Plant Archives Volume 20 No. 2, 2020 pp. 6470-6474 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

COMPARATIVE FREE RADICAL SCAVENGING ABILITIES AND PHYTOANTIOXIDANT ACTIVITIES OF THREE SELECTED VEGETABLE, FRUIT AND SPICE JUICE

Sayeed Abdul Hamid Patel[1*], Mudasir Yaqoob[2] and Balogun Adedeji Suleimon[3,4]

[1*]Department of Vegetable Science, Punjab Agricultural University, Ludhiana-141004 (Punjab) India.
[2]Department of Food science and Nutrition, Lovely Professional University, Phagwara-144411 (Punjab) India.
[3]Department of Animal production technology, School of animal and Fisheries technology, Igboora, Oyo state, Nigeria.
[4]Department of Animal Genetics and Breeding, Guru Angad Dev Veterinary and Animal Science University, PAU Campus,-141004 (Punjab), India.

Abstract

The inhibition of free radical scavenging ability and natural antioxidants present in plant kingdom can be an important holistic plan in preventing epigenetic diseases. Hence this study aims to analyze three groups *i.e.* vegetable (tomatoes), fruits (oranges) and spice (garlic) to find best source to reduce free radical activity as these crops are consumed at larger amount. Fresh juice and extract were obtained by screw type juice extractor and further processed for study free radical scavenging ability using DPPH assay and other phytoantioxidant activity. DPPH assay revealed that orange juice (602.48mg/100ml) had significant highest free radical scavenging ability compared to tomato and garlic juices (307.46 and 157.01mg/100ml) due to ferulic acid. Total phenol was significantly higher in tomato (523.33mg/100ml) owing to few major phenolic compounds. Total Carotenoid and β-carotene were statistically significant ($P>0.05$) in garlic juice extract (5.39 µg/100g; 4.25mg/100ml), followed by tomato (4.36µg/100g; 3.67mg/100ml) and orange (3.33µg/100g; 2.64mg/100ml), respectively. Total flavonoids content has non-significant difference in tomato (29.05mg/100ml) and orange (29.03mg/100ml) juice. Percentage of ascorbic acid content was highest in orange juice (356.35mg/100 ml) as *Citrus* family has the characteristics of higher ascorbic acid content. This confirms that these juices might protect human as well as animal body from oxidative damage and may cure epigenetic diseases. Our experiment concluded that orange followed by tomato and garlic juice will be best for human health.

Key words: DPPH assay, Epigenetic, Free radical, Phytoantioxidant.

Introduction

Natural source of deriving antioxidants by human and animals depends solely on what we consume (food). The natural antioxidants are a stable part of nutrition as they occur in almost all edible plant products (Aleksandra and Tomasz, 2007). Cereals, legumes and tubers crops constitute major part of human and animal consumption, since they serve as bulk and mostly fillers, which constitute the energy and some important essential amino acids. However, for maintenance and effective body functioning system, vegetables, fruits and spices plays an important role, due to its abundant antioxidant enzymes especially at fresh harvest state. Polyphenols, are known to be numerous group of antioxidant components and largest group known as 'secondary plant products' synthesized by higher plants, that are present in fruits, vegetables, leguminous plants, grains, teas, herbs, spices and wines (Horubała, 1999; Borowska and Szajdek, 2003). The antioxidant activity is defined as the ability of an organism to protect itself against free radicals by either through innate or external means (Kurutas, 2016). Phytoantioxidant includes terpenes or polyphenols (vital role in plant metabolism and defense system) (Jadoon *et al.,* 2015). The three bioactive compounds present in various phytoextracts include phenols, flavonoids and carotenoids which have both antioxidant and UV protection effects

***Author for correspondence :** E-mail: sayeed-coavc@pau.edu

(Kole *et al.,* 2005). Phytoantioxidant are classified as primary or chain-breaking, synergist and secondary antioxidants (Rajalakshmi and Narasimhan, 1996).

Free radicals are responsible for many pathological processes, or they can be generated as the result of the pathological stage and cause important secondary damage to biological systems and cells (Beklova *et al.,* 2008; Blazekovic *et al.,* 2010). Strong connections between free radicals and some serious diseases, including Parkinson´s, Alzheimer´s disease, atherosclerosis, heart attacks, cancer and chronic fatigue syndrome have been discussed (de Diego-Otero *et al.,* 2009; Patel and Thakur, 2017). Consumption of food containing a lot of polyunsaturated fatty acids may expedite unnecessarily ROS production in the human body. It justifies harnessing and comparing the significance and usage of substances that protect system against oxidation with respect to the class of food to which they belongs.

Due to high demand, during four decades world crop area of tomato increased by 164% in relation with 314% in world consumption (Nicola *et al.,* 2009; Chaudhary *et al.,* 2018). Tomato is known as protective food as it contains carotenoids, flavonoids, glycosides which act as an antioxidant (Waheed *et al.,* 2020). Similarly oranges have high antioxidant activity as it is good source of vitamin C, carotenoids and phenolic compounds and thus have free radicals scavenging activity (Yoo *et al.,* 2004; Cirmi *et al.,* 2016; Silva *et al.,* 2019). Eighty eight per cent of world orange consumption is in North America (Pollack *et al.,* 2003) and Europe (Spreen, 2010). Whereas garlic is also known to contain natural antioxidant that binds with free radical and reduce oxidation of lipids and lipoprotein (Saravanan and Prakash, 2004; Ide and Lau, 1997) due to allicin, diallyl sulfides and other sulfur compounds (Amagase *et al.,* 2001; Chung, 2006). This necessitates the need for choosing them among the family they belong to for evaluation and comparison.

Harnessing their antioxidant and free radicals scavenging abilities may be a beneficial approach to recommend which of them has a better antioxidant food component that can effectively scavenge the free radicals by providing better health status for human, this experiment therefore aim at harnessing and evaluating the phytoantioxidant properties and free radicals scavenging abilities of tomato, orange and garlic extract.

Materials and Methods

The ripe tomatoes, oranges and matured garlic were procured from the farms of Punjab Agricultural University, Ludhiana, Punjab, India (30.90°N and 75.81°E).

Preparation of the Extracts

Juice was extracted from tomatoes and oranges with screw type juice extractor and was stored at -20°C till further analysis. While fresh mature garlic cloves without papery skin were blended and soaked for three days in aqueous solution for extraction of the juice. These juices were further subjected to relevant analysis of phytoantioxidant properties.

Phytoantioxidant analysis

The aqueous extracts of tomato, oranges and garlic were subjected to phytochemical analysis for the identification of their active constituents such as phenols, carotenoids, flavonoids, β-carotene and ascorbic acid by following the reported procedure (Singleton and Rossi, 1965; Thimmaih, 1999; Willet, 2002; Watanabe *et al.,* 1991; Heinze *et al.,* 1944)

***In Vitro* evaluation of antioxidant**

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds (standards) was measured from the bleaching of the purple-coloured methanolic solution of 1,1-diphenyl-2-picryl-hydrazylhydrate (DPPH) assay described by Bursal and Gulcin, (2011).

The statistical analysis was done using SPSS software (version 16), where means were compared using one way anova. The mean differences were considered significant at $p<0.05$.

Results and Discussion

Oxidative damage leads to succession of many epigenetic diseases along with aging in human as well as animal body (Guillaumet-Adkins *et al.,* 2017). Antioxidants and free radical scavenging activity were reported in different parts of vegetable, fruits and spice crop that reduces reactive ions to prevent the changes in

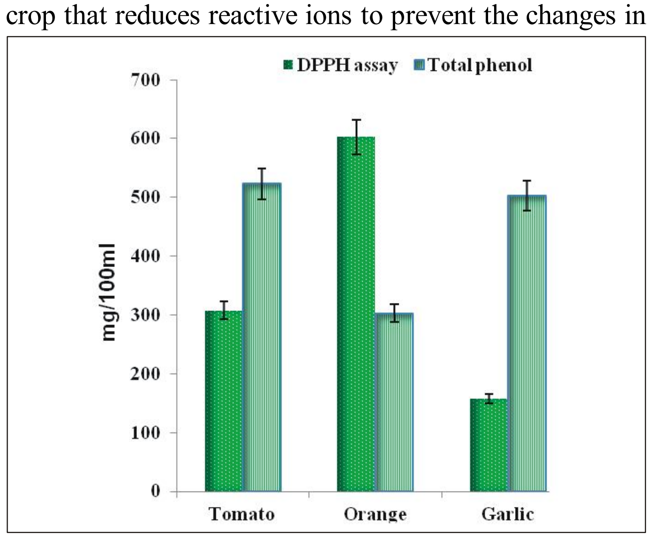

Fig. 1: DPPH assay and total phenols content in vegetable, fruit and spice juice extract.

basic DNA structure (Bajpai *et al.,* 2005; Yadav and Bhatnagar, 2007; Najafabad and Jamei, 2014; Ali and Naz, 2017).

The DPPH assay revealed that there is significant different (P>0.05) among the juice (Fig. 1). Moreover, orange juice had significant highest free radical scavenging ability of 602.48mg/100ml compared to tomato and garlic juices (307.46 and 157.01mg/100ml respectively). Free radical scavenging ability of orange juice may be traceable to the contribution of ferulic acid, a major phenolic compound in orange juice (Augustin and Williams, 2000), it accounts especially for its neutralizing the free radicals known as superoxide, hydroxil radical and nitric oxide and it acts synergistically with other antioxidant giving them extra potency and greatly reduces free radical damage to the external and internal membranes of cells (Zuo *et al*., 2002).

The total phenol analysis has significant difference (P>0.05) among the three juices (Fig. 1). Even though phenols do possess good free radical scavenging/ antioxidant property (Dirar *et al.,* 2019), still in our study total phenol was significantly higher in tomato (523.33mg/ 100ml), followed by garlic (503.33mg/100ml) and orange (302.67mg/100ml). Tomato contains caffeic, chlorogenic, sinapic and p-coumaric as major phenolic compounds (Chaudhary *et al.,* 2018). Keskin-Šašić *et al.,* (2012) observed similar trend where black currant and black chokeberry have highest antioxidant capacity but they have significantly lower total phenol content than cranberry. Total phenol content in tomato or other crop may vary with cultivar and the extrinsic growing factors especially UV radiation (Sharma *et al.,* 2018).

Total flavonoids was statistically similar (Fig. 2A) in both tomato juice (29.05 mg/100ml) and orange (29.03 mg/100ml) and significantly higher (P<0.05) as compare to garlic extract (19.83 mg/100ml). Tomato contains flavonoids i.e quercetin and chlorogenic acid as most abundant (Sharma *et al.,* 2018) and else are kaempferol, naringenin and rutin. Its accumulation increase with reduction in chlorophyll and ripening of fruit (Chaudhary *et al.,* 2018). The normal range of flavonoids is 15-30mg/ kg fresh weight of fruit or vegetable. Among vegetable onion is the richest source of flavonoids (350-1200mg/L) probably due to high quercetin content (Manach *et al.,* 2004) and provides color too.

The plant carotenoid pigment synthesized by isoprenoid biosynthesis pathway where it act as major source of antioxidant and trapping light in plant kingdom (Singh *et al.,* 2016). β-carotene work as a chain breaking antioxidant (Liebler and McClure, 1996). Total Carotenoid (Fig. 2D) and β-carotene (Fig. 2B) was significantly different (P>0.05) among the studied vegetable, fruit and spice. However, Garlic juice had highest significant value (5.39 µg/100g) for total carotenoids, followed by tomato juice (4.36 µg/100g) and orange juice (3.33µg/ 100g). Similarly, Garlic juice had highest significant value of (4.25mg/100ml) for β-carotene, followed by tomato juice (3.67mg/100ml) and orange juice (2.64mg/100ml). Conversely, Ziegler *et al.,* 1996, reported that intake of fruits and vegetables, as major source of carotenoids in the diet, however, lower carotenoids content observed in tomato and orange juice may be traceable to the magnitude of ripeness of tomato and orange. Similarly, Raffo *et al.,* (2002) reported that carotenoids content of tomato were very low at the breaker stage (1.08 mg/100g) which increased 10-fold during ripening and reached 12.705 mg/100g at full ripening stage. Garlic having high carotenoid content than its counterpart vegetable and fruit samples in the present study is suspected a range of diverse biologic functions and actions and has an essential role in human

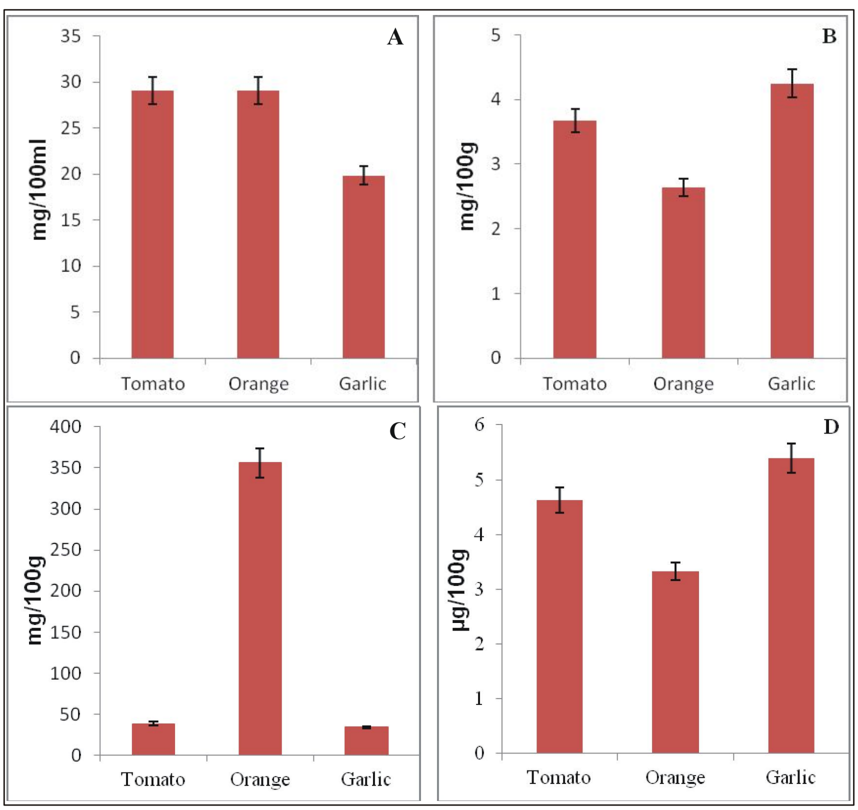

Fig. 2: Total flavonoid (A), β-carotene (B), ascorbic acid (C) and total carotenoid (D) content in vegetable, fruit and spice juice extract.

health such as immune enhancement and chemopreventive actions.

Ascorbic acid (Vitamin C) content was highest in Orange juice (356.35 mg/100 ml) (Fig. 2C) and in the range documented by Mennah-Govela and Bornhorst, (2017). Among *Citrus* genus, oranges have higher ascorbic acid while lemon has the lowest content (Khosravi and Asadollahzadeh, 2014). Raw (unprocessed) orange juices had higher ascorbic acid than any processed orange juices (Mennah-Govela and Bornhorst, 2017). While in tomato and garlic, it was 38.71 mg/100g and 34.10mg/100g respectively. The parallel range of results was reported by Sharma *et al.,* (1996) in different tomato genotypes i.e. from 11.21-53.29mg/100g. Ascorbic acid reduces when stored for longer period under normal room temperature (El-Ishaq and Obirinakem, 2015; Pavlović *et al.,* 2019; Martinsen *et al.,* 2020).

Based on the consumption pattern and observation of studied crops, it can be confirmed that these juices will protect human as well as animal body from oxidative damage and may cure epigenetic diseases. Our experiment concludes that orange followed by tomato and garlic juice will be best for human health based on free radical scavenging and phytoantioxidant activity.

References

Aleksandra, D. and T. Tomasz (2007). Antioxidant properties of different fruit seeds and peels. *Acta Sci. Pol. Technol. Aliment.,* **6(3):** 29-36.

Ali, H. and N. Naz (2017). Free radical scavenging activity of pulps and peels of some selected vegetables commonly used in Pakistan. *Pakistan J. Agric. Res*., **30:** 55-66.

Amagase, H., B.L. Petesch, H. Matsuura, S. Kasuga and Y. Itakura (2001). Recent advances on the nutritional effects associated with the use of garlic as a supplement: intake of garlic and its bioactive components. *J. Nutr.,* **131(3):** 955S-962S. Doi: 10.1093/jn/131.3.955S.

Augustin, S. and G. Williams (2000). Dietary intake and bioavailability of polyphenols. *J. Nutr.,* **130:** 2073-2085.

Bajpai, M., A. Mishra and D. Prakash (2005). Antioxidant and Free Radical Scavenging Activities of Some Leafy Vegetables. *Int. J. Food. Sci. Nutr.,* **56:** 473-481. Doi: 10.1080/09637480500524299.

Beklova, M., O. Zitka, Z. Gazdik, V. Adam, P. Hodek, M. Stiborova, A. Horna and R. Kizek (2008). Electroanalytical techniques for determination of flavonoids. *Toxicol. Lett.,* **180:** S230.

Blazekovic, B., S. Vladimir-Knezevic, A. Brantner and S.M. Bival (2010). Evaluation of Antioxidant Potential of Lavandula × intermedia Emeric ex Loisel. 'Budrovka': A Comparative Study with *L. angustifolia* Mill. *Molecules,* **15:** 5971-5987.

Bursal, E. and I. Gulcin (2011). Polyphenol contents and in vitro antioxidant activities of lyophilised aqueous extract of kiwifruit (*Actinidia deliciosa*). *Food Res. Int.,* **44:** 1482-1489.

Borowska, J. and A. Szajdek (2003). Antioxidant activity of berry fruits and beverages. *Pol. J. Natur. Sci.,* **14:** 521-528.

Chaudhary, P., A. Sharma, B. Singh and A.K. Nagpal (2018). Bioactivities of phytochemicals present in tomato. *J. Food Sci. Technol.,* **55(8):** 2833-2849. doi:10.1007/s13197-018-3221-z

Chung, L.Y. (2006). The antioxidant properties of garlic compounds: Allyl cysteine, Alliin, Allicin and Allyl disulfide. *J. Med. Food,* **9(2):** 205-213.

Cirmi, S., N. Ferlazoo, G.E. Lombardo, A. Maugeri, G. Calapai, S. Gangemi and M. Navarra (2016). Chemopreventive agents and inhibitors of cancer hallmarks: may Citrus offer new perspectives? *Nutrients,* **8(11):** 698.

de Diego-Otero, Y., Y. Romero-Zerbo, R. el Bekay, J. Decara, L. Sanchez, F.F. Rodriguez-de and I. del Arco-Herrera (2009). alpha-Tocopherol Protects Against Oxidative Stress in the Fragile X Knockout Mouse: An Experimental Therapeutic Approach for the Fmr1 Deficiency. *Neuropsychopharmacology,* **34:** 1011-1026.

Dirar, A.I., A. Adhikari-Devkota, M.M. Hassan, M. Wada, T. Watanabe and H.P. Devkota (2019). Phenolic compounds as potent free radical scavenging and enzyme inhibitory components from the leaves of *Guiera senegalensis*. *Nat. Prod. Commu*., **14(6):**1934578X19857364. Doi: 10.1177/1934578X19857364.

El-Ishaq, A. and S. Obirinakem (2015). Effect of temperature and storage on vitamin C content in fruits juice. *Int. J. Chem. Biomol. Sci.,* **1(2):**17-21.

Guillaumet-Adkins, A., Y. Yañez, M.D. Peris-Diaz, I. Calabria, C. Palanca-Ballester and J. Sandoval (2017). Epigenetics and Oxidative Stress in Aging. *Oxid. Med. Cell. Longev*., **2017:** 9175806. doi:10.1155/2017/9175806.

Heinze, P.H., S.K. Margaret, B.L. Wade and R.L. Foster (1944). Ascorbic acid content of 39 varieties of snap beans. *Food Res.,* **9:** 19-26.

Horubala, A. (1999). Antioxidant Capacity and their changes In Fruit and Vegetables Processing. *Food Technology Symposium, Przem. Ferm. Owoc. Warz.,* **3:** 30-31.

Ide, N. and B.H. Lau (1997). Garlic Compounds Protect Vascular Endothelial Cells from Oxidized Low Density Lipoprotein-induced Injury. *J. Pharm. Pharmacol.,* **49(9):** 908-911. Doi: 10.1111/j.2042-7158.1997.tb06134.x.

Jadoon, S., S. Karim, M.H. Asad, M.R. Akram, A. Kalsoom Khan, A. Malik, C. Chen and G. Murtaza (2015). Anti-aging potential of phytoextract loaded-pharmaceutical creams for human skin cell longetivity. *Oxi. Med. Cellular longevity*., **2015:** 1-17. https://doi.org/10.1155/2015/709628.

Keskin-Šašić, I., I. Tahirović, A. Topèagiæ, L. Klepo, M. Salihović, S. Ibragić, J. Toromanović, A. Ajanović and E.

Velispahić (2012). Total phenolic content and antioxidant capacity of fruit juices. *Glas. Hem. Technol. Bosne. Herceg*., **39:** 25-28.

Khosravi, F. and H. Asadollahzadeh (2014). Determination of ascorbic acid in different citrus fruits under reversed phase conditions with UPLC. *Euro. J. Exp. Bio.,* **4(1):** 91-94.

Kole, P.I., H.R. Jadhav, P. Thakurdesai and A.N. Nagappa (2005). Cosmetics potential of herbal extracts. *Nat. Prod. Rad.,* **4(4):** 315-321.

Kurutas, E.B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/ nitrosative stress: current state. *Nutr. J.,* **15(1):** 71. https://doi.org/10.1186/s12937-016-0186-5.

Liebler, D.C. and T.D. McClure (1996). Antioxidant reactions of β-carotene: identification of carotenoid" radical adducts. *Chem. Res. Toxicol.,* **9:** 8-11. Doi: 10.1021/tx950151t.

Manach, C., A. Scalbert, C. Morand, C. Rémésy and L. Jiménez (2004). Polyphenols: food sources and bioavailability. *Am. J. Clinic. Nutr.,* **79(5):** 727-747. DOI: https://doi.org/10.1093/ajcn/79.5.727.

Martinsen, B.K., K. Aaby and G. Skrede (2020). Effect of temperature on stability of anthocyanins, ascorbic acid and color in strawberry and raspberry jams. *Food Chem.,* **316:** 126297. DOI: 10.1016/j.foodchem.2020.126297.

Mennah-Govela, Y.A. and G.M. Bornhorst (2017). Fresh-squeezed orange juice properties before and during *In Vitro* digestion as influenced by orange variety and processing method. *J. Food Sci.,* **82(10):** 2438-2447. Doi: 10.1111/1750-3841.13842.

Najafabad, A.M. and R. Jamei (2014). Free radical scavenging capacity and antioxidant activity of methanolic and ethanolicextracts of plum (*Prunus domestica* L.) in both fresh and dried samples. *Avicenna J. Phytomed*., **4:** 343-353.

Nawar, W.W. (1996). Lipids. In: Food Chemistry, Fennema R.O. (ed.)., Marcel Dekker Inc., New York, 225-320.

Nicola, S., G. Tibaldi and E. Fontana (2009). Tomato production systems and their application to the tropics. Proc. IS on tomato in the tropics. *Acta Hortic.,* **821:** 27-33.

Patel, S.A.H. and P. Thakur (2017). Anti-cancer: A vegetable prospective. *Kisan world*. 33-34.

Pavlović, N.V., J.D. Mladenović, N.M. Zdravković, D.Z. Moravèević, D. Ž. Poštić and J.M. Zdravković (2019). Effect of tomato juice storage on vitamin C and phenolic compounds and their stability over one-year period. *Bulg. Chem. Commun.,* **51(3):** 400-405. DOI: 10.34049/bcc.51.3.5088.

Pollack, S.L., B.H. Lin and J.E. Allshouse (2003). Characteristics of US orange consumption. US Department of Agriculture, Economic Research Service.

Raffo, A., C. Leonari, V. Fogliano, P. Ambrosino, M. Salucci, L. Gennaro, R. Bugianesi, F. Giuffrida and G. Qualgia (2002). Nutritional value of cherry tomatoes (*Lycopersicon esculantum* cv. Naomi F_1) harvested at different ripening stages. *J. Agri. Food Chem.,* **50:** 6550-6556.

Rajalakshmi, D. and S. Narasimhan (1996). Food antioxidants: sources and methods of evaluation. In: Food Antioxidants: Technological, Toxicological and Health Perspectives. Marcel Dekker Inc., New York, 65 -157.

Saravanan, G. and J. Prakash (2004). Effect of garlic (*Allium sativum*) on lipid peroxidation in experimental myocardial infarction in rats. *J. Ethnopharmacol.,* **94(1):** 155-158. Doi: 10.1016/j.jep.2004.04.029.

Sharma, A., M. Kaur, J.K. Katnoria and A.K. Nagpal (2018). Polyphenols in food: cancer prevention and apoptosis induction. *Curr. Med. Chem.,* **25(36)**: 4740-4757. Doi: 10.2174/0929867324666171006144208.

Sharma, S., R. Mahajan and K.L. Bajaj (1996). Biochemical evaluation of some tomato varieties. *Veg. Sci.,* **23(1):** 42-47.

Silva, A.F.D., B. Silva, A.S.B.D. Sousa, V. Figueiredo, M.A.R.I.A. De Aquino, R. Mendonça, M. Nunes and S.D.M. Silva (2019). Quality, bioactive compounds and antioxidant activity during maturation of oranges produced in the borborema territory. *Rev. Caatinga.,* **32(2):** 526-536.

Singh, B., J.P. Singh, A. Kaur and N. Singh (2016). Bioactive compounds in banana and their associated health benefits-A review. *Food Chem.,* **206:** 1-11. Doi: 10.1016/j.foodchem.2016.03.033.

Singleton, V.L. and J.A. Rossi (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.,* **16:** 144-158.

Spreen, T. (2010). Projections of World Production and Consumption of Citrus to 2010, Food and Agriculture Organisation of the United Nations (FAO) website. Economic and Social Department. Available at http://www.fao.org.

Thimmaiah, S.K. (1999). Lipids and fats. Standard Methods of Biochemical Analysis. 130-150.

Waheed, K., H. Nawaz, M.A. Hanif and R. Rehman (2020). Tomato. In: Medicinal Plants of South Asia, Elsevier. 631-644.

Watanabe, K., T. Saito, S. Hirota, B. Takahashi and N. Fujishita (1991). Carotenoid pigments in orange, light orange, green and white flesh colored fruits of melon (*Cucumis melo* L.). *J. Jpn. Soc. Food. Sci. Tech.,* **38:** 153-159.

Willet, W.C. (2002). Balancing life-style and genomics research for disease prevention. *Science,* **296:** 695-698.

Yadav, A.S. and D. Bhatnagar (2007). Free radical scavenging activity, metal chelation and antioxidant power of some of the Indian spices. *Biofactors.,* **31:** 219-227. Doi: 10.1002/biof.5520310309.

Yoo, K.M., K.W. Lee, J.B. Park and I.K. Hwang (2004). Variation in major antioxidants and total antioxidant activity of Yuzu (*Citrus junos* Sieb ex Tanaka) during maturation and between cultivars. *J. Agric. Food. Chem.,* **52(19):** 5907-5913.

Ziegler, R.G., S.T. Mayne and C.A. Swanson (1996). Nutrition and lung cancer. *Cancer Causes Control.,* **7:** 157-177.

Zuo, Y., C. Wang and H. Zhang (2002). Separation, characterization and quantitation of benzoic and phenolic antioxidant in American Cranberry fruit by GC-MS. *J.*