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Research Article

In Vitro Anticancer Effect of Some Novel Functional Juice Blends

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Abstract

This study aims to produce functional juices prepared from strawberry, blackberry, pomegranate juices and ginger extract with different ratios and then the physio-chemical properties, bioactive compounds, sensory quality and cytotoxic effects against liver (HEPG-2), colon (HCT-116) and breast (MCF-7) cancer cell lines of juice blends were evaluated *in vitro*. The chemical analysis results showed that both pomegranate and blackberry juices contained high levels of crude protein, ash, sugars, total solids and acidity, while the strawberry juice was characterized by its high content of crude fibers, fat and vitamin C. Blackberry juice also showed a higher content of total phenols, flavonoids and anthocyanins, as well as antioxidant activity followed by pomegranate and strawberry juices. Amongst the nine juice blends, the best four blended juices prepared with the control sample were selected, blend (3) being the most acceptable sensory experience. A non-significant difference ($P \geq 0.05$) was also observed between the juice blends in crude protein, fat and ash content, with a significant increase in total solids and acidity values, while pH decreased compared to the control sample. The results also showed a significant increase in the sugars and vitamin C contents of the juice blends, with a positive improvement in the content of total phenols, flavonoids and anthocyanins and antioxidant activity compared to the control sample. All juice blends exhibited antitumor activity against cancer cells in a juice concentration-dependent manner. Blend (9) showed the highest inhibitory effect on liver and colon cancer cell growth, while both blends (3) and (6) showed the strongest inhibitory effect on breast cancer cell growth. Therefore the prepared fruit juices can be used as antitumor agents because they contain some biologically active components with high antioxidant activity and anticancer effects.

Keywords: Fruit Juices; Physico-Chemical Properties; Cytotoxicity; Bioactive components; Anticancer

Introduction

Cancer is an increasingly health issue worldwide, accounting for nearly 9.6 million deaths annually, referring to a large group of diseases characterized by abnormal, uncontrolled cell division in various tissues of the body that can lead to a lump called a tumor, which has the ability to invade, spread and destroy adjacent normal body cells [28].

Strawberry juice is characterized by its sweet taste, attractive red color and pleasant aroma. It is also a rich source of a wide variety of micronutrients such as minerals, sugars and vitamins as well as phytochemicals such as anthocyanins, flavonoids and phenolic acids. All these compounds exert a cumulative and synergistic effect on the promotion of human health and the prevention of chronic ailments such as cardiovascular disease, cancer as well as diabetes mellitus [10,22].

Pomegranate juice is primarily characterized by its distinctive color, exclusive taste and fresh aroma. It offers a convenient way to consume a lot of ingredients with the biological activity of pomegranate. Among the latter, minerals, vitamins, as well as polyphenols, mainly phenolics and flavonoids with antiatherogenic, anti-cancer and anti-inflammatory activities, which have powerful effects against health disorders such as heart attacks, cancer, obesity and diabetes mellitus [9].

Blackberry juice contains a diverse range of bioactive compounds, including minerals, phenolic acids, vitamin C and flavonoids and anthocyanins, which play a pivotal role in curing and preventing against degenerative diseases owing to their pharmacological properties like anti-carcinogenic, antioxidant and anti-diabetic [25].

Ginger extract possesses a high rate of antioxidant activity, which is mainly due to the polyphenol compounds such as 6-gingerol, shogaol, curcumin, flavonoids, which are responsible for providing unique therapeutic properties, including prevention of indigestion, malaria, cancer and reduced insulin resistance [43].

The functional juice industry is expanding worldwide, mainly due to the growing popularity of health-conscious consumers and the desire for nutritious foods. Fruits juice blending is an ideal vehicle for the development of attractive products that add flavour alongside the elevated levels of functional bioactive compounds and essential nutrients that promote nutritional and health benefits to consumers [11].

In recent years, the continually rising global demand for high-quality, fresh and functional healthy fruit juices has led to their inclusion in the human diet instead of soft drinks. Fruit juices, which are naturally rich in bioactive compounds containing polyphenols such as flavonoids, tannins and phenolic acids with health-promoting and disease-reducing properties such as maintaining normal blood pressure and protecting the bones, cardiovascular system and nervous system as well as anti-diabetic and anti-cancer properties, make an important contribution to human nutrition [3].

So, this work is a trial to produce novel functional fruit juices, evaluate the functional and quality characteristics of produced samples and study their effects as anticancer *in vitro*.

Materials and Methods

Materials

Fresh strawberry (*Fragaria ananassa*) cv. 'Festival' were collected from a special farm, El-Mansoura city, El-Dakahlia Governorate, Egypt.

Fresh Pomegranate (*Punica granatum*) cv. 'Manfaloty' were purchased from local market (Fathalla Hypermarket), El-Mansoura city, El-Dakahlia Governorate, Egypt.

Fresh Blackberry (*Rubus fruticosus*) were obtained from Nemat Allah farm, El-Nubaria City, Beheira Governorate, Egypt.

Ginger (*Zingiber officinale*) root powder package was purchased from Bliss of Earth Company, Dubai, United Arab Emirates.

All chemicals utilized in this study were obtained from El-Gomhouria Pharmaceutical Company, El-Mansoura city, El-Dakahlia Governorate, Egypt.

Methods

Processing methods

Preparation of different raw materials for juice processing

- **Strawberry juice (SJ):** After removing the green calyx, fresh strawberries were washed under running tap water, gently rubbed by hand for 5 min, then cut into small pieces and homogenized with water (2:1 w/v) using a clean Marlex blender according to [22].
- **Pomegranate juice (PJ):** Ripened pomegranate fruit was accurately washed with tap water, left to dry, peeled using a sharp knife and then cut into quarters. After the arils were separated from the peels, the juice was extracted by manually squeezing the seeds as described by [2].

- **Blackberry juice (BJ):** Fresh blackberries were cleaned and washed thoroughly utilize tap water and then lightly dried with paper towels. Blackberry juice is often obtained manually by squeezing the fruits, as mentioned by [7].
- **Ginger extract (GE):** The aqueous extract was obtained by using 5 g of ginger powder diluted with 100 ml distilled water and hitting using a clean Marlex blender according to [17].

Ginger extract and each fruit juice were diligently filtered through cotton gauze to remove fruit debris, seeds and sediments and then stored in a refrigerator at 4 ± 1°C until further processing. All preparation of different raw materials for juice processing was conducted in the chemistry laboratory of the Food Industries Department, Faculty of Agriculture, Mansoura University.

Preparation of fruit juice blends

Initially, blended fruit juices were produced from ginger extract, strawberry, pomegranate and blackberry juices at varied proportions with potassium sorbate (E202) in the ratio (0.2%) as presented in Table (A). After that, the juices were homogenized using an electric blender, packed into 250 ml clear glass bottles, pasteurized at 90 Cº for 10 min in a thermostatically water bath, closed immediately, cooled and then stored in a refrigerator at 4 ± 1°C until required for analysis, as mentioned by [30]. Finally, the best sensory samples were selected based on the sensory evaluation results.

Treatments	SJ (%)	BJ (%)	PJ (%)	GE (%)	Potassium sorbate (%)
Control sample	90	-	-	10	0.2
Blend (1)	80	10	-	10	0.2
Blend (2)	75	15	-	10	0.2
Blend (3)	70	20	-	10	0.2
Blend (4)	80	-	10	10	0.2
Blend (5)	75	-	15	10	0.2
Blend (6)	70	-	20	10	0.2
Blend (7)	70	10	10	10	0.2
Blend (8)	60	15	15	10	0.2
Blend (9)	50	20	20	10	0.2

Table A: Formulation of studied fruit juice blends.

SJ: Strawberry Juice; BJ: Blackberry Juice; PJ: Pomegranate juice; GE: Ginger Extract.

Analytical methods:

Physico-chemical analyses:

Physicochemical characterization of each raw juice as well as selected juice blends was analyzed at the Food Tech. Res. Institute, Agric. Res. Center, El-Giza, Egypt. All results were expressed as mean values of triplicate samples.

Proximate and minerals analysis:

Moisture, crude fibers, fat, ash and protein (as nitrogen) were determined according to the method described in [1]. Available carbohydrates was calculated by differences as follows:

Available carbohydrates (%) = 100 – {Protein (%) + Fat (%) + Crude fibers (%) + Moisture (%)}

Minerals content were performed according to [1]. The total quantities of iron, magnesium, calcium, sodium, zinc and potassium were measured by atomic absorption spectrophotometry. Whereas phosphorus was determined by spectrophotometer according to the method described by [5] using Sens AA "GBC scientific equipment" model" Sens AA Dual" made in Dandenong, Victoria, Australia, Micro-Analysis unit, Faculty of Science, Mansoura University, El-Dakahlia Governorate, Egypt.

Determination of sugars content:

Percent total sugars and reducing sugars were measured according to [1]. Non-reducing sugar was calculated by differences.

Determination of pH value and titratable acidity (TA)

The pH values of juice samples were measured by using a digital pH-meter (MVx100. Beckman, USA) and the titratable acidity was determined according to [1]. The results were expressed as % of citric acid or tartaric acid. Maturity index (TSS/acid ratio) was calculated for each sample.

Determination of total soluble solids (TSS), Viscosity and Vitamin C

The total soluble solids was measured using a digital refractometer (MA871, Milwaukee Instruments, USA) from the refractive index of a drop of juice at room temperature (20°C) based on the method of [27]. Viscosity was measured using Brookfield DV-III UlTRA in centipoises (cP) unit according to [19]. Vitamin C was titrimetrically estimated by using the indicator dye 2,6 di-chlorophenolindophenol as mentioned by [32].

Bioactive compounds assessment

The antioxidants activity and total phenolics, flavonoids and anthocyanins contents were determined in raw and selected fruit juice samples at Food Tech. Res. Institute, Agric. Res. Center, El-Giza, Egypt. All tests were performed as means values of triplicates.

Preparation of extracts

The extracts from fruit juices were prepared using methanol as mentioned by [4].

Quantitative evaluation of total phenolics content (TPC)

The total phenolics content was determined based on the Folin – Ciocalteu (FC) assay according to [37].

Determination of total flavonoids content (TFC)

Total flavonoids content was investigated according to the method described by [46].

Determination of total anthocyanins content (TAC)

Estimation of total anthocyanins content was carried out according to [1] by the differential pH method.

Determination of antioxidants activity (DPPH)

The ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by tested samples was determined by the method proposed by [6].

Sensory evaluation

Samples of nine blended juices were applied to sensory tests and evaluated by thirteen panelists from the Food Industries Dept., Fac. of Agric., EL-Mansoura University. Organoleptic evaluation for color (10), taste (10), odor (10), mouth feel (10), appearance (10), overall acceptability (10) and total 60 degrees as stated by [11].

Anti-carcinogenic Activity

Sample extraction

Five juice blends [Control sample, Blend (3), Blend (6), Blend (8) and Blend (9)] extracted with ethanol according to the method described by [15].

Cell viability assay

Human liver (HEP-G2), colon (HCT-116) and breast (MCF-7) cancer cell lines were purchased from cell bank of Vacsera Co., Dokki, El-Giza, Egypt were used for screening the anti-cancer activity of the juice extracts using the microculture tetrazolium (MTT) assay as mentioned by [38,44] at the Science Way Lab., Nasr City, Cairo, Egypt. In brief, the 96 well tissue culture plate was injected with 1×10^5 cells/ ml (100 µl/well), which were tested with various concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/ml) of juice extracts and incubated at 37 °C. 20 µL MTT solution (5 mg/

ml in phosphate-buffered saline (PBS)) was added to each well, shaken (150 rpm for 5 min) and then incubated (37°C, 5% CO_2) for 4 hours. After removing the media, the formazan was solubilized (MTT metabolic product) with 200 µl of dimethyl sulfoxide (DMSO) and shaken. Optical density (OD) was read at 560 nm and subtract background at 620 nm. Absorbance should be directly related to cell quantity and the concentrations desired to kill 50% of cancer cells (IC_{50}) were calculated. Cell viability percentage was calculated using the following formula:

Cell viability (%) = [(Control OD – Sample OD)/Control OD] X 100

Cytotoxicity (%) = 100 – cell viability

Optical density (OD) = Absorbance at 560 nm.

Statistical analysis

Data were statistically analyzed using the producer of the SAS software system program [31]. Analysis of variance (ANOVA) was conducted using General Liner Model (GLM) procedure [34]. Means were separated using Tukey's test where significant differences existed ($P \leq 0.05$).

Results and Discussion

Physico-chemical properties of raw juices and ginger extract

The physico-chemical characteristics of strawberry juice (SJ), pomegranate juice (PJ), blackberry juice (BJ) and ginger extract (GE) are summarized in Table (1). The obtained results indicated that, the highest value of moisture was recorded for GE (97.14%), while PJ had the lowest moisture content being (84.82%).The highest values of ash and crude protein were recorded for BJ (0.42%) and (1.83%), respectively followed by PJ (0.39%) and (1.80%), respectively. Meanwhile, SJ had the highest fat and crude fibers content being (0.22%) and (0.35%), respectively, while GE had the lowest value of fat and crude fibers being (0.07%) and (0.03%). PJ contained the highest value of available carbohydrates followed by BJ being (12.71%) and (4.77%), respectively. These results are closed to those obtained by [26] who found that the content of moisture, crude protein, ash, crude fibers, fat and total carbohydrate of PJ were 84.71, 1.48, 0.38, 0.92, 1.31 and 11.20 (%), respectively. Also, [16] who outline that, SJ contained 91% moisture, 1.23% crude protein, 0.27% ash, 0.20% fat and 1.00% crude fibers.

Concerning the sugars content presented in the same Table (1), BJ and SJ contain the highest percentage of total sugars (TS), which amounted in (8.53 and 8.42 mg/100 ml), respectively. On the other hand, the content of reducing sugars (RS) was highest in PJ (6.88 mg/100 ml), whereas SJ contained the highest amount of non-reducing sugars (NRS), being 2.08 mg/100 ml. Alternatively, GE showed the lowest content of total, reducing and non-reducing sugars. These results are in accordance with those obtained by [8,35] they reported that the total, reducing and non-reducing sugars of SJ and PJ were (8.91, 6.57 and 2.34) and (7.46, 6.71 and 0.75) (%), respectively.

From the data given in Table (1) it could be ascertained that, the pH values of the fruit juices lie between 3.57 and 4.52. The highest pH values was shown by GE (4.52), followed by PJ (3.68), while the lowest value was 3.57 for SJ. This low pH value indicates that these juices are relatively rich in organic acids. The TSS is an indicator of the sweetness of juices, which is related to their inner quality. In general, the TSS content of PJ and BJ were higher than those of other materials, which recorded 14.3 and 12.2%, respectively, while the lowest content was 0.9% for GE. A reverse in values was detected for titratable acidity (TA) where in BJ had the highest value of 0.92% followed by PJ (0.62%). The maturity index (MI), which regarded as a measure of fruit quality, varied from 11.15 to 23.06 in the different juices. Viscosity varied in different types of fruit juices, which ranged from 6.80 and 80.00 (cP). Vitamin C was found to be maximum (23.49 mg/100 ml) in SJ and decremental equally in the other fruit juices. Previous results comparatively agree with the finding of [29,40] they outline that, PJ contained vitamin C ranged from (5.96 – 7.1 mg/100 ml), pH values from (2.28 – 3.71), TSS from (12.23 to 15.67) and TA from (0.51 – 3.52%), while the viscosity, TSS and pH value of BJ were 7.12 cP, 10.93% and 3.21, respectively. Also, [39, 21, 33] they found that, the pH value, TSS, TA and vitamin C of GE were 4.23, 0.3% and 0.08 %, respectively, while SJ had 3.73 pH, 6.3%TSS, 0.52 % TA, 72.50 cP viscosity and 19.2 mg/100 ml vitamin C.

Physico-chemical properties	Raw materials			
	SJ	PJ	BJ	GE
Chemical composition (%)				
Moisture	95.23 ± 0.84^a	84.82 ± 0.34^c	92.69 ± 0.94^b	97.14 ± 0.68^a
Crude Protein	1.52 ± 0.11^{ab}	1.80 ± 0.19^a	1.83 ± 0.30^a	0.99 ± 0.17^b
Fat	0.22 ± 0.12^a	0.16 ± 0.05^a	0.19 ± 0.02^a	0.07 ± 0.03^a
Crude fibers	0.35 ± 0.07^a	0.12 ± 0.02^b	0.10 ± 0.03^b	0.03 ± 0.02^b
Ash	0.17 ± 0.08^b	0.39 ± 0.03^a	0.42 ± 0.09^a	0.15 ± 0.06^b
Available carbohydrates	2.51 ± 1.40^{bc}	12.71 ± 0.63^a	4.77 ± 1.38^b	1.63 ± 0.94^c
Sugars content (%)				
Total sugars (TS)	8.42 ± 1.11^a	7.12 ± 0.77^{ab}	8.53 ± 0.88^a	1.44 ± 0.31^b
Reducing sugars (RS)	6.35 ± 0.76^a	6.88 ± 0.94^a	6.77 ± 1.12^a	1.35 ± 0.26^b
Non-reducing sugars (NRS)	2.07 ± 0.35^a	0.23 ± 0.17^b	1.76 ± 0.24^a	0.09 ± 0.05^b
Physical properties				
pH	3.57 ± 0.31^b	3.68 ± 0.29^b	3.67 ± 0.16^b	4.52 ± 0.15^a
TSS (%)	5.8 ± 0.10^c	14.3 ± 0.10^a	12.2 ± 0.10^b	0.9 ± 0.06^d
Titratable acidity (%)	0.52 ± 0.02^c	0.62 ± 0.01^b	0.92 ± 0.01^a	0.07 ± 0.00^d
Maturity index (TSS/acid)	11.15 ± 0.15^c	23.06 ± 0.20^a	13.26 ± 0.03^b	13.02 ± 0.27^b
Viscosity (cP)	80.00 ± 3.00^a	9.60 ± 0.20^b	6.80 ± 0.05^b	7.20 ± 0.20^b
Vitamin C (mg/100g)	23.49 ± 0.61^a	6.83 ± 0.31^b	6.63 ± 0.37^b	6.59 ± 0.52^b

Table 1: Physico-chemical properties of raw juices and ginger extract (on fresh weight basis).

Mean of triplicate ± SD; a, b, c, d= Means followed by different letters in the same row are significantly different by the Tukey's test ($P < 0.05$). SJ: Strawberry Juice. BJ: Blackberry Juice. PJ: Pomegranate Juice. GE: Ginger extract.

Phytochemicals composition of raw juices and ginger extract

The total phenolics content (TPC), total flavonoids content (TFC), total anthocyanins content (TAC) and antioxidants activity (AA) of the raw juices and ginger extract are presented in Table (2). Data indicated that, the TPC in BJ (274.85 mg GAE/100ml) was one, two and eight fold higher than in PJ, SJ and GE (171.08, 124.56, 31.97 mg GAE/100ml), respectively. The calculated TFC of the fruit juices lie between 112.04 and 26.33, which the highest TFC (112.04 mg QE/100ml) was observed in BJ followed by SJ (97.10 mg QE/100ml), while GE showed the lowest TFC (26.33 mg QE/100ml). On the other hand, the highest TAC value (30.82 mg/100ml) was cited in BJ, which was two and three folds higher than in SJ and PJ (14.26 and 8.98 mg/100ml), respectively. High antioxidants activity was observed in BJ (41.80%) followed by PJ (36.04%), while the lowest AA value was recorded for GE (14.03%). These results exhibited closeness to those obtained by [18,30] they reported that the antioxidant capacity (DPPH) of PJ ranged from 31.16% to 66.82%. As well as, TPC and TFC of PJ were 172.68 and 45.07 (mg/100 ml), respectively. Also, [23] proved that BJ was a relatively rich source of bioactive compounds, especially TPC and TFC, which were recorded 256.10 and 117.23 mg/100ml, respectively.

Sensory evaluation of selected juice blends

The sensory qualities of juice play a vital role in assessing consumer attitudes and their influence on food decision and acceptance. Thus, the best formulated juices and control sample were

Phytochemicals composition	Raw materials			
	SJ	PJ	BJ	GE
Total phenolics (mg GAE/100ml)	124.56 ± 1.32[c]	171.08 ± 0.12[b]	274.85 ± 0.11[a]	31.97 ± 0.06[d]
Total flavonoids (mg QE/100ml)	97.10 ± 1.63[b]	57.06 ± 0.08[c]	112.04 ± 0.11[a]	26.33 ± 2.13[d]
Total anthocyanins (mg/100ml)	14.26 ± 1.76[b]	8.98 ± 0.33[c]	30.82 ± 0.33[a]	0.40 ± 0.33[d]
Antioxidant Activity (%)	35.49 ± 0.20[b]	36.04 ± 0.88[b]	41.80 ± 0.24[a]	14.03 ± 1.22[c]

Table 2: Phytochemicals content of used raw juices and ginger extract (on fresh weight basis).

Mean of triplicate ± SD; a, b, c, d= Means followed by different letters in the same row are significantly different by the Tukey's test ($P < 0.05$). SJ: Strawberry juice. BJ: Blackberry juice. PJ: Pomegranate juice. GE: Ginger extract.

sensorial evaluated and presented in Table (3). Overall, there were no significant differences ($P \leq 0.05$) in color and odor between the control sample and all blends. Significant increase for taste, mouthfeel, appearance, overall acceptability and total score were observed for all blends as compared to control sample. This may be attributed to the mixing of the juice with PJ or BJ, or both with the decrease of SJ. The blend (3) amongst all blends was found to be the most preferred one, which recorded (53.15) for total studied attributes followed by blend (8) (52.23), while the control sample had the least acceptability (46.69). Juice of blend (3) is characterized with its highest score in sensory parameters in terms of mouth-feel and overall acceptability, whereas taste, odor and appearance were detected in high scores for blend (6), blend (8) and blend (9), respectively. The high TSS value in PJ and BJ may explain the sweet taste of these blends compared to the control sample (Table 1, 4). High sensorial acceptance was reported by [45] for pomegranate/blackberry juice blends; [36] for pomegranate/orange/ginger juice blends and [33] for strawberry/blackberry juice blend.

Sensory evaluation	Juice blends				
	Control sample	Blend (3)	Blend (6)	Blend (8)	Blend (9)
Color (10)	8.69 ± 0.75[ab]	9.46 ± 0.66[a]	8.61 ± 0.87[a]	9.23 ± 0.83[a]	9.46 ± 0.51[a]
Taste (10)	7.15 ± 1.28[b]	8.76 ± 0.92[a]	8.84 ± 0.80[a]	8.61 ± 0.87[a]	8.69 ± 0.94[a]
Odor (10)	8.07 ± 1.32[a]	8.46 ± 0.87[a]	7.92 ± 1.55[a]	8.53 ± 0.96[a]	8.38 ± 1.04[a]
Mouth feel (10)	7.23 ± 1.42[b]	8.69 ± 0.75[a]	8.46 ± 1.05[a]	8.00 ± 1.87[a]	8.23 ± 1.23[a]
Appearance (10)	8.00 ± 1.15[b]	8.84 ± 0.55[a]	8.69 ± 0.85[a]	9.23 ± 0.83[a]	9.15 ± 0.68[a]
Overall acceptability (10)	7.61 ± 1.04[b]	8.84 ± 0.51[a]	8.76 ± 0.59[a]	8.76 ± 0.83[a]	8.76 ± 0.92[a]
Total (60)	46.69 ± 4.47[b]	53.15 ± 2.94[a]	51.31 ± 4.28[a]	52.23 ± 4.28[a]	51.92 ± 4.92[a]

Table 3: Sensory evaluation of selected juice blends.

Mean of triplicate ± SD; a, b, c, d= Means followed by different letters in the same row are significantly different by the Tukey's test ($P < 0.05$). Control sample = Strawberry+ Ginger. Blend (3) = Strawberry+ Blackberry+ Ginger. Blend (6) = Strawberry+ Pomegranate+ Ginger. Blend (8) = Strawberry+ Blackberry+ Pomegranate +Ginger. Blend (9) = Strawberry+ Blackberry+ Pomegranate +Ginger.

Physico-chemical properties of selected juice blends

The assayed physico-chemical properties of selected juice blends with various ratios are presented in Table (4). The significant differences ($P < 0.05$) were more pronounced in moisture and available carbohydrates than in crude protein, fat, crude fibers, and ash contents across samples. The data indicated a gradual decrease in moisture content for all juice samples compared with control sample, which consequently led to an increase in the total solids content. The blend (3) recorded the highest content of crude protein being (1.91%) with a non-significant ($P \geq 0.05$) difference with the other blends. Fat and ash contents of all tested juice blends ranged from (0.14 to 0.23 %) and (0.29 to 0.45 %), respectively. The highest moisture and crude fibers content was observed in control sample (94.88 and 0.73%, respectively), such a finding may be due to the high content of crude fibers in SJ (Table 1). In contrast, available carbohydrates were increased progressively in all juice blends, which reached to (4.81 and 5.49 %) in the blends mixed with PJ and BJ, namely blend (9) and blend (8), respectively. The obtained data are confirmed by [41] they noticed that fruit juices were rich in moisture and carbohydrates, but low in crude fibers, fat, crude protein and ash.

Also, results given in the above mentioned Table (4), it could be noticed that, the total sugars content of juice blends varied from 5.63 to 7.35% with a non-significant ($P \geq 0.05$) difference. Blend (6) recorded the highest total and reducing sugars content being (7.35 and 5.90%), respectively, as compared to other blends. Similarly, the highest amount of non-reducing sugars was found in blend (8) (2.06%) followed by blend (6) (1.45%), while the lowest value was 0.60% for blend (9), such finding may be attributed to higher sugar content in raw juices (Table 1).

The same tabulated data shown in Table (4) it could be concluded that, the TSS content of juice blends varied from 5.4% to 8.2% and was higher for blend (9) followed by blend (8). The amount of TSS increased when the juice was mixed with PJ or BJ or both due to their high content of TSS (Table, 1). The pH value is essentially a measure of taste and quality control of fruit juice. Thus, verification was made that control sample showed the highest pH (4.09) and consequently the lowest acidity (0.45%) and blend (9) had the lowest pH (3.85) and the highest acidity (0.69%) as compared to other blends. The TSS/acidity ratio of juice blends ranged from 10.92 to 12.09. Also, the blends showed a significant decrease in viscosity, ranging from 22.01 to 74.00 (cP), when the juice was mixed with PJ or BJ or both with the decrease of SJ. This can be attributed to the lower viscosity present in PJ and BJ compared to SJ (Table, 1). These results are in the line with [39] who reported that the syrups prepared from SJ and blood orange juice recorded a decrease of viscosity than that of 100% SJ. Vitamin C was found to be maximum (26.63 mg/100 ml) in blend (6) and minimum (14.91 mg/100 ml) for blend (9) that may be attributed to rich source of vitamin C in SJ (Table, 1). Our data is in agreement with [12] they found that the vitamin C content was higher in the blended juices compared with a single fruit juice, reporting the occurrence of a synergistic effect. In a previous study [30] who developed mixed fruit juices from PJ, SJ and orange juice, noted that the concentrations of vitamin C, TA, TSS and pH in the juice blend were 26.54 mg/100g, 0.67%, 15.5 % and 3.76, respectively.

Phytochemicals composition of selected fruit juice blends

Phytochemicals, including polyphenols, anthocyanins and flavonoids, are the main antioxidants in fruits, which provide their flavor and color as well as play a crucial role in health protection due to their antioxidants properties [20]. Table (5) showed a significant differences ($P < 0.05$) in total phenolics (TPC) between all the tested blends. The blend (3) showed the highest TPC (156.50 mg/100 ml), followed by blend (8) (142.45 mg/100 ml), while the control sample has the lowest TPC (128.66 mg/100 ml) as compared to other blends. Similar trend was observed in the total flavonoids (TFC) among juice blends, where they were 99.01 followed by 96.14 than 87.43 (mg QE/100 ml), in blend (8), blend (3) and control sample, respectively. As for (TAC), the blends containing a mixture of PJ and BJ recorded the highest TAC, which were (16.96 and 16.43 mg/100ml) for blend (9) and blend (8), respectively compared to the other blends. Figure (1) illustrated that blend (9) and blend (8) exhibited the highest antioxidants activity (AA) values (35.35 and 34.95%, respectively), while control sample contained low AA value (31.40%). Finally, this increase of bioactive compounds across all tested blends may be attributed to higher content of these compounds in PJ and BJ (Table, 2). The obtained data are confirmed by [13,33], they reported that the high content of polyphenols and anthocyanins characterizes the juice blends

Physico-chemical properties	Juice blends				
	Control sample	Blend (3)	Blend (6)	Blend (8)	Blend (9)
Chemical composition (%)					
Moisture	94.88 ± 0.04[a]	94.10 ± 0.90[ab]	93.14 ± 0.27[bc]	92.30 ± 0.18[cd]	91.91 ± 0.19[d]
Crude protein	1.87 ± 0.05[a]	1.91 ± 0.21[a]	1.81 ± 0.09[a]	1.82 ± 0.12[a]	1.66 ± 0.06[a]
Fat	0.23 ± 0.08[a]	0.21 ± 0.09[a]	0.18 ± 0.05[a]	0.20 ± 0.03[a]	0.14 ± 0.02[a]
Crude fibers	0.73 ± 0.07[a]	0.59 ± 0.02[a]	0.64 ± 0.07[a]	0.58 ± 0.05[a]	0.41 ± 0.08[b]
Ash	0.29 ± 0.02[a]	0.33 ± 0.08[a]	0.36 ± 0.06[a]	0.45 ± 0.06[a]	0.39 ± 0.08[a]
Available carbohydrates	2.02 ± 0.26[d]	2.86 ± 0.88[cd]	3.87 ± 0.54[bc]	4.81 ± 0.44[ab]	5.49 ± 0.43[a]
Sugars content (%)					
Total sugars (TS)	6.72 ± 0.41[a]	5.63 ± 0.91[a]	7.35 ± 0.76[a]	6.59 ± 0.68[a]	6.16 ± 1.15[a]
Reducing sugars (RS)	5.36 ± 0.95[a]	4.87 ± 0.63[a]	5.90 ± 0.52[a]	4.53 ± 0.18[a]	5.55 ± 0.83[a]
Non-reducing sugars (NRS)	1.36 ± 0.54[ab]	0.76 ± 0.28[b]	1.45 ± 0.24[ab]	2.06 ± 0.50[a]	0.61 ± 0.32[b]
Physical properties					
pH	4.09 ± 0.09[a]	4.00 ± 0.17[a]	3.86 ± 0.13[a]	3.91 ± 0.10[ab]	3.85 ± 0.25[a]
TSS (%)	5.4 ± 0.15[e]	5.9 ± 0.10[d]	7.2 ± 0.05[c]	7.5 ± 0.05[b]	8.2 ± 0.10[a]
Titratable acidity (%)	0.45 ± 0.01[d]	0.54 ± 0.01[c]	0.60 ± 0.01[b]	0.62 ± 0.01[b]	0.69 ± 0.01[a]
Maturity index (TSS/acid)	12.00 ± 0.08[a]	10.92 ± 0.43[a]	12.00 ± 0.10[a]	12.09 ± 0.12[a]	11.88 ± 0.03[a]
Viscosity (cP)	74.00 ± 2.00[a]	60.10 ± 1.60[b]	36.01 ± 0.66[c]	30.33 ± 2.52[d]	22.07 ± 0.61[e]
Vitamin C (mg/100g)	23.63 ± 0.48[ab]	21.94 ± 1.69[bc]	26.63 ± 0.85[a]	18.52 ± 1.68[c]	14.91 ± 1.65[d]

Table (4): Physico-chemical properties of selected juice blends (on fresh weight basis)

Mean of triplicate ± SD; a, b, c, d, e= Means followed by different letters in the same row are significantly different by the Tukey's test ($P < 0.05$). Control sample = Strawberry+ Ginger. Blend (3) = Strawberry+ Blackberry+ Ginger. Blend (6) = Strawberry+ Pomegranate+ Ginger. Blend (8) = Strawberry+ Blackberry+ Pomegranate +Ginger. Blend (9) = Strawberry+ Blackberry+ Pomegranate +Ginger.

Phytochemicals composition	Juice blends				
	Control sample	Blend (3)	Blend (6)	Blend (8)	Blend (9)
Total phenolics (mg GAE/100ml)	128.66 ± 0.34[d]	156.50 ± 0.10[a]	138.24 ± 1.26[c]	142.45 ± 0.87[b]	137.15 ± 0.42[c]
Total flavonoids (mg QE/100ml)	87.43 ± 1.85[c]	96.14 ± 2.60[ab]	88.33 ± 1.35[c]	99.01 ± 1.26[a]	93.40 ± 2.02[b]
Total anthocyanins (mg/100ml)	12.42 ± 2.34[d]	13.55 ± 2.00[c]	15.76 ± 2.67[b]	16.43 ± 2.33[a]	16.96 ± 1.00[a]

Table 5: Phytochemicals composition of selected juice blends (on fresh weight basis).

Mean of triplicate ± SD; a, b, c, d= Means followed by different letters in the same row are significantly different by the Tukey's test ($P<0.05$). **Control sample =** Strawberry+ Ginger. Blend (3) = Strawberry+ Blackberry+ Ginger. Blend (6) = Strawberry+ Pomegranate+ Ginger. Blend (8) = Strawberry+ Blackberry+ Pomegranate+Ginger. Blend (9) = Strawberry+ Blackberry+ Pomegranate +Ginger.

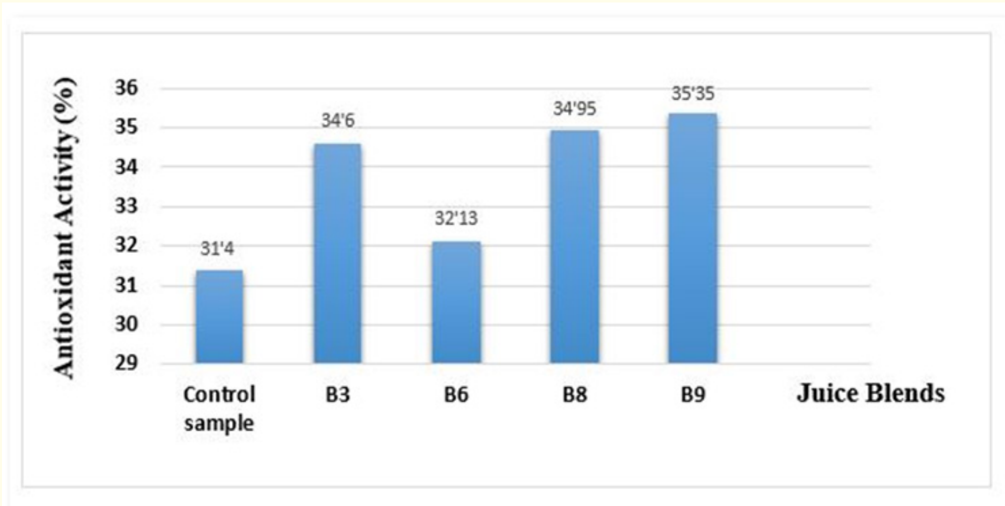

Figure 1: Antioxidant activity of fruit juice blends (%).

based on PJ, BJ and SJ. [30] declared that the juice blend of PJ, SJ and orange juice showed a remarkable increase in the content of TPC, TFC, TAC and AA being 149.17, 82.37, 35.21 (mg/100ml) and 37.39%, respectively.

Anti-cancer activity of selected juice blends

In vitro cytotoxicity assessment of selected juice blends against liver cancer (HEPG-2), colon cancer (HCT-116) and breast cancer (MCF-7) cell lines were evaluated using the microculture tetrazolium (MTT) assay. Cancer cell lines were exposed to various concentrations of juice blends (31.25, 62.5, 125, 250, 500, 1000 µg/ml) and the results were evidently presented in Table (6) and illustrated in Figures (3). Data clearly observed that, the cytotoxicity of juice blends on cancer cell lines, differs in a dose-dependent manner, which the minimum inhibiting activity was obtained with 31.25 µg/ml concentration, and there was a steady increase in the inhibition activity against cell viability with the rise in the dose. Concerning the liver cancer (HEPG-2) cell line, the highest viability percentage at the concentration of 1000µg/ml was marked (2.84, 2.65, 2.65 and 2.60%) for blend (8), blend (3), blend (6) and blend (9), respectively, as compared to the control sample (3.09%). While, the blend (9) exhibited the maximum inhibition effect in IC_{50}= 83.41µg/ ml, followed by blend (8) (91.16µg/ ml), while the lowest inhibition was in the control sample (179.54µg/ ml). The obtained data are confirmed by [14,25] they suggested using BJ and GE as effective agents in inhibiting liver cancer (HEPG-2) cell proliferation according to the higher contents of the phytochemical.

Accomplish with special concentrations of juice blends emphasized that a considerably changeable cytotoxicity against colon cancer (HCT-116) cell line as shown in Table (6). An analogous effect of cytotoxicity was shown at level of (1000µg/ml) as the viability percentages were 3.53, 3.47 and 3.15 (%) for blend (9), blend (6) and blend (3), respectively. Furthermore, the highest inhibition effects were recorded in IC_{50}= 106.24 µg/ ml for the blend (9), followed by blend (8) (116.88 µg/ ml), while the lowest inhibition was in the control sample (161.31µg/ ml).

Handling of breast cancer by means of particular concentrations of selected juice blends showed that a significant variable cytotoxicity against breast cancer (MCF-7) cell line, as observed in the Table (6). The cytotoxicity of blend (8), blend (9), blend (3) and blend (6) of (1000µg/ml) leads to viability percentages of 2.55, 2.50, 2.27 and 2.14 (%) respectively, whereas it was 2.91 % in the control sample on breast cancer (MCF-7) cell lines. Also, the blend (3) exhibited the best inhibition effect with IC_{50}= 102.95µg/ml followed by blend (6) (123.05µg/ml) compared to the control sample

(206.12µg/ml). These results are similar to those obtained by [42] who found that PJ exhibited a cytotoxic effect against breast cancer cells. Also. [3,11] they observed that, functional juice mixtures exhibited strong antitumor activities as evidenced by increased cancer cell death of liver (HEPG-2), colon (HCT-116) and breast (MCF-7), which was related to the high content of bioactive components and their inhibitory effect. Figure (2) illustrated that, all photomicrographs showed a pronounced induction of cell death and a significant change in the morphology of all treated cancer cells at a dose of 1000 µg/ml. These morphological changes include blabbing, cell shrinkage and nuclear condensation.

From the previous results, it could be concluded that blend (9) had a superior inhibition growth on colon (HCT-116) and liver (HEPG-2) cancer cell lines with the best IC_{50} (106.24 and 83.41µg/ml) followed by blend (8) (116.88 and 91.16 µg/ml), while the best inhibition of the breast cancer (MCF-7) cell line was found in blend (3) (102.95 µg/ml). Also, the anticancer activity of all the tested juice blends, especially blend (9), blend (8) and blend (3) could be ascribed to the synergistic effect of the combination of anthocyanins and phenolic compounds, which were found in high proportions in these blends and PJ, BJ and SJ (Table 2,5).

Conclusion

From the above-mentioned results, it could be denoted that, the possibility to develop nutritious healthy fruit juices from a combination of strawberry, pomegranate, blackberry juices and ginger extract that positively improve the content of important bioactive components for human health, such as minerals, vitamin C, phenolic compounds, anthocyanins, flavonoids and antioxidants with protective effects against liver (HEPG-2), colon (HCT-116) and breast (MCF-7) cancer by inhibiting cell growth and inducing apoptosis plus having high palatable organoleptic properties. Therefore, it could be advisable to prepare such juice blends as a functional juice.

Concentration µg/ml	Juice blends				
	Control sample	Blend (3)	Blend (6)	Blend (8)	Blend (9)
% Viability HEPG-2					
31.25	100	99.60	99.41	99.50	99.85
62.5	99.95	99.36	67.05	94.99	61.65
125	94.64	54.59	44.32	13.40	16.35
250	10.60	8.44	21.25	6.18	8.78
500	3.87	2.89	3.48	2.79	3.83
1000	3.09	2.65	2.65	2.84	2.60
% Viability HCT-116					
31.25	99.93	99.81	99.62	99.05	96.15
62.5	99.62	94.32	86.74	85.29	76.57
125	70.45	45.39	47.09	44.44	38.57
250	3.85	4.16	4.35	14.33	3.66
500	3.72	3.21	3.91	4.16	3.53
1000	3.53	3.15	3.47	3.47	3.53
% Viability MCF-7					
31.25	100	100	99.81	100	100
62.5	99.77	95.86	98.32	100	99.86
125	98.86	30.60	48.47	99.04	98.45
250	28.96	3.05	2.77	15.55	23.83
500	4.23	2.32	2.18	2.95	3.45
1000	2.91	2.27	2.14	2.55	2.50

Table 6: Cytotoxicity of selected juice blends on liver cancer (HEPG-2), colon cancer (HCT-116) and breast cancer (MCF-7) cell lines *in vitro*.

Control sample = Strawberry+ Ginger. Blend (3) = Strawberry+ Blackberry+ Ginger. Blend (6) = Strawberry+ Pomegranate+ Ginger. Blend (8) = Strawberry+ Blackberry+ Pomegranate +Ginger. Blend (9) = Strawberry+ Blackberry+ Pomegranate +Ginger.

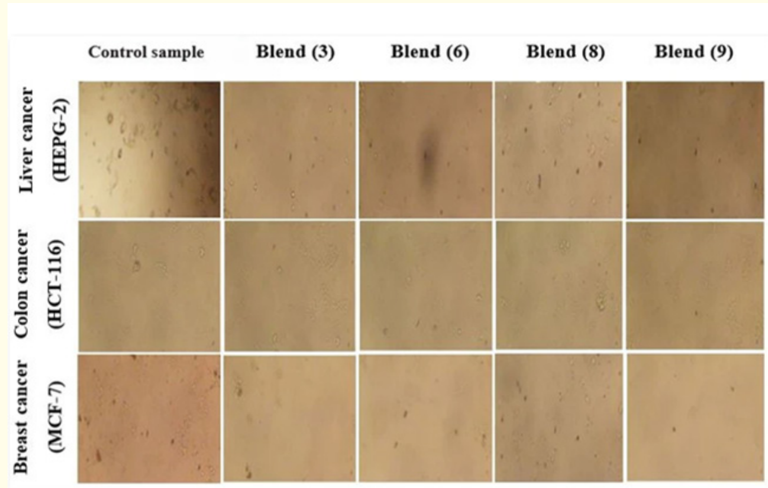

Figure 2: Morphological changes observed in human cancer cells after treatment with fruit juice blends at 1000 μg/ml as visualized under the inverted microscope.

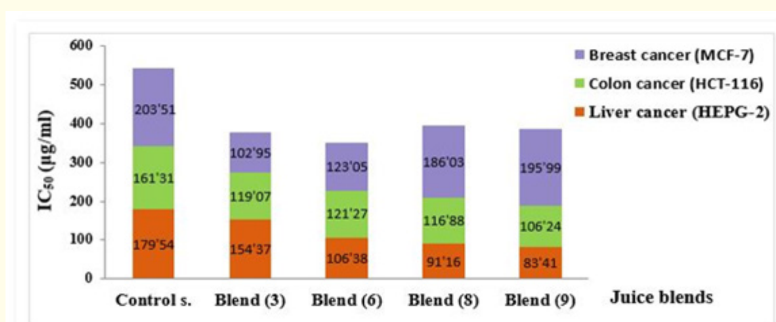

Figure 3: The IC_{50} values (μg/ml) of fruit juice blends against three cancer cell lines.

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