Comparative Antioxidant Study of Different Fruits and Vegetables Commonly Consumed in Odisha, India

Sahoo SK¹, Gangopadhyay A¹, Kar D², Bhuyan R², Bose A¹*

¹School of Pharmaceutical Sciences, Siksha O Anusandhan (Deemed to be University), Kalinga Nagar, Bhubaneswar, Odisha-751003, India; ²IMS & SUM Hospital, Siksha O Anusandhan (Deemed to be University), Kalinga Nagar, Bhubaneswar, Odisha-751003, India.

ABSTRACT

Introduction: Reactive oxygen species produced in our body may result in the pathogenesis of different degenerative diseases like atherosclerosis, carcinogenesis, neurodegenerative diseases, ageing etc. Antioxidant-rich foods can protect us by neutralizing these free radicals reducing.

Objective: This study determined the total flavonoid and phenolic content of various fruit and vegetable samples collected from a different region of Bhubaneswar, India.

Methods: The methanolic extract of twelve fruits and vegetables were evaluated for their total phenolic content, total flavonoid content and antioxidant activity by DPPH scavenging assay.

Results: The results showed high flavonoid and phenolic contents present in all the fruits and vegetable samples tested. These samples also exerted good antioxidant activity in the DPPH scavenging assay. There was a clear positive correlation between total flavonoid and phenolic content and a negative correlation between total flavonoid and phenolic content with IC50 value of DPPH radical scavenging assay based on the results.

Conclusion: This work might be helpful to local consumers and nutritionists of the covered locality in selecting proper fruits and vegetables in their daily diet.

Key Words: Oxidation, Diet, Flavonoid, Phenolic content, DPPH

INTRODUCTION

Reactive oxygen species (ROS) are produced in our body naturally as a byproduct of metabolism or by exposure to toxins.¹ Our body’s natural antioxidant system always remains vigilant to keep their amount in control.² However, when ROS are produced in excess and not eliminated by the internal antioxidant system, they result in pathogenesis of different degenerative diseases like atherosclerosis, carcinogenesis, neurodegenerative diseases, including Parkinson’s and Alzheimer’s diseases, ageing etc.³,⁴ Previous studies have consistently shown that consumption of fruits and vegetables leads to prevention of many degenerative diseases due to presence of phytoconstituents like flavonoids and related phenolic compounds.⁵ Synthetic antioxidants reduce oxidation but have various adverse reactions.⁶ On the other hand, natural antioxidant-rich foods neutralize free radicals reducing the damage. Hence nowadays scientists recommend the inclusion of antioxidant-rich foods in our normal diet. The objective of this study was to analyze a comparative study of anti-oxidant models and determine the total flavonoid and phenolic content of extract of various fruit and vegetable samples collected from a different region of Bhubaneswar, India. The results of this study will guide the people of this region in the selection of the best vegetables and fruits for inclusion in their daily food habits.

MATERIALS & METHODS

Materials

Fruit and vegetable samples such as Orange (Citrus × Sinensis), Banana (Musa acuminate), Apple (Podophyllum pelletatum), Grapes (Vitis vinifera), Pomegranate (Punica granatum), Amla (Phyllanthus Emblica), Lemon (Citrus limon),
Capsicum (Capsicum annuum), Tomato (Solanum Lycopersicum), Karela (Momordica charantia), Green chilli (Capsicum annuum) and Carrot (Daucus carota) were randomly collected from local market of Bhubaneswar during December 2018 were store in a clean dry sterile bowl in the refrigerator until they were analyzed.

**Extract preparation**

Fruits and Vegetables were cleaned with distilled water. Blended dried samples were mixed with methanol (1:1) properly through agitation by magnetic stirrer in low rpm for 30 min at room temperature. The total mixture was filtered by a clean white cotton cloth. Refiltration was done by Whatman filter paper. Finally, a concentration of 0.5gm/ml for each extract was achieved by dilution with the solvent. 7

**Total flavonoid content determination (TFC)**

50 µg/ml concentration of each sample was prepared separately from the stock solutions. 1ml 2% ammonium chloride was added to each solution. Then total solutions were mixed properly by sonication and the absorbance was recorded at 434 nm using a UV-Visible spectrophotometer. For estimating the flavonoid content, a standard curve of quercetin in the concentration range of 20-50 µg/ml was prepared. Total flavonoids were expressed as quercetin equivalents (µg) per 100gram of extract. 8

**Total phenolic content determination (TPC)**

50 µg/ml concentration of each sample was prepared separately from 1mg/ml stock solution and mixed with 1.0 ml of Folin Ciocalteu reagent and 2.0 ml of 20% w/v sodium carbonate solution. Absorbance was recorded at 641 nm using a UV-Visible spectrophotometer. A standard curve of gallic acid was constructed in the concentration range of 50-250 µg/ml. TPC was expressed as gallic acid equivalents (µg) per 100gram of extract. 8

**In-vitro antioxidant study of DPPH radical scavenging activity method**

50 µg/ml conc of the sample was prepared separately from 1mg/ml stock solution. 1ml of different concentration of the sample was mixed with 500µl of 0.004% w/v solution of 2,2-diphenyl-1-picryl-hydrazine-hydrate solution (DPPH) and 4 ml of methanol in sequence and then the absorbance of the sample was recorded at 516 nm using a UV-Visible spectrophotometer. 9 Percentage of DPPH radical scavenging (IC_{50}) for the samples were recorded using a calibration curve of quercetin using the following formula:

\[
\% \text{DPPH radical scavenging activity} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100, \text{where } \text{Abs}_{\text{control}} \text{ and } \text{Abs}_{\text{sample}} \text{ are the absorbance readings of the solvent (control) and sample respectively.}
\]

**Method Validation**

All the antioxidant methods were validated through wavelength selection, linearity, precision and % of recovery by standard spectrophotometric methods. 10-12

**Statistical analysis**

All study was performed in triplicate and report presented as mean±standard deviation format. ANOVA was done by Origin-pro software where P values less than 0.05 were considered for significance.

**RESULTS**

The quantitative estimations of the total flavonoid and phenolics of the methanolic extracts of the different fruits are summarized in Figures 1 and 2 respectively. In our study, a considerable amount of flavonoid and phenolic compounds were found in the fruit and vegetable samples tested. The TFC values of fruits and vegetables ranged about 3.6-33.43 µg of quercetin equivalents per 100 gm of the extract with karela having the highest content with banana being least (Figure 1). On the other hand, the TPC values of the tested samples varied from 9.2 µg (Tomato) to 26 µg (Amla) of gallic acid equivalents per 100 gm of extract (Figure 2). These samples also exerted good antioxidant activity in the DPPH scavenging assay (Figure 3). Banana has the highest IC_{50} value in the antioxidant study by DPPH radical scavenging assay, with tomato also having a similar high capacity of DPPH radical scavenging (Figure 3). However, both banana and tomato had low phenolic and flavonoid content. Amla, which is considered one of the best natural antioxidants, showed poor IC_{50} in the DPPH scavenging assay.

**DISCUSSION**

These results indicated that the flavonoid and phenolic components in common fruits and vegetable have a major contribution to their antioxidant capacity. In most cases, greater TPC or/and TFC value of a fruit or vegetable resulted in better antioxidant capacity in DPPH radical scavenging assay. These findings are similar to previously reported literature which stated that the phenolic or flavonoid content makes an ingredient more potent as a functional or dietary antioxidant. 13 These functional foods should be consumed regularly to prevent oxidative stress-related diseases like ageing, cancer, neurodegeneration, cardiovascular diseases etc.
CONCLUSION

Our work aimed at comparison of total phenolic and flavonoid content of commonly consumed plant foods in Bhubaneswar (India) and correlated with antioxidant capacity by DPPH radical scavenging assay. To the best of our knowledge, this kind of work is the first time evaluated in this locality. The comparison of the results showed a positive correlation between total flavonoid and phenolic content and a negative correlation between total flavonoid and phenolic content with IC_{50} value of DPPH radical scavenging assay. This work would be very useful to local consumers and nutritionists of the covered locality in selecting proper fruits and vegetables in their diet.

ACKNOWLEDGEMENT

The authors are thankful to the authorities of Siksha O Anusandhan (Deemed to be University), Bhubaneswar for providing facilities for the successful completion of this research work.

Author’s contribution

SKS – Extraction and evaluation, preparation of the manuscript
AG – Extraction and evaluation, preparation of the manuscript
DK– Extraction and evaluation, preparation of the manuscript
RB and AB–Concept and planning, interpretation of results, preparation of the manuscript

Conflict of Interest: Nil

Source of Funding: Nil

REFERENCES

1. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J. 1996;313(1):17-29.
2. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative Stress and Antioxidant Defense. World Allergy Organ J. 2012;5(1):9-19.
3. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol. 2009;7(1):65-74.
4. Neeraj, Pramod J, Singh S, Singh J. Role of free radicals and antioxidants in human health and disease. Int J Curr Res Rev. 2013;5(19):14-22.
5. Harris PS, Ferguson LR. Dietary fibre: its composition and role in protection against colorectal cancer. Mutat Res. 1993;290(1):97-110.
6. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine – A move towards nature. Biotechnol Mol Biol Rev. 2007;1(4):97-104.
7. Sharat S, Xuming H, Munir HS, Arshad MA. Evaluation of Polyphenolics Content and Antioxidant Activity in Edible Wild Fruits. Bio Med Res Int 2019;2019:1381989.
8. Luzia DMM, Jorge N. Study of antioxidant activity of non-conventional Brazilian fruits. J Food Sci Technology. 2014;51(6):1167-1172.
9. Saha MR, Hasana SMR, Aktera R, Hossaina MM, Alam MS, Alam MA, et al. In vitro free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* linn. Bangl J Vet Med. 2008;6(2):197-202.
10. Almeida MGJ, Chiari BG, Correa MA, Chung MC, Isaac V. Validation of an Alternative Analytical Method for the Quantification of Antioxidant Activity in Plant Extracts. Lat Am J Pharm. 2013;32(1): 90-95.
11. Hussain AI, Anwar F, Sherazi ST, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem. 2008;108:986-995.
12. Isaac KA, Emmanuel O, Abraham YM, Francis MS, Francis AA, Linda MS. Development and validation of a radical scavenging antioxidant assay using potassium permanganate. J Sci Innov Res. 2016;5(2):36-42.
13. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. J Funct Foods. 2015;18(B):820-897.