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Physiochemical Properties, Anti-pathogenic and Anti-tumour Activity of Whey based Probiotic Muskmelon Health Beverage

V. Krishnaprabha[1], Lalitha Ramakrishnan[1], R. Vijayaraghavan[2], Gayathri Unnikrishnan[3] 10.18805/ajdfr.DR-2018

ABSTRACT

Background: Muskmelon (*Cucumis melo*) was a beautiful, juicy, tasty fruit of the Cucurbitaceae family which includes 825 species in 118-119 genera. It is also rich in antioxidant flavonoids such as carotene, Vitamin C, lutein, adenosine, zea-xanthin and cryptoxanthin and the folic acid present in the fruit is very good for pregnant women and makes the baby healthy and it helps to prevent the neural tube defects. Whey and whey based products had relatively high lactose content which forms a suitable substrate for probiotics in the intestine and it increased the absorption of calcium.

Methods: A probiotic beverage was prepared by using whey water and muskmelon incorporation.

Result: Product developed with whey protein at 60% incorporation received highest acceptability score. No statistically significant difference was found between the control and Whey protein incorporated muskmelon beverage. The nutrient content of V2 was higher than other variations. Conclusively, the incorporation of whey water, muskmelon (rich in antioxidants) and probiotic could prevent our body from various disease conditions and it has potential health benefits disease.

Key words: Anti-pathogenic, Anti-tumour activity, Physiochemical properties, Probiotic beverage, Whey water.

INTRODUCTION

Muskmelon is a beautiful, juicy, tasty and delicious fruit popular for its nutritive and medicinal properties. Muskmelon is recommended for the treatment of cardiovascular disorders, as a diuretic, stomachic, anti-tussive and as a vermifuge (Parle and Singh, 2011; Bhalekar *et al.,* 2022). The nutrient content of muskmelon is low in calories, fat and sodium but good sources of potassium and Vitamin C. In addition muskmelon is an excellent source of beta carotene, Vitamin C and low in folic acid, iron and calcium. It is no table that one cup of serving of muskmelon provides the recommended daily dietary allowance for Vitamins A and Vitamin C (Gene Lester, 1997). Whey which is obtained as a by product during the manufacture of products such as paneer, chhana, shrikhand and cheese is considered to be a reliable source of a number of high quality and biologically active proteins, carbohydrates and minerals (Seethalakshmi *et al*., 2010). Probiotics are friendly or good bacteria described as “living drug” are considered as an army of microorganism running inside our body and fighting against a variety of digestive disorders (Anushree and Sen, 2014). Probiotics stimulates the immune system by increasing antibodies production. It also increases macrophage activity shown by the enhanced ability to phagocytes microorganisms (Neish *et al*., 2000). Foods used with the incorporation of probiotics are present in infant formula, fruit drinks, whey drinks and sweet milk. Probiotic LAB (Lactic acid bacteria), especially lactic acid bacteria and *Bifido bacterium*, are known to enhance the capacity of host to fight against intestinal infections by stimulating the mucosal immune system (Saha *et al.,* 2017; Erickson and Hubbard, 2000). Introducing a nutrient rich ‘functional beverage’ can be an important dietary intervention to decrease the risk of cancer and cardiovascular diseases. Formulation of functional beverages fortified with antioxidants should be intended to provide nutritional and health benefits. The overall objective of this study was to develop and evaluate a nutritionally efficient beverage, formulated by combining a selected phytonutrient and antioxidant rich fruit, with whey and probiotic microorganism.

[1]Department of Nutrition and Dietetics, PSG College of Arts and Science, Coimbatore-641 014, Tamil Nadu, India.
[2]Department of Microbiology, PSG College of Arts and Science, Coimbatore-641 014, Tamil Nadu, India.
[3]Department of Microbiology, Nehru Arts and Science College, Coimbatore-641 104, Tamil Nadu, India.

Corresponding Author: V. Krishnaprabha, Department of Nutrition and Dietetics, PSG College of Arts and Science, Coimbatore-641 014, Tamil Nadu, India. Email: prabhavijay2007@gmail.com

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MATERIALS AND METHODS

Preparation of muskmelon juice and whey water

The study was conducted during the period of October 2013 to December 2016 at PSG college of Arts and Science, Coimbatore, Tamil Nadu. Selected fresh, ripened musk melon fruits were washed well and chopped into small pieces

to prepare the pulp from the fruit after the removal of seeds. The juice was extracted from the pulp with the help of juicer and stored in the refrigerator. Whey water was prepared with the help of standard procedures. The musk melon squash was standardized in three variations were prepared by adding 50%, 40% and 30% of whey water to 50%, 60% and 70% of muskmelon juice. The prepared beverage was pasteurized at 80°C for 15 mins to destroy the pathogenic microorganisms. The probiotic microorganism (*L. bulgaricus*) was inoculated and incubated at 37°C for 48 hrs and added in the beverage. The prepared beverages were filled in bottles (200 ml) which were sterilized with boiling water and then filled aseptically and sealed again dipped in hot water for few seconds inorder to avoid any contamination. Filled bottles were cooled and stored in refrigerated conditions for storage studies.

Organoleptic evaluation of the formulated beverage

Fruit juices contain high amounts of sugars which could encourage probiotic growth and the decrease in the sugar content could easily be monitored using arefractometer. Sensory attributes like color and appearance, flavor, consistency, taste and overall acceptability were evaluated using nine point hedonic scale as described by Ranganna (1993). A nine-point Hedonic scale score card was provided tothe panelists to adjudge the quality of the product with respect toappearance, odor, taste and overall acceptability. Prepared beverage was given to 25 semi trained panel members for evaluating the organoleptic characteristics of the product at a regular interval of 15 days.

Nutrients analysis of the formulated beverage

The nutrient analysis was analyzed for control sample and most accepted scores of Whey water incorporated muskmelon squash. The parameters selected for the analysis like energy, protein, fat, calcium, iron, phosphate, β-carotene, Vitamin C, casein and total antioxidant activity. The physico-chemical constituents like acidity, pH, TSS, total sugar, reducing sugar were analyzed with standard procedures of AOAC (1995).

Microbial analysis of the formulated beverage

Total plate count (TPC) and gram staining was recorded over a 15 days interval for 60 days for shelf life analysis.

Antioxidant activity of the formulated beverage

The antioxidant content was determined by using the method of Braca *et al*., (2001). The muskmelon juice was applied on a TLC plate as spots (100 µg/ml) using mobile phase with methanol: acetonitrile in 7:1 ratio. Itwas allowed to develop the chromatogram for 30 min. After the completion of the chromatogram, the platewas sprayed with DPPH (0.2% w/v). The colour change (yellow spot on purple background on TLC plate) is an indication of the presence of antioxidants.

The antioxidant activity of muskmelon and probiotic incorporated beverage was estimated with DPPH method. Antioxidant capacity DPPH radical was used as a stable free radical to determined antioxidant activity of natural compounds. DPPH is considered a valid and easy assay to evaluate scavenging activity of antioxidants. The antioxidant activity was determined in terms of the ability of the antioxidants in the fruit to inhibit oxidation.

Antibacterial assay

The antibacterial activity of the beverages was assessed against seven bacterial species: *Staphylococcus aureus* (MTCC737), *Escherichia coli* (MTCC1560), *Proteus vulgaris* (MTCC426), *Salmonella typhi* (MTCC734), *Streptococcus pyogenes* (MTCC1923), *Pseudomonas aeruginosa* (MTCC424) and *Enterococcus faecalis* (MTCC439), maintained in Brain Heart Infusion broth (BHI) at -20°C; 3 ml of each stock-culture were added to 300 ml of BHI broth. Overnight cultures were kept for 24 hr at 36°C±1°C and the purity of cultures was checked after 8 hr of incubation. After 24 hr of incubation, bacterial suspension (inoculum) was diluted with sterile physiological solution, for the diffusion and indirect bioautographic tests, to 10^8 CFU/ml (turbidity= McFarland barium sulphate standard 0.5) as recommended by WHO (2009).

The bacterial inoculum was uniformly spread using sterile cotton swab on a sterile Petri dish Muller Hinton (MH) agar. An antibacterial activity of the selected beverage was determined by cup diffusion method as described by Anonymous 1996. Wells are made in Muller Hinton agar plate using cork borer (5 mm diameter). The wells were filled with 20 µl of V2 RTS beverage. The systems were incubated for 24 hr at 36°C±1°C, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm. Reference commercial discs were used (Ampicillin 10 mg). Tests were performed in triplicate and the values expressed as mean±S.D.

Antitumor assay (MTT assay)

The human breast cancer cell line (MCF-7) and human hepatic carcinoma cell line (HepG2) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37°C, 5% CO_2, 95% air and 100% relative humidity. Maintained cultures were passage weekly and the culture medium was changed twice in a week.

The number of viable cells was determined according to the method described by Monks *et al.,* (1991) using MTT dye (3-4,5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) with slight modifications. The assay was carried out as follows. The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions. The viable cells were counted by 0.4% trypan blue dye exclusion test and the cell count was adjusted to 1×10^5 cells/ml. A pilot experiment was performed using a cell concentration of 1×10^5 cells/ml (100 µl per well) in quadruplicate wells in a 96-well microculture plate. The culture plate was incubated at 37°C in a CO_2 incubator for 24 hr. After incubation, the cells were treated with serial

concentrations of test samples which were pre-sterilized by using 0.45 µm syringe filter. Then the plates were incubated at 37°C in a CO_2 incubator for 48 hr. The medium containing without test sample was served as control. All the steps were done in triplicate.

MTT 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma catalog no. M2128) was dissolved in PBS at 5 mg/ml and filtered to sterilizeand remove a small amount of insoluble residue present in some batches of MTT method by Mosmann (1983). At the times indicated below, stock MTT solution (10 µl per 100 µl medium) was added to all wells of an assay and plates were incubatedat 37°C for 4 hr. Acid-isopropanol (100 µl of 0.04 N HCl in isopropanol) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After a few minutes at room temperature to ensure that all crystals were dissolved, the plates were measured the absorbance at 570 nm. The percentage cell viability was then calculated with respect to control as follows:

$$\% \text{ Cell viability} = \frac{[A] \text{ Test}}{[A] \text{ Control}} \times 100$$

Statistical analysis

The data generated in the experiments such as sensory evaluation and nutrient analysis was subjected for statistical analysis by using standard deviation and Annova one way.

RESULTS AND DISCUSSION

Organoleptic evaluation of the RTS beverage

On comparing all the variations, it was observed that the beverage sample V2 prepared by addition of 60% muskmelon juice scored higher in sensory parameters. The color and appearance, flavor, consistency, taste and overall acceptability of V2 beverage was better than V1 and V3 beverages respectively as reflected by scores. The organoleptic evaluation of the prepared RTS beverages is found in Table 1.

The fresh juice of V2 beverage sample (60% muskmelon juice and 40% whey) obtained high scores for flavor (7.44±1.04) than other muskmelon juice blends such as V1 and V3. This might be due to prominence of volatile flavoring compounds. Maximum score (7.34±1.12) was found in muskmelon juice and whey (60:40) blended V2 RTS beverage. This might be due to decrease in cloudiness and viscosity due to the blending of muskmelon with whey water. Taste scores were maximum in muskmelon and whey (60:40) blended RTS beverages as 7.61±0.912, 7.58±0.898, 7.52±0.892 during 0, 15 and 30 days respectively when compared with other experimental samples such as V1 and V3. The taste score of 100% muskmelon juice (control) was higher because the delicious taste provided by muskmelon predominated well than all experimental samples (V1, V2 and V3). The experimental sample V2 beverage with 60% muskmelon juice and 40% whey was better acceptable (7.67±1.224, 7.65±1.220, 7.51±1.213) on different storage periods.

The pH of the V2 [whey: muskmelon beverage (60:40) RTS beverage] was higher (4.7±0.56) than control sample (4.1±0.12). The pH of prepared beverage decreased from 4.7±0.56to 4.2±0.31 in V2 beverage after 30 days but remarkable changes in pH were not found in control sample. Decrease in pH during storage may be attributed to simultaneous increase in titrable acidity. The acidity content was higher in control sample beverage (1.2±0.03) than the

Table 1: Organoleptic evaluation of the prepared RTS beverages.

Storage period (days)	Criteria	Samples			
		Control	V1	V2	V3
0	Color and appearance	8.54±1.073	6.78±1.507	7.35±0.78	7.16±1.27
15		8.50±1.070	6.72±1.501	7.35±0.78	7.16±1.27
30		8.43±1.062	6.62±1.493	7.29±0.70	7.07±1.27
0	Flavor	8.16±1.06	6.75±1.24	7.44±1.04	6.43±1.38
15		8.11±1.06	6.71±1.20	7.40±1.00	6.40±1.38
30		8.01±1.002	6.71±1.20	7.30±1.00	6.32±1.32
0	Consistency	8.45±1	7.04±1.20	7.34±1.12	7.26±1.02
15		8.41±1	7.02±1.18	7.18±1.04	7.30±1.10
30		8.33±1.008	7.00±1.16	7.15±1.01	7.31±1.10
0	Taste	8.34±1.322	6.78±1.392	7.61±0.912	6.36±1.075
15		8.32±1.320	6.72±1.389	7.58±0.898	6.36±1.075
30		8.28±1.313	6.72±1.389	7.52±0.892	6.28±1.075
0	Overall acceptability	8.38±1.220	6.88±1.0967	7.67±1.224	6.56±1.260
15		8.31±1.218	6.85±1.0963	7.65±1.220	6.53±1.256
30		8.13±1.203	6.81±1.0960	7.51±1.213	6.49±1.252

Control - 100 % Muskmelon juice.

V1 - 50% Muskmelon juice and 50% Whey wate.

V2 - 60% Muskmelon juice and 40% Whey water.

V3 - 70% Muskmelon juice and 30% Whey water.

experimental sample (0.42±0.06) due to the reaction of ascorbic acid with sugar and amino acids. Significant increase in acidity was observed in control than V2 [whey: muskmelon beverage (60:40) RTS beverage] during 30 days of storage. The control sample contained more TSS (46±2.3) than the V2 RTS beverage [muskmelon juice 60%: whey 40% -30±1.8]. The control sample prepared with 100% muskmelon juice. The reducing sugar value for V2 beverage [muskmelon juice 60%: whey 40%) RTS beverage] ranged from 10.9±2.23 to 11.7±2.21 per cent during the 30 days of storage. There were no significant changes in reducing sugar content in control sample during storage period. Total sugar content varied considerably between the samples such as control and Variation 2. Total sugars were higher in fresh control sample (38±1.11). There was a slight decrease in content of control sample after 30 days of storage. The physicochemical parameters of formulated RTS beverage is depicted in Table 2.

Nutrient analysis of the formulated RTS beverage

The nutrient analysis was performed for control sample and most acceptable form of muskmelon juice V2 (60% muskmelon juice and 40% whey water). The parameters selected for analysis were energy, protein, calcium, iron and phosphorus. The nutrient analysis of muskmelon beverage is depicted in Table 3.

The energy content of V2 sample during the storage period was comparatively lower than control (136.97±1.07) beverage sample (100 % muskmelon juice). The decrease in energy value of V2 RTS beverage sample (112.80±1.06) may be due addition of whey water in muskmelon juice that leads to dilution of total sugar content. There was an increase in protein with the addition of whey. V2 sample (muskmelon juice 60%: whey 40%) recorded high protein value (2.00±0.32 g) than control sample (0.56±0.15). The increase in protein was to the extent 1.5 g in V2 RTS beverage. The calcium content was more in V2 beverage sample (49.00±3.04 mg) than the control (38.00±2.07). The high level of calcium content in V2 RTS beverage sample may be due to the incorporation of whey. Phosphorus content was higher in the V2 beverage sample (48.00±1.03 mg) than the control (16.00±0.57) but minute changes were noted in experimental sample during storage. Generally milk is a good source of minerals. Hence, the whey water incorporation in muskmelon juice promoted phosphorous level in V2 RTS beverage sample. The iron content was slightly more (0.28 mg) in V2 RTS beverage compared to control (0.16 mg).

Muskmelon fruit juice generally contains β-carotene and Vitamin C. The β-carotene content of the V2 RTS beverage sample was 2965.00±37.12v and considerably higher than the control (2569.10±95.15c**). Muskmelon juice is a natural source of provitamin A because of its high β-carotene content. Vitamin C content of V2 RTS beverage was 27.00±1.07b**. The vitamin C value observed in V2 beverage sample was lower when compared with control sample this may be due to the acidity of whey water. Total antioxidant value increased when the muskmelon juice is mixed with whey water (60:40). Muskmelon juice contains large amounts of polyphenols and phenolic acids and it may help to reduce the risk of chronic disease due to antiproliferative and cell signalling effects. Moreover, when compared to control, the V2 RTS beverage sample contains more of total antioxidants. Total antioxidant value was increased when the muskmelon juice is mixed with whey water [3410.0± 3.43c**(control) to 5400.0±7.84b** (V2 RTS) beverage] which may due to hydrolysis of whey proteins by protease enzymes in muskmelon juice. The antioxidant content of formulated RTS beverage is depicted in Table 4.

Table 2: Physicochemical parameters of formulated RTS beverage.

Parameter	Storage period (days)	Control	V2
pH	0	4.1±0.12	4.7±0.56
	15	4.1±0.12	4.3±0.42
	30	4.0±0.11	4.2±0.31
Titrable acidity (%)	0	1.2±0.03	0.42±0.06
	15	1.2±0.03	0.46±0.01
	30	1.12±0.02	0.49±0.01
TSS (°Brix)	0	46±2.3	30±1.8
	15	46±2.3	30±1.8
	30	45±2.3	31±1.9
Reducing sugar (%)	0	11.6±3.21	10.9±2.23
	15	11.6±3.21	11.3±2.23
	30	11.3±3.19	11.7±2.21
Total sugar (%)	0	38±1.11	34±0.98
	15	37±1.11	34.3±0.98
	30	35±1.09	34±0.98

Control - 100% Muskmelon juice.

V2 - 60% Muskmelon juice and 40% whey water.

Table 3: Nutrient analysis of muskmelon beverage.

Parameter	Storage period (days)	Control	V2
Energy (kcal)	0	136.97±1.07	112.80±1.06
	15	134.97±1.07	110.80±1.03
	30	134.97±1.07	113.80±1.06
Protein (g)	0	0.56±0.15	2.00±0.32
	15	0.55±0.15	2.09±0.32
	30	0.56±0.15	2.00±0.32
Calcium (mg)	0	38.00±2.07	49.00±3.04
	15	39.00±2.07	48.00±3.03
	30	38.00±2.07	49.00±3.04
Iron (mg)	0	0.16	0.28
	15	0.16	0.28
	30	0.16	0.28
Phosphorus (mg)	0	16.00±0.57	48.00±1.03
	15	16.00±0.57	47.00±1.00
	30	16.00±0.57	47.05±1.02

Total bacterial counts in the prepared beverage sample V2 were 1.92×10^4 CFU/mL, 2.34×10^4 CFU/mL and 2.62×10^4 CFU/mL with respect to 0, 15 and 30 days of storage period. Yeast and mould count was not detected in V2 sample at zero-day storage whereas it increased to 7 and 23 CFU/mL respectively after 30 days of storage. Microbial loads in the beverage sample V2 was comparatively less than the control during different stages of storage period. This may be due to acidifying effect and presence of some antimicrobial components in whey water. Moreover, the microbial content increased with increase of storage period. Initially the muskmelon juice blended with whey which is in acidic pH. So at starting stage of storage, yeast and mold would predominate. Later the blended RTS beverage pH becomes nearly neutral because of the end product produced by yeast and mold after hydrolysis of complex sugars. Upon hydrolysis of complex sugar degradation, bacteria will predominate. Under any circumstances, the storage temperature cannot arrest completely the growth of microorganisms but it could only delay the microbial growth. The microbial load (bacteria, yeast and mold) increased with the storage period. The shelf life study of formulated beverage is depicted in Table 5.

Table 4: Antioxidant contents of formulated RTS beverage.

Parameter	Control	V2
Âeta carotene (mcg)	2569.10±95.15c**	2965.00±37.12v**
Vitamin C (mg)	35.00±1.01c**	27.00±1.07b**
Total antioxidant value	3410.0±3.43c**	5400.0±7.84b**

Table 5: Shelf-life study of the formulated beverage.

Parameter	Storage period (days)	Control	V2
Total bacteriological count (CFU/mL)	0	2.00×10^4	1.92×10^4
	15	7.22×10^4	2.34×10^4
	30	8.27×10^4	2.62×10^4
Yeast/mold count (CFU/mL)	0	9	ND
	15	15	7
	30	24	23

Table 6: Antibacterial assay (Well diffusion method.

Test organism	Zone of inhibition in diameter (mm)		
	Control	V2	Standard drug (Ampicillin)
Staphylococcus aureus	8±1	10±1	3±1
Escherichia coli	7±1	8±1	4±1
Proteus vulgaris	5±1	6±1	5±1
Salmonella typhi	9±1	10±1	2±1
Streptococcus pyogens	7±1	9±1	1±1
Pseudomonas aeruginosa	9±1	11±1	1±1
Enterococcus faecalis	9±1	6±1	2±1

Antibacterial activity of most acceptable form of muskmelon RTS beverage (V2) was carried out by cup diffusion method as described by Anonymous (1996). The pathogens *Staphylococcus aureus* (MTCC737), *Escherichia coli* (MTCC1560), *Proteus vulgaris* (MTCC426), *Salmonella typhi* (MTCC734), *Streptococcus pyogenes* (MTCC1923), *Pseudomonas aeruginosa* (MTCC424) and *Enterococcus faecalis* (MTCC439) were tested for antimicrobial assay. The observed results of growth inhibition were exhibited in Table 6.

The findings of the present study showed that fractions of all samples had higherantibacterial activity against six pathogenic strains. The higher antimicrobial activity showed by whey based *Cucumis melo* RTS beverage may be due to the presence of phenyl and organic acids present in muskmelon and whey water respectively.

Anti-tumor assay (MTT assay)

Analysis on human breast cancer cell line (MCF 7)

After treatment with various concentrations (3.125 µl, 6.25 µl, 12.5 µl, 25 µl, 50 µl and 100 µl) of V2 RTS beverage sample for MTT assay, the parameters such as cell viability and inhibition of growth of the MCF 7 cell line were noted and compared with control cell sample (Fig 1). The percentage of growth inhibition of the treated cells with different doses of V2 RTS beverage was seen in Table 7.

From the results, it was observed that the cell viability decreased due to increased growth inhibition by V2 RTS beverage sample. With respect to different concentrations of the beverage, significant decreases in cell viability were observed in the concentrations of 50 µl and 100 µl. The inhibitory activity of the cell growth was more significant.

Analysis on human hepatic carcinoma cell line (HepG2)

After treatment with various concentrations (3.125 µl, 6.25 µl, 12.5 µl, 25 µl, 50 µl and 100 µl) of V2 RTS beverage sample for MTT assay, the parameters like cell viability and inhibition of growth of the HepG2 cell line were noted and compared with control cell sample. From the results, it was observed that the cell viability was decreased in inhibition of growth by the V2 RTS beverage sample (Fig 1). With respect to different concentrations of the enzyme, significant decreases in cell viability were observed in the concentrations of 25 µl, 50 µl and 100 µl (Table 7 and Fig 2).

Table 7: MCF-7 cell line viability of V2 RTS beverage treated.

Conc.	Control	3.125 µl	6.25 µl	12.5 µl	25 µl	50 µl	100 µl
Abs.	0.378	0.397	0.378	0.368	0.365	0.318	0.198
	0.381	0.394	0.378	0.377	0.342	0.307	0.182
	0.362	0.399	0.379	0.382	0.35	0.314	0.179
Avg.	0.373	0.396	0.378	0.375	0.352	0.313	0.186

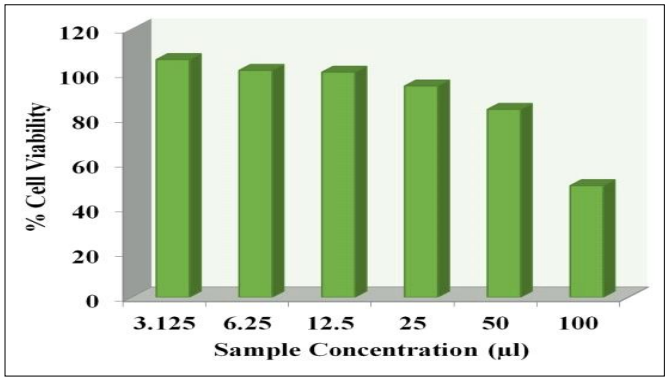


Fig 1: Percentage of cell viability at different sample concentrations of V2 RTS beverage sample.

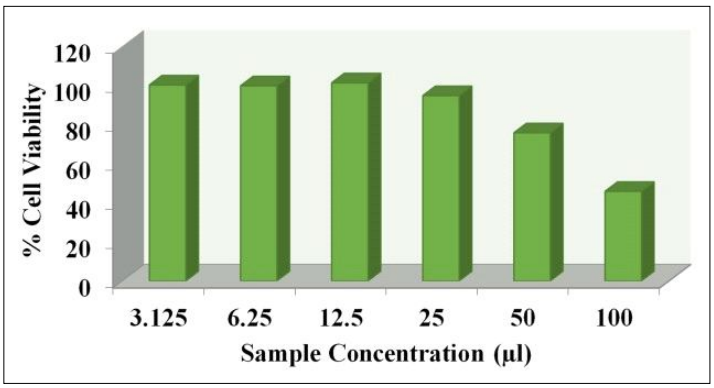


Fig 2: Percentage of cell viability at different sample concentrations of enzyme.

The growth inhibitory activity was more significant than the percentage viability. The percentage of growth inhibition of the treated cells with different doses of V2 RTS beverage sample was seen in figure.

CONCLUSION

Fruit beverages receive a considerable amount of attention reflecting a growing awareness of the potential of these products in the market place. These beverages have high nutritional quality,functional properties and increased energy value.The changes observed in the experimental sample was decrease in pH, ascorbic acid, total sugar content with theconcomitant increase in acidity, TSS andreducing sugar content. The prepared RTS beverage V2 had highest value of calcium, protein, phosphate, vitamin C and antioxidant content. The musk melon with whey beverage in this study will be gainful, reach the weaker sections of people who are deprived of such nutritious beverage and extents storage life. Hence, muskmelon fruit juice blended with whey water can be successfully utilized for the production of good quality and nutritionally enriched products with remunerative cost on commercial scale. Therefore, it can be concluded that muskmelon juice may be incorporated in whey water up to the ratio of 60: 40 to prepare RTS beverage, having reasonable amount of vitamins, minerals and high quality protein and will be acceptable to consumers and may serve as protein rich health drink.

Conflict of interest

All authors declared that there is no conflict of interest.

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