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Der Chemica Sinica, 2014, 5(3):30-33



**Pelagia Research  
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ISSN: 0976-8505  
CODEN (USA) CSHIA5

### Cardinal investigation on phenolic and antioxidant content analysis of mixed fruit juices marketed in Bangladesh

Md. D. Sohel<sup>1</sup>, Faisal Asif<sup>\*1</sup>, Tania Sultana<sup>1</sup>, Habiba Mohammadi<sup>1</sup>, Md. H. U. Sumon<sup>2</sup> and Md. A. Rahman<sup>3</sup>

<sup>1</sup>Department of Pharmacy, State University of Bangladesh, Dhaka, Bangladesh

<sup>2</sup>Department of Pharmacy, University of Development Alternative, Dhaka, Bangladesh

<sup>3</sup>Department of Pharmacy, East West University, Dhaka, Bangladesh

#### ABSTRACT

Fresh fruits convenient source of natural phenols and antioxidants which are healthy for human. At present many marketed fruit juices claims to be salubrious also full of phenols and antioxidants. In this study enlighten on investigation of phenols and antioxidant. Five mixed fruits juices were collected from Bangladeshi market for investigation. For the phenolic contents determination modified Folin–Ciocalteu and 1,1-diphenyl-2-picrylhydrazyl (DPPH) modified method for antioxidant analysis was applied. The value of phenolic content was S1 (43.88±0.51), S2 (54.29±0.55), S3 (79.97±1.15), S4 (69.97±1.02) and S5 (57.70±0.78) respectively. On basis of the IC<sub>50</sub> value was S1 (81.59±1.20), S2 (49.91±0.85), S3 (28.75±0.76), S4 (24.44±0.67) and S5 (61.075±0.95) respectively. This study signifies the determination of phenolic contents and antioxidants presence in mixed fruit juices with substantial activeness.

**Keywords:** Antioxidant, Mixed fruit juices, Phenolic content, Free radical scavenging activity.

#### INTRODUCTION

Consumption of vegetables, fruits and red wines etc on a regular basis prior to eminent nutritive properties also provides protection against illnesses including cancer, cerebrovascular, cardiovascular and numerous other illnesses [1-2]. Fruit provides a clean visual sense of purity and free of injurious preservative but presently, Fruit is substituted by fruit juice due to all season availability and extended shelf-life. So, it becomes more convenient for individuals, especially for the children [3].

The most contributing antioxidants properties of food, juices and beverages are polyphenol compounds including flavonoids, flavonols, flavonolols, flavonones, flavones, flavanalols, isoflavones, anthocyanidins, chalcones, anthocyanin, chlorogenic acid isomers, cinnamic and benzoic acid families, stilbenes, lignins, lignans, tannins, tocopherol and tocotrienol[4].

Consumers can daily intake polyphenols is near about 1000 mg in dietary, which is around 10 times higher than the intake of vitamin C and 100 times higher than vitamin E and vitamin A also. Consumption of polyphenols helps to prevent cardiovascular diseases, cancer, and osteoporosis, neurodegenerative diseases and diabetes mellitus [5,6]. In commercial fruit juices manufacturer normally promotes that their fruit juices are rich in antioxidants including synthetic vitamins A, C and E. Not only synthetic vitamins but also manufacturer mixes several synthetic antioxidants including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertbutyl hydroxyquinone (TBHQ) [7-9]. Synthetic antioxidants are less active than natural antioxidant, In addition this

fortified commercial fruit juices carcinogenic and toxic for the human health [10-11]. In this Study, focuses on polyphenolic contents and free radical scavenging of marketed fruit juices.

## MATERIALS AND METHODS

### Instruments

All analytical absorbance measurements were performed by a double beam Shimadzu UV-visible spectrometer (UV mini-1700, Shimadzu Corporation, Kyoto, Japan) with 1 cm quartz cell.

### Collection of fruit juices

Five samples of mixed fruit juices (freshly prepared not exceeding two weeks after manufacturing) were collected in Dhaka, Bangladesh area marketplace. All samples were stored in 4°C through study. The samples were decently checked for their license number, manufacturing batch number, manufacturing date and expiring dates before collecting. They were randomly numbered such as S1, S2, S3, S4 and S5 etc. The marketed fruit juices were filtered through 4-fold muslin cloth for existence of pulp in fruit juice contents.

### Reagents and materials

All analytical chemicals Folin–Ciocalteu’s phenol reagent (Sigma-Aldrich, Germany), gallic acid (Sigma–Aldrich Germany), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Merck, Darmstadt, Germany), Ascorbic acid (ASA) (Merck, Darmstadt, Germany), tert-butyl-1-hydroxytoluene (BHT) (Merck, Darmstadt, Germany) and other reagents were provided from Pharmaceutical Analysis Lab. State University of Bangladesh.

### Phenolic content analysis

The modified Folin–Ciocalteu procedures (Marjorie,2011)[12]. Gallic acid ranging from 500 µg / ml to 0 µg / ml prepared in distilled water. The modified Folin–Ciocalteu procedure consisted of transferring 50 µl standard or sample into a 4–5 ml test tube, followed by additions of 430 µl distilled water and 20 µl Folin–Ciocalteu reagent. After mixing the fruit juice samples, 50 µl 20% Na<sub>2</sub>CO<sub>3</sub> and 450 µl distilled water were added. The sample mixtures were allowed to stand for 60 minutes at room temperature and were mixed after every addition of reagents. Aliquots of 200 µl were transferred to clear microtitre wells in duplicate and the absorbance was measured at 725 nm. The phenolic content of samples was measured against the gallic acid (GA) calibration standard (0–500 µg / ml) and the results were expressed as gallic acid equivalents (GAE).

### Antioxidant Activity Analysis

At first ascorbic acid (ASA) and tert-butyl-1-hydroxytoluene (BHT) as positive control was dissolve in methanol with serial dilution ranging from 500.0 to 0.977 µg /ml. The fruit juices sample (200 µl) assorted at different concentration (500 µg/ml to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min of reaction at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. So the Inhibition of free radical DPPH in percent (I %) was calculated as following:

$$(I \%) = (1 - A(\text{Fruit juices samples})/A(\text{Blank})) \times 100$$

where A (Blank) the absorbance of the control reaction. Mixed fruit juices concentration providing 50% inhibition (IC<sub>50</sub>) was calculated from the graph plotted with inhibition percentage against extractive/standard concentration[13-14].

## RESULT AND DISCUSSION

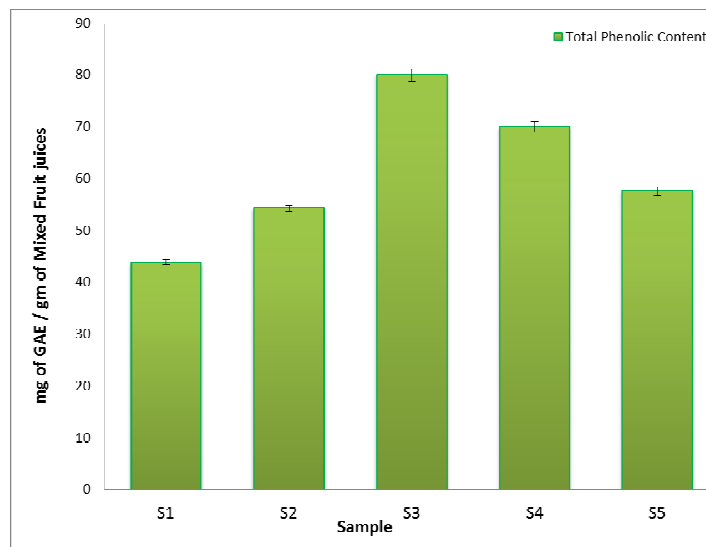
The measuring gallic acid curve R<sup>2</sup> = 0.998 (Not mentioned) was used as reference standard for phenolic content assay.

**Table 1: Total phenolic content and free radical Scavenging activity**

| Sample | Total Phenolic Content (mg of GAE/gm of Mixed fruit juices) | free radical scavenging activity (DPPH) IC <sub>50</sub> (µg/ml) |
|--------|---|--|
| S1     | 43.88±0.51  | 81.59±1.20   |
| S2     | 54.29±0.55  | 49.91±0.85   |
| S3     | 79.97±1.15  | 28.75±0.76   |
| S4     | 69.97±1.02  | 24.44±0.67   |
| S5     | 57.70±0.78  | 61.075±0.95  |
| ASA    | NM  | 5.90±0.32  |
| BHT    | NM  | 28.33±0.42   |

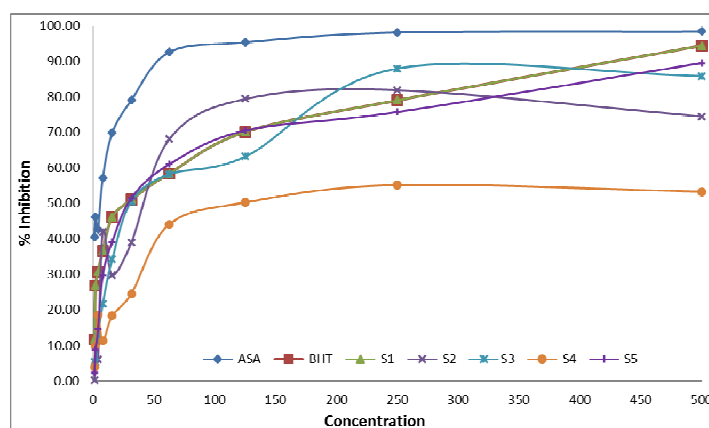
All data were calculate triplet mean±SD, ASA= Ascorbic acid, BHT = tert-butyl-1-hydroxytoluene, NM = Not measured.

In figure 1 and Table 1 was shown about total phenolic content of mixed fruit juices where the value of S3 ( $79.97 \pm 1.15$ ) was higher than other sample. Following sample S4 ( $69.97 \pm 1.02$ ), S5 ( $57.70 \pm 0.78$ ), S2 ( $54.29 \pm 0.55$ ) and S1 ( $43.88 \pm 0.51$ ) was gradually shown high amount of phenolic content.



**Fig. 1: Total Phenolic Content of Fruit juices**

In Figure 2 was shown the % inhibition of mixed fruit juices. Where Ascorbic acid (ASA) and tert-butyl-1-hydroxytoluene (BHT) shown as comparison standard.



**Fig.2: Comparison free radical scavenging activity of different mixed fruit juices including ASA and BHT**

In Table 1 also explained about the free radical scavenging activity (DPPH) where S1 ( $81.59 \pm 1.20$ ) was the highest  $IC_{50}$  value relative to other sample. Other Sample such as S5 ( $61.075 \pm 0.95$ ), S2 ( $49.91 \pm 0.85$ ), S3 ( $28.75 \pm 0.76$ ) and S4 ( $24.44 \pm 0.67$ ) was gradually higher free radical scavenging activity.

## CONCLUSION

On the basis of study some mixed fruit juices contains reasonable amount of phenols and antioxidant. Due to commercial fruit juices using synthetic phenols and antioxidant there is pertaining issue of the activity on human body. Also high amount of synthetic phenols and antioxidant are vulnerable for human health. Further analysis and chemical studies is needed for the proper justification of mixed fruit juices.

## Acknowledgments

Special thanks to the Pharmaceutical Technology Lab, State University of Bangladesh for rendering facilities and proficient support.

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