



ISSN (E): 2277-7695
 ISSN (P): 2349-8242
 NAAS Rating: 5.23
 TPI 2023; 12(4): 940-944
 © 2023 TPI
www.thepharmajournal.com
 Received: 07-01-2023
 Accepted: 11-02-2023

Priti Kumari
 Department of Horticulture
 (Fruit & Fruit Technology),
 BAU, Sabour, Bhagalpur, Bihar,
 India

Pawan Kumar
 Department of Horticulture
 (Fruit & Fruit Technology),
 BAU, Sabour, Bhagalpur, Bihar,
 India

Kumari Karuna
 Department of Horticulture
 (Fruit & Fruit Technology),
 BAU, Sabour, Bhagalpur, Bihar,
 India

Ahmar Aftab
 Department of Food Science &
 Post Harvest Technology, BAU,
 Sabour, Bhagalpur, Bihar, India

Anand Kumar
 Department of Plant Breeding
 and Genetics, BAU, Sabour,
 Bhagalpur, Bihar, India

Feza Ahmad
 Department of Horticulture,
 Fruit & Fruit Technology, BAU,
 Sabour, Bhagalpur, Bihar, India

Corresponding Author:
Kumari Karuna
 Department of Horticulture,
 Fruit & Fruit Technology, BAU,
 Sabour, Bhagalpur, Bihar, India

Effect of pre-harvest application of salicylic acid on yield and physico-chemical parameters of litchi (*Litchi chinensis* Sonn.) cv. China

Priti Kumari, Pawan Kumar, Kumari Karuna, Ahmar Aftab, Anand Kumar and Feza Ahmad

Abstract

An experiment was conducted on litchi cv. China at Horticulture garden in the Department of Horticulture (Fruit and Fruit Technology), Bihar Agricultural University, Sabour, Bhagalpur, Bihar, to study the effect of pre-harvest spray of salicylic acid on yield and physico-chemical parameters of litchi. The experiment was laid out in randomized block design with five treatments and four replications. Salicylic acid was sprayed twice i.e. 15 days and 30 days before anticipated harvesting time with different concentrations of salicylic acid (T₁- 50 ppm, T₂-100 ppm, T₃-150 ppm, T₄-200 ppm and T₅-control). Different concentrations have different effect on physical and biochemical parameters. Salicylic acid with T₃-150 ppm has highest fruit length (3.46 cm), breadth (3.32 cm), volume (19.22 ml), weight (21.12 g), pulp (68 %), no. of fruits (4274 fruits/plant) and yield (71.25 kg/plant), TSS (20.02 °Brix), total sugar (13.10 %), ascorbic acid (38.21 mg/100 g pulp). Highest antioxidant (4.84 μ mol Trolox Eq./g pulp) and anthocyanin (21.54 mg/100 g pulp) was observed in T₄-200 ppm salicylic acid.

Keywords: Litchi, salicylic acid, physico-chemical parameters, yield

Introduction

As India has such a diverse climate, it is ideal for cultivation of different varieties of litchi. Litchi (*Litchi chinensis* Sonn.) belongs to sapindaceae family. It is native of Southern China. Its chromosome no. 2n=30 and type of fruit is single seeded nut and its edible part is fleshy aril. It is highly cross-pollinated crop. Its name is derived from Chinese word "lee chee" which means "one who gives the pleasure of life". It is grown in tropical and sub-tropical regions. Due to its bright red peel colour and pleasant flavoured juicy aril this fruit is highly valued in national and international markets. Litchi fruit is very much liked as a table fruit as well as in dried and canned forms. Jam, jelly, squash and cordial are also prepared from this fruit. It is very nutritious and good source of vitamin C (64 mg/100 g pulp). It also contains vitamin E, B complex and trace amount of protein (0.7%), fat (0.3%) and minerals like phosphorous, calcium and iron. It is non climacteric type fruit; it does not ripe after harvesting from plants so it is harvested at ripe stage. Litchi is grown in different countries like China, West Indies, Myanmar, Japan, South Africa, Florida, Hawaii, etc. in the world; India is one among them. China is first and India is second largest producer of litchi fruits in the world. Litchi is very perishable in nature and it has very short shelf life. After harvesting, the fruit turn brown within a couple of days due to loss of water from the pericarp. (Panwar *et al.* 2018) [23] reported that salicylic acid decreases respiration rate by the closure of stomata. Salicylic acid (SA) is a natural plant hormone and it acts as signaling molecule. It makes the plants resistance against biotic and abiotic stress (Khan *et al.* 2012) [15]. SA helps in plant growth, ion uptake and nutrient transport in plants. It is phenolic compound, and in plants it provides systemic resistance to fungal pathogens (Meena *et al.* 2014) [18]. Pre harvest application of various chemicals have been reported to enhance the shelf life of fruits by reducing physiological loss in weight and decay losses during storage (Gupta and Metha, 1988) [8].

Materials and Methods

The present investigation was carried out at the Horticulture Garden under the Department of Horticulture (Fruit & Fruit Technology), Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur during the year of 2021-2022 with a view to study the pre-harvest application of salicylic acid on yield and physico-chemical parameters of litchi.

Litchi plants of uniform age, spread and bearing behaviour were selected for experiment purpose. Variety used for this experiment was "China". The experiment was laid out with five treatments (T₁-50 ppm SA, T₂- 100 ppm SA, T₃- 150 ppm SA, T₄- 200 ppm SA and T₅- control) and four replications in randomized block design. Fruits taken for analysis were of uniform size and free from disease, pest and injuries.

Physical parameters such as length and breadth were measured by digital vernier calipers, volume by water displacement method, fruit weight, pulp weight, stone weight were measured with the help of electronic weighing balance, specific gravity was calculated as per standard methods whereas number of fruits were counted manually and total weight of the marketable fruits per tree was taken using pan balance of 5 kg capacity. Biochemical parameters namely titrable acidity (%) was calculated by the given titration method (AOAC, 2000) [31], ascorbic acidity (mg/100 g pulp) was estimated by using 2-6-dichlorophenol indophenols dye method (Jones and Hughes, 1983) [32], TSS (°Brix) was recorded with the help of digital hand refractometer, TSS: Acid ratio was calculated by dividing TSS with titrable acidity, total sugar (%) was estimate by Lane and Eyon (1923) [33] copper titration method, anthocyanin (mg/100 g pulp) was calculated by peel pH- differential method (Wrolstad *et al.* 2005) [34] using two different buffer systems: potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.4 M, pH 4.5), antioxidant (μ mol Trolox Eq. /g pulp) was determined by cupric reducing antioxidant capacity (CUPRAC) method (Apak *et al.* 2008) [35].

Statistical analysis

The experiment was laid out in RBD. Statistical analysis was performed in two-way factorial RBD method. Least significant difference was calculated following significant F-test ($p \leq 0.05$). Effect of different treatments on various parameters and their interactions were assessed with ANOVA. Standard errors were computed by MS-Excel. This RBD design was adopted as suggested by Panse and Sukhatme (1967) [22].

Result and Discussion

Physical parameters

Fruit length (cm): Highest (3.46 cm) fruit length was found in plants treated with T₃- 150 ppm salicylic acid. It may be due to increased nutrition and rapid cell division and enlargement caused by the presence of growth substances. This cell division gives way to cell enlargement which enhanced different fruit quality such as length and breadth (Malik and Singh, 2004) [16].

Fruit breadth (cm)

It was revealed that maximum (3.32 cm) fruit breadth was recorded in T₃-150 ppm salicylic acid. Reason for maximum fruit breadth may be due to increased nutrition and rapid cell division and enlargement caused by the presence of growth substances. SA showed in enhancing the productivity of fruits by increasing total leaf area and photosynthesis (Hayat *et al.*, 2010) [10]. These results were supported by (Kassem *et al.*, 2011) [36].

Fruit volume (ml)

Among all the treatments fruit volume was found significantly different from each other. Maximum (19.22 ml) volume was

found in T₃- 150 ppm salicylic acid. It was due to rapid cell division and development of fruits as their initial requirements was met with foliar application of nutrients. Similar results were also observed by (Roychoudhury *et al.* 1992) [26], (Haq *et al.*, 2013) [9] in litchi.

Fruit weight (g)

There was significant variation in fruit weight among different treatments. The highest (21.12 g) fruit weight was found in T₃-150 ppm salicylic acid. Maximum fruit weight was recorded in those fruits which were treated with T₃-150 ppm salicylic acid over control. It was due to rapid cell division and development of fruits as their initial requirements was met with foliar application of nutrients. For weight increment similar results were obtained by (Singh and Tiwari, 1994) [30] in peach and (Meena *et al.* 2014) [18] in aonla.

Pulp (%)

Salicylic acid affected pulp percentage significantly different with different dose. Pulp percentage was maximum (68.00%) in T₃-150 ppm salicylic acid treated fruits. Pre-harvest spray of salicylic acid influenced fruit pulp. Similar results were revealed by Rani and Brahmachari, (2001) [24] in litchi cv. China.

Peel (%)

Peel percentage was significantly reduced (15.83 %) in T₄- 200 ppm salicylic acid treated fruits. Pre-harvest spray of salicylic acid influenced fruit peel. Similar results were revealed by Rani and Brahmachari, (2001) [24] in litchi cv. China.

Stone (%)

Among all the treatments stone percentage of litchi fruits differed from each other. It was significantly high (20.29%) in T₅-control and minimum (16.97 %) in T₄- 200 ppm salicylic acid treated fruits. Pre-harvest spray of salicylic acid influenced stone percentage of litchi. Similar results were revealed by Rani and Brahmachari, (2001) [24] in litchi cv. China.

Specific gravity of fruit (g/cm³)

Specific gravity (1.07 g/cm³) was found in control whereas (1.09 g/cm³) found in T₃-150 ppm salicylic acid treated fruits, treatment T₃ was recorded significantly higher specific gravity of the fruits over control and rest of the treatments were at par with control, hence this data is non-significant. Similar effects were also reported by Dutta, (2004) in guava.

Yield per tree (kg)

It was recorded that maximum (71.25 kg) fruit was found in T₃-150 ppm salicylic acid treated plants. These results were in congruent with (Metwally *et al.* 2013) [37] in strawberry. The positive effect of SA on yield may be due to its effect on plant hormones (Shakirova, 2007) [29].

Biochemical Parameters

Titrable acidity (%): The acidity of litchi fruits was significantly affected by salicylic acid of different doses. It was revealed that titrable acidity was the highest in T₅-control (0.760 %) while lowest (0.440) in T₃-150 ppm. It is a fact that taste is mainly made up of sugars and acids combination. It was found that titrable acidity in fruits was increased due to

break down of sugars to acids during respiration (Ball, 1997) [4].

Ascorbic acid (mg/100 g)

Highest (38.21 mg/100 g) ascorbic acid content was recorded in fresh litchi fruits in T₃-150 ppm salicylic acid concentration while lowest (33.01 mg/100 g) in T₅-control. SA treated peach fruits maintained higher ascorbic acid content as compared with control. It is very sensitive to degradation ascribable to its oxidation in comparison to other nutrients during processing and storage (Akhtar *et al.* 2010) [1]. It has been revealed that SA had helped to maintain higher ascorbic acid in peach fruits. (Kalarani *et al.* 2002) [12] reported that tomato fruits treated with SA were observed with highest ascorbic acid content.

TSS (°Brix)

TSS of fruits was significantly affected by foliar application of different treatments of salicylic acid. The highest (20.02 °Brix) TSS was found in T₃-150 ppm salicylic acid whereas lowest (18.75 °Brix) was in T₅-control. Foliar application of SA had a significant effect on TSS of fruits. The results indicated that all the treatments increased the TSS compared to control. In some non-climacteric fruits such as pomegranate (Sayyari *et al.* 2009) [27] and grape (Ranjbaran *et al.* 2011) [25] non-significant effect of SA on TSS in fruits during cold storage have been reported.

TSS: acid ratio

Among all the treatments TSS: Acid ratio was found significantly different from each other. Highest (45.52) TSS: Acid ratio was found in T₃-150 ppm salicylic acid treated fruits while lowest (24.67) was recorded in T₅-control. TSS: acid ratio is considered to be the main feature affecting the taste of fruits (Sen *et al.* 2016) [28]. Highest TSS: Acid ratio of T₃-150 ppm SA treated fruits might be due to highest soluble solids due to delaying metabolic activities of fruits during ripening and lowest acidity due to increased respiration rate and ethylene biosynthesis and more utilization of acids in biochemical activities leading to depletion of organic acids. SA suppressed rate of respiration and ethylene biosynthesis (Bablar *et al.* 2007) [27].

Total sugar (%)

It was shown that highest (13.10 %) total sugar was present with T₃-150 ppm salicylic acid treatment and lowest (10.84 %) was recorded in T₅-control. Foliar application of SA influenced total sugar of fruits. Polysaccharides converted into water soluble sugar might be a reason for an increase in the sugar content. Matto *et al.* (1975) [17] indicated that starch is completely hydrolyzed into soluble sugars such as glucose, fructose and sucrose during ripening process

Anthocyanin of peel (mg /100 g pulp)

Highest (21.54 mg/100 g pulp) anthocyanin was observed in T₄-200 ppm while it was recorded lowest (18.72 mg/100 g pulp) in T₅-control. There was no significant difference in anthocyanin values among the different treatments of SA used. The results of this study correspond to the findings of

(Huang *et al.* 2008) [11] in orange. (Obinata *et al.* 2003) [21] reported that SA could markedly increase the production of procyanidin in grape. Salicylic acid increased the synthesis of plant hormone, enzymes and photosynthesis and thus accumulates pigments such as carotenoids and anthocyanin (Capitani *et al.* 2005) [5].

Antioxidant capacity (µmol Trolox equivalent/g pulp)

In case of antioxidant capacity, it was recorded that highest (4.84 µmol TE eq./g FW) antioxidant was present in T₄-200 ppm concentration of salicylic acid whereas lowest (4.48 µmol TE eq./g FW) antioxidant was recorded in T₅-control. Pre harvest application of salicylic acid affected the antioxidant of fruits. The antioxidant activity was low in the untreated fruits in comparison to SA treated fruits, which might be due to lower retention of ascorbic acid content in untreated fruits. This effect of salicylic acid treatment was due to maintained total phenolics and ascorbic acid (Davarynejad *et al.* 2015) [6].

Table 1: Effect of pre-harvest spray of salicylic acid on physical parameters of litchi cv. China

Treatment	Fruit length (cm)	Fruit breadth (cm)	Fruit volume (ml)	Fruit weight (g)
T ₁ -SA 50 ppm	3.19	2.85	17.38	18.92
T ₂ -SA 100 ppm	3.21	2.94	18.55	20.15
T ₃ -SA 150 ppm	3.46	3.32	19.22	21.12
T ₄ -SA 200 ppm	3.16	3.17	19.08	20.90
T ₅ -Control	2.74	2.76	17.18	18.53
Sem(±)	0.11	0.10	0.51	0.75
CD (p≤0.05)	0.34	0.30	1.56	1.75
CV (%)	7.00	6.52	5.53	5.70

Table 2: Effect of pre-harvest spray of salicylic acid on other physical attributes of litchi cv. China

Treatment	Pulp (%)	Peel (%)	Stone (%)	Specific gravity (g/cm ³)
T ₁ -SA 50 ppm	64.28	16.02	18.01	1.08
T ₂ -SA 100 ppm	66.40	16.35	17.20	1.08
T ₃ -SA 150 ppm	68.00	16.11	17.32	1.09
T ₄ -SA 200 ppm	67.19	15.83	16.97	1.09
T ₅ -Control	61.40	18.81	20.29	1.07
Sem(±)	1.48	0.46	0.48	0.03
CD (p≤0.05)	4.54	1.40	1.49	0.09
CV (%)	4.51	1.00	5.38	5.37

Table 3: Effect of pre-harvest spray of salicylic acid on yield of litchi fruits cv. China

Treatment	Yield (kg/plant)
T ₁ -SA 50 ppm	62.40
T ₂ -SA 100 ppm	67.75
T ₃ -SA 150 ppm	71.25
T ₄ -SA 200 ppm	69.00
T ₅ -Control	58.85
Sem(±)	2.15
CD (p≤0.05)	6.61
CV (%)	6.52

Table 4: Effect of pre-harvest spray of salicylic acid on biochemical parameters of litchi cv. China

Treatments	Titration acidity (%)	Ascorbic acid (mg/100 g pulp)	TSS (°Brix)	TSS : Acid ratio	Total sugar (%)	Anthocyanin (mg/100 g pulp)	Antioxidant Capacity (µ mol Trolox Eq. / g pulp)
T ₁ -SA 50 ppm	0.605	34.86	19.21	31.77	11.66	19.54	4.53
T ₂ -SA 100 ppm	0.580	35.44	19.56	33.72	12.75	19.96	4.59
T ₃ -SA 150 ppm	0.440	38.21	20.02	45.52	13.10	20.88	4.71
T ₄ -SA 200 ppm	0.520	36.15	19.85	38.17	12.86	21.54	4.84
T ₅ -Control	0.760	33.01	18.75	24.67	10.84	18.72	4.48
Sem(±)	0.014	0.71	0.32	0.49	0.36	0.41	0.06
CD ($p \leq 0.05$)	0.043	2.18	1.00	1.50	1.11	1.26	0.20
CV(%)	4.790	3.98	3.32	2.81	5.88	4.05	2.79

Conclusion

From the above findings it may be concluded that pre-harvest spray of salicylic acid on litchi plant can enhance the yield and physico-chemical properties of fruits. Salicylic acid spray on plants with 150 ppm concentration improved the physical parameters such as fruit length, breadth, fruit weight and pulp in litchi fruits and biochemical parameters like titration acidity, ascorbic acidity, TSS, TSS: Acid ratio and total sugar. Treatment T₄-200 ppm concentration performed better in case of anthocyanin and antioxidant. On the basis of above findings it may be concluded that the concentration of 150 ppm salicylic was the best among all treatments.

References

- Akhtar A, Abbasi NA, Hussain A. Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage. *Pakistan Journal Botany*. 2010;42(1):181-188.
- Meena B, Marimutha T, Velazhan R. Salicylic acid induce systematic resistance in groundnut against late leaf spot caused by (*Cercosporidium personatum*). *J Mycol. Plant Pathol*. 2001;31:139-145.
- Babler M, Asghari M, Talaei A, Khosroshahi A. Effect of pre harvest and post-harvest salicylic acid treatment on ethylene production, fungal decay and overall quality of selva strawberry fruit. *Food Chemistry*. 2007;105(2):449-453.
- Ball JA. Evaluation of two lipid based edible coating for their ability to preserve post-harvest quality of green bell peppers. Master Diss., Faculty of the Virginia Polytechnic Institute and State University. Blacksburg, Virginia, USA. 1997.
- Capitani F, Biondi S, Falasca G, Ziosi V, Balestrazzi A, Carbonera D, et al. Methyl jasmonate disrupts shoot formation in tobacco thin cell layers by over inducing mitotic activity and cell expansion. *Planta*. 2005;220:507-519.
- Davarynejad HG, Zarei M, Nasrabadi ME, Ardakani E. Effects of salicylic acid and putrescine on storability, quality attributes and antioxidant activity of plum cv. Santa Rosa. *Journal Food Science Technology*. 2015;52:2053-2062.
- Dutta P. Foliar application spray in improving the quality of Sardar guava (*Psidium guajava* L.). *Orissa Journal Horticulture*. 2004;32(1):153-156.
- Gupta OP, Metha N. Effect of pre harvest applications on the shelf life of ber (*Zizyphus mauritiana* Lamk) fruits cv. Gola. Haryana. *J Hort. Sci*. 1988;17:183-189.
- Haq I, Rab A, Sajid M. Foliar application of calcium chloride and borax enhance the fruit quality of litchi cultivars. *Journal of Animal and Plant Science*. 2013;23:1385-1390.
- Hayat Q, Hayat S, Irtan M, Ahmad AA. Effect of exogenous salicylic acid under changing environment. A review *Environmental and Experimental Botany*. 2010;68:14-25.
- Huang R, Xia R, Y Lu, Hu L, Xu Y. Effect of pre harvest salicylic acid spray treatment on post-harvest antioxidant in the pulp and peel of Cara caranavel orange (*Citrus sinensis* L. Osbeck). *Journal Science Food Agriculture*. 2008;88:229-236.
- Kalarani MK, Thangaraj M, Shivakumar S, Mallika R. Effects of salicylic acid on tomato (*Lycopersicon esculentum* Mill) Productivity. *Crop Research (Hisar)*. 2002;23:486-492.
- Karlidge H, Yildirim E, Tarun M. Exogenous application of salicylic acid affect quality and yield of strawberry growth under antifrost heated greenhouse conditions. *Journal of Plant Nutrition and Soil Science*. 2009;172:270-276.
- Kaseem HA, Al-Obeed RS, Ahmed MA, Omar AKH. Productivity, Fruit quality and profitability of jujube trees improvement by pre harvest application of agro-chemicals. *Middle-East Journal Science Research*. 2011;9(5):628-637.
- Khan N, Nazar R, Iqbal N, Anjum NA. *Phytohormones and Abiotic Stress Tolerance in Plants*. Springer, Berlin, Heidelberg; c2012.
- Malik A, Singh U, Singh Z. Endogenous free polyamines of mangoes in relation to development and ripening. *Journal American Society Horticulture Science*. 2004;129:280-286.
- Matto AK, Murata T, Pantastico EB, Ogata KK, Phan CT. Chemical changes during ripening and senescence, in postharvest physiology, handling and utilization of tropical and sub-tropical fruit and vegetables, AVI Publishing, Westport, CT; c1975. p. 13-15.
- Meena D, Tiwari R, Singh OP. Effect of nutrient spray on growth, fruit yield and quality of aonla. *Annals Plant and Soil Research*. 2014;16:242-245.
- Metwaaly AA, Youssef SM, EI-Miniawy SM, Ragab ME. Effect of foliar spraying of salicylic acid on growth, yield quality of cold stored strawberry fruits. *Journal Biochemistry Environment Science*. 2013;8:1-17.
- Muneshwar P, Manorama M, Kumar R, Das B. Effect of mulching and plant growth regulators on growth, yield and economics of strawberry cv. Douglas. *Journal Interacad*. 2012;16(1):44-55.
- Obinata N, Yamakawa T, Takamiya M, Tanaka N, Ishimaru K, Kodama T. Effects of salicylic acid on the

- production of procyanidin and anthocyanin in cultured grape cells. *Plant Biotechnology*. 2003;20:105-111.
22. Panse VG, Sukhatme PV. Statistical methods for agricultural workers, Indian Council of Agricultural Research, New Delhi; c1967.
 23. Panwar N, Rai PN, Kumar J, Shankar D, Singh MDP. Effect of different chemicals on litchi (*Litchi chinensis* Sonn.) cv. rose scented. *Journal of Pharmacognosy and Phytochemistry*. 2018;7(4):1418-1422.
 24. Rani R, Brahmachari. Effect of plant bioregulators on cracking and physico-chemical composition on fruit in China litchi. *Progressive Horticulture*. 2001;33(1):32-36.
 25. Ranjbaran E, Sarikhani H, Wakana A, Bakhshi D. Effect of salicylic acid on storage life and postharvest quality of grape (*Vitis vinifera* L. cv. 'Bidaneh Sefid'). *Journal Faculty of Agriculture*. 2011;56:263-269.
 26. Roychoudhury R, Kabir J, Ray S, Dutta K, Dhua RS. Effect of calcium on fruit quality of litchi. *International Journal Horticulture*. 1992;49:27-30.
 27. Sayyari M, Bablar M, Kalantari S, Serrano M, Valero D. Effect of salicylic acid treatment on reducing chilling injury in stored pomegranates. *Post-harvest Biology Technology*. 2009;53:152-154.
 28. Sen F, Oksar R, Kesgin M. Effects of shading and covering on "Sultana Seedless" grape quality and storability. *Journal of Agricultural Science and Technology*. 2016;18:245-254.
 29. Shakirova FM. Role of hormonal system in the manifestation of growth promoting and antistress action of salicylic acid. In S. Hayat and A. Ahamad (Eds), *Salicylic acid A plant hormone*. Dordrecht, Netherland: Springer. 2007.
 30. Singh J, Tiwari JP. Effect of ethephon on the post-harvest quality of guava (*Psidium guajava* L.) cv. Sardar. *Progressive Horticulture*. 1994;26:189-193.
 31. [AOAC] Assn. of Official Analytical Chemists. Coffee and tea. In: *Official methods of analysis*. 17th ed. Gaithersburg, Md.: AOAC; c2000.
 32. Tree M, Atrash B, Donovan B, Hallett A, Hughes M, Jones DM, *et al.* New inhibitors of human renin tested in vitro and in vivo in the anaesthetized baboon. *Journal of Hypertension*. 1983 Dec 1;1(4):399-403.
 33. Lane JH, Eynon L. Methods for determination of reducing and nonreducing sugars. *Journal of Science*. 1923;42:32-37.
 34. Wrolstad RE, Durst RW, Lee J. Tracking color and pigment changes in anthocyanin products. *Trends in Food Science & Technology*. 2005 Sep 1;16(9):423-428.
 35. Apak R, Güçlü K, Özyürek M, Celik SE. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchimica Acta*. 2008 Apr 1;160(4).
 36. Eskildsen T, Taipaleenmäki H, Stenvang J, Abdallah BM, Ditzel N, Kassem M. MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. *Proceedings of the National Academy of Sciences*. 2011 Apr 12;108(15):6139-6144.
 37. El-Metwally S, Hamza T, Zakaria M, Helmy M. Next-generation sequence assembly: four stages of data processing and computational challenges. *PLoS computational biology*. 2013 Dec 12;9(12):e1003345.