiple range test.

RESULTS AND DISCUSSION

Bioactive compounds contents: Bioactive compounds in commercial apple varieties are given in (Table 1). The flavonoid contents were ranged from 36.86-55.52 mg QE g^{-1} fw; Apricot apples recorded the highest followed by Jester and Black. Medium and Big Ariane varieties had lower flavonoid contents 36.86 and 39.15 mg QE g^{-1} fw than the other varieties. Total phenolic content is considered as an evaluation of apple nutrients, varied obviously from 21.65 mM (TEAC) in Big Ariane to 35.11 mM (TEAC) in Apricot. Black and Medium Ariane total phenol values were similar to each other, 23.04 and 23.17 mM (TEAC), respectively. The bioactive compounds amounts depend on lots of factors, as, climate, soil, water core, varieties, season, harvest date, geographic region, storage, bitter pit and irradiation moreover additional conditions[16]. Medium Ariane reported the highest total antioxidant (16.16 µg AAE mg^{-1} fw), while Jester reported the lowest (µg AAE mg^{-1} fw) as the total antioxidant value for the previous reports[21,22].

Antioxidant activities: The antioxidant activities of apple varieties were determined by (FRAP) assay exhibited strongly as (17.98, 85.98 µmol TE g^{-1} fw) for Big Ariane and Apricot, respectively (Fig. 1). Vierira *et al.*[23] recorded similar antioxidant values using FRAP assay 22.90-83.33 µmol TE g^{-1} dw. The antioxidant activities exhibited highly scavenging against (DPPH) radicals, which ranged 76.44-93.67 µmol TE g^{-1} fw. Results were in an arrangement with other studies[24,25]. The results exposed that the capacity to scavenge an ABTS+ radical reached a level of (5.33 and 7.71 µmol TE g^{-1} fw) for Big Ariane and Apricot, respectively. Vieira *et al.*[26] had evaluated six apple varieties grown under open field conditions from an orchard located in Santa Catarina, which reported from 3.41-7.40 µmol TE g^{-1} fw.

Anti-inflammatory activity: Peritoneal macrophages were incubated with different methanolic apple extracts at a concentration range of 0-200 µg mL^{-1} of LPS for anti-inflammatory activity by using MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) for assessing cell metabolic activity to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials, while NO synthesis determines nitric oxide composition through measurement of nitrate (NO3) and nitrite (NO2) levels *in vitro*. According to (Fig. 2), significant concentration-dependent inhibition was detected when cells were co-treated with various concentrations of the five apple variety extracts and LPS. Big Ariane apples recorded the highest values compared with other apple extracts with various concentrations as follows (25 µg mL^{-1}, 94.33%; 50 µg mL^{-1}, 83.45%; 100 µg mL^{-1}, 91.31%; 200 µg mL^{-1}, 82.02%). On the other hand, Black apples established the lowest values with concentrations of 25-100 µg mL^{-1}, while Medium Ariane reported the lowest for (200 µg mL^{-1}, 72.21%). Hence, results displayed that the concentration range used did not confirm any significant cytotoxicity against the macrophages by using MTT assay.

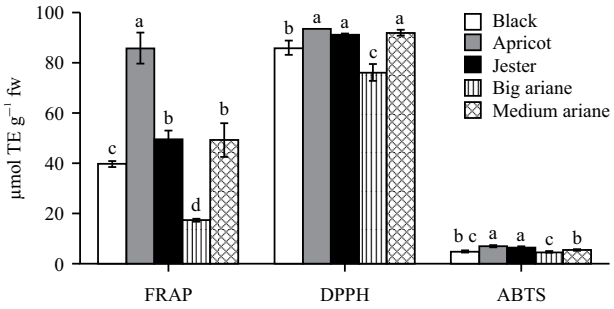

Fig. 1: Antioxidant Activities (FRAP, DPPH and ABTS) values in (µmol TE/g fw)

Values are the average of three replications of each apple varieties with (±SD) standard deviations. Various uppercase letters indicate the significant differences ($p < 0.05$) that analyzed by the test of Duncan's multiple-range

Table 1: Bioactive compounds, total antioxidant µg (AAE mg^{-1}), total phenol mM (TEAC) and total flavonoid mg (Qe g^{-1}) contents of apple varieties

	Total antioxidant µg (AAE mg^{-1})	Total phenol mM (TEAC)	Total flavonoid mg (Qe g^{-1})
Black	14.64±0.31^{c}	23.04±1.28^{c}	44.87±3.15^{b}
Apricot	15.36±0.27^{b}	35.11±0.42^{a}	55.52±1.95^{a}
Jester	13.89±0.19^{d}	28.72±0.28^{b}	53.22±4.19^{a}
Big ariane	15.88±0.61^{ab}	21.65±0.88^{c}	39.15±2.05^{c}
Medium ariane	16.16±0.37^{a}	23.17±3.66^{c}	36.86±2.10^{c}

Values within a column (lowercase) are significantly different ($p = 0.05$) that analyzed by the test of Duncan's multiple-range

NO production: All extracts presented highly anti-inflammatory activities with the range of 0-100 µg, where Medium Ariane, Jester and Apricot were the best varieties followed by Big Ariane and Black varieties. NO synthesis

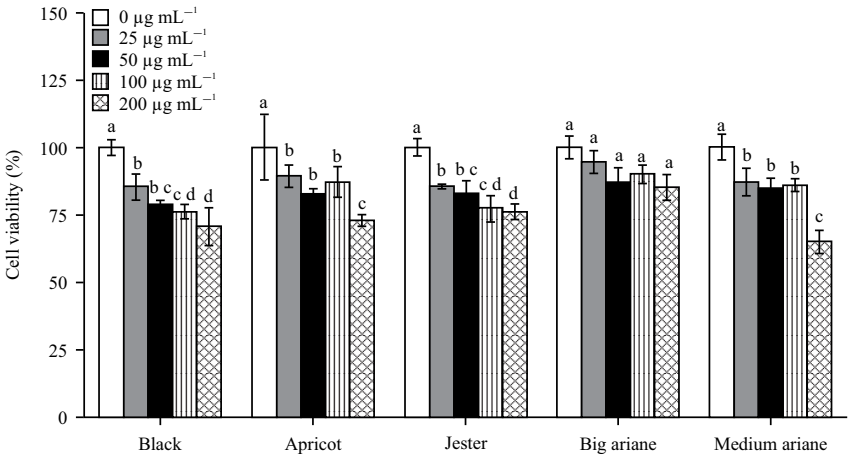

Fig. 2: Cell viability of different apple varieties (%)

Values are the average of three replications of each apple varieties with (±SD) standard deviations. Various uppercase letters indicate the significant differences ($p < 0.05$) that analyzed by the test of Duncan's multiple-range

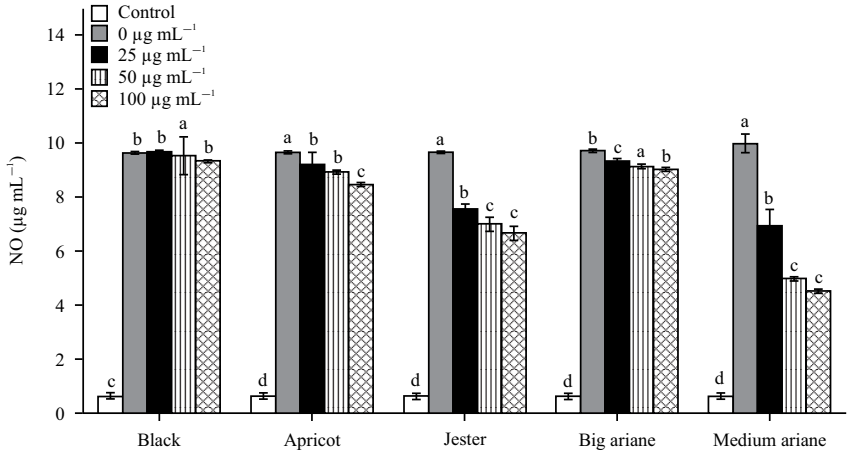

Fig. 3: No production by RAW 264.7 cells in apple varieties

Values are the average of three replications of each apple varieties with (±SD) standard deviations. Various uppercase letters indicate the significant differences ($p < 0.05$) that analyzed by the test of Duncan's multiple-range

exposed that (RAW 264.7) cells generated a low amount of (NO_2). After incubation with apple extracts for 24 hrs, NO increased obviously. According to Fig. 3, Medium Ariane reported the lowest values in concentrations in range 25-100 µg mL^{-1} as follows (25, 7.01, 50, 5.47, 100 and 4.36 µg mL^{-1}). Black apples established the highest values for (25, 9.73 µg mL^{-1}). Black and Big Ariane apples reported similar values with concentrations of 50-100 µg mL^{-1} as (9.32-9.21 µg mL^{-1}) and (9.29-9.17 µg mL^{-1}), respectively. The extreme NO production denotes a possibly toxic effect which can cause many pathologies disease[27]. These results established that apple verities have a noticeable effect on scavenging free radicals due to its high phenolic compound contents which is in a link to hydroxyl groups substitution of phenolics aromatic rings of thus contributing to their hydrogen denoting ability[28]. A similar sequence was detected for anti-inflammatory with Jester and Medium Ariane, which resulted in a little high capacity to NO production according to the previous studies[20,28].

Correlation between apple activities: Apple activities (antioxidant, anti-inflammatory) both refer to fruit nutrients concerning its biological functions. Jester recorded the lowest antioxidant and slight high anti-inflammatory values (13.89 µg AAE mg^{-1} fw, 3.01 µg mL^{-1}), respectively (Fig. 4). Apricot, Big Ariane and Black recorded a slight high antioxidant and low anti-inflammatory activities. Medium Ariane reported the highest antioxidant and anti-inflammatory levels (16.16 µg AAE mg^{-1} fw, 5.46 µg mL^{-1}), respectively.

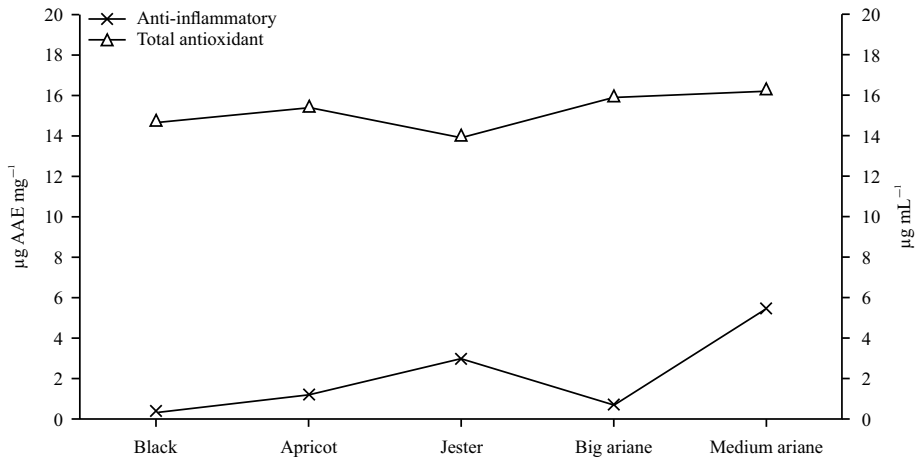

Fig. 4: Correlation between anti-inflammatory and anti-oxidant activities
Two independent trials were achieved of three replications of apple varieties

CONCLUSION

The results recommend that apples, especially Apricot variety had the highest bioactive compounds, antioxidant and anti-inflammatory activities, so apples in general might act as sources of important anti-inflammatory and antioxidant to reduce the oxidative stresses and inflammations.

SIGNIFICANCE STATEMENT

The results of this study offer for the event of providing many pharmacological and biochemical properties. Even though the results demand further *in vivo* studies, the *in vitro* data recommend apple varieties to reduce the oxidative stresses and inflammations. This work can support the investigators to increase recovering views on the critical area of the phytochemical bioavailability which many investigators were not capable to explore.

ACKNOWLEDGMENT

The authors would like to thank the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia for their support. This research was funded by the Deanship of Scientific Research at Princess Nourah Bint Abdulrahman University through the Fast-track Research Funding Program.

REFERENCES

1. Griffiths, K., B. Aggarwal, R. Singh, H. Buttar, D. Wilson and F. De Meester, 2016. Food antioxidants and their anti-inflammatory properties: A potential role in cardiovascular diseases and cancer prevention. Diseases, 4: 28-42.
2. Lobo, V., A. Patil, A. Phatak and N. Chandra, 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn. Rev., 4: 118-126.
3. Lonkar, P. and P.C. Dedon, 2011. Reactive species and DNA damage in chronic inflammation: Reconciling chemical mechanisms and biological fates. Int. J. Cancer, 128: 1999-2009.
4. El Jemli, M., R. Kamal, I. Marmouzi, A. Zerrouki, Y. Cherrah and K. Alaoui, 2016. Radical-scavenging activity and ferric reducing ability of *Juniperus thurifera* (L.), *J. oxycedrus* (L.), *J. phoenicea* (L.) and *Tetraclinis articulata* (L.). Adv. Pharmacol. Sci., Vol. 2016. 10.1155/2016/6392656.
5. Qiao, G., D. Wenxin, R. Sami, E. Khojah and S. Amanullah, 2020. Antioxidant and anti-inflammatory capacities of pepper tissues. Ital. J. Food Sci., 32: 265-269.
6. Chen, L., H. Teng, Z. Jia, M. Battino and A. Miron *et al*., 2017. Intracellular signaling pathways of inflammation modulated by dietary flavonoids: The most recent evidence. Crit. Rev. Food Sci. Nutr., 58: 2908-2924.
7. Chu, Y.H., C.L. Chang and H.F. Hsu, 2000. Flavonoid content of several vegetables and their antioxidant activity. J. Sci. Food Agric., 80: 561-566.
8. Magalhaes, L.M., M.A. Segundo, S. Reis and J.L. Lima, 2008. Methodological aspects about *in vitro* evaluation of antioxidant properties. Anal. Chim. Acta, 613: 1-19.
9. Kårlund, A., U. Moor, M. Sandell and R. Karjalainen, 2014. The impact of harvesting, storage and processing factors on health-promoting phytochemicals in berries and fruits. Processes, 2: 596-624.
10. Peiretti, P., M. Karamać, M. Janiak, E. Longato, G. Meineri, R. Amarowicz and F. Gai, 2019. Phenolic composition and antioxidant activities of soybean (*Glycine max* (L.) Merr.) plant during growth. Agronomy, Vol. 9. 10.3390/agronomy9030153.

11. Mohib, M., N. Chowdhury, J. Sarker, N. Hasan and S. Khan *et al*., 2018. A comprehensive review on effective role of apple polyphenols in the treatment of obesity, diabetes and liver dysfunctions with some possible molecular mechanisms. Oxid. Antioxid. Med. Sci., 7: 9-27.
12. LeBaron, T.W., B. Kura, B. Kalocayova, N. Tribulova and J. Slezak, 2019. A new approach for the prevention and treatment of cardiovascular disorders molecular hydrogen significantly reduces the effects of oxidative stress. Molecules, Vol. 24. 10.3390/molecules24112076.
13. Rokayya, S., C.J. Li, Y. Zhao, Y. Li and C.H. Sun, 2014. Cabbage (*Brassica oleracea* L. var. capitata) phytochemicals with antioxidant and anti-inflammatory potential. Asian Pac. J. Cancer Prev., 14: 6657-6662.
14. Charrier, G., J. Ngao, M. Saudreau and T. Améglio, 2018. Effects of environmental factors and management practices on microclimate, winter physiology, and frost resistance in trees. Front. Plant Sci., Vol. 6. 10.3389/fpls.2015.00259.
15. Tan, B.L., M.E. Norhaizan, W.P.P. Liew and H.S. Rahman, 2018. Antioxidant and oxidative stress: A mutual interplay in age-related diseases. Front. Pharmacol., Vol. 9. 10.3389/fphar.2018.01162.
16. Pavun, L., P. Đurđević, M. Jelikić-Stankov, D. Đikanović and S. Uskoković-Marković, 2018. Determination of flavonoids and total polyphenol contents in commercial apple juices. Czech J. Food Sci., 36: 233-238.
17. Kaur, C., S. Walia, S. Nagal, S. Walia and J. Singh *et al*., 2013. Functional quality and antioxidant composition of selected tomato (*Solanum lycopersicon* L.) cultivars grown in northern India. LWT-Food Sci. Technol., 50: 139-145.
18. Lee, Y.J., D.B. Kim, J. Lee, J.H. Cho and B. Kim *et al*., 2013. Antioxidant activity and anti-adipogenic effects of wild herbs mainly cultivated in Korea. Molecules, 18: 12937-12950.
19. Tonder, V.A., A.M. Joubert and A.D. Cromarty, 2015. Limitations of the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. BMC. Res. Notes, 8: 1-10.
20. Mueller, M., S. Hobiger and A. Jungbauer, 2010. Anti-inflammatory activity of extracts from fruits, herbs and spices. Food Chem., 122: 987-996.
21. Drogoudi, P.D. and G. Pantelidis, 2011. Effects of position on canopy and harvest time on fruit physico-chemical and antioxidant properties in different apple cultivars. Sci. Hortic., 129: 752-760.
22. Tungmunnithum, D., A. Thongboonyou, A. Pholboon and A. Yangsabai, 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines, Vol. 5, No. 3. 10.3390/medicines5030093.
23. Vieira, F.G.K., G.D.S.C. Borges, C. Copetti, P.F. Di Pietro, E. da Costa Nunes and R. Fett, 2011. Phenolic compounds and antioxidant activity of the apple flesh and peel of eleven cultivars grown in Brazil. Sci. Hortic., 128: 261-266.
24. Rufino, M.S.M., R.E. Alves, E.S. de Brito, J. Perez-Jimenez, F. Saura-Calixto and J. Mancini-Filho, 2010. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chem., 121: 996-1002.
25. Bouayed, J., L. Hoffmann and T. Bohn, 2011. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. Food Chem., 128: 14-21.
26. Vieira, F.G.K., G.D.S.C. Borges, C. Copetti, R.D.D.M.C. Amboni, F. Denardi and R. Fett, 2009. Physico-chemical and antioxidant properties of six apple cultivars (*Malus domestica* Borkh) grown in southern Brazil. Sci. Hortic., 122: 421-425.
27. Evans, D.A., J.B. Hirsch and S. Dushenkov, 2006. Phenolics, inflammation and nutrigenomics. J. Sci. Food Agric., 86: 2503-2509.
28. Nowacka, M., M. Śledź, A. Wiktor and D. Witrowa-Rajchert, 2014. Changes of radical scavenging activity and polyphenols content during storage of dried apples. Int. J. Food Prop., 17: 1317-1331.