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RESEARCH ARTICLE

Development of Triple Component Aqueous Blend Derived from *Stevia rebaudiana*, *Musa acuminata* and *Solanum lycopersicum*

Shom Prakash Kushwaha[1,2], Sujeet Kumar Gupta[3], Munendra Mohan Varshney[4], Deepanshu Sharma[5], Irfan Aziz[2], Hemendra Mishra[6], Syed Misbahul Hasan[2], Kuldeep Singh[2], Arun Kumar[2] and Sushil Kumar[1*]

[1]School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Moradabad - 244102, Uttar Pradesh, India; drsushiliftm@gmail.com

[2]Faculty of Pharmacy, Integral University, Dasauli, Kursi Road, Lucknow - 226026, Uttar Pradesh, India

[3]Hygia Institute of Pharmaceutical Education and Research, Lucknow - 226020, Uttar Pradesh, India

[4]Raj Kumar Goel Institute of Technology (Pharmacy), Ghaziabad - 201003, Uttar Pradesh, India

[5]Advanced Institute of Pharmacy, Delhi Mathura Road, Palwal - 121105, Haryana, India

[6]Department of Pharmacy, Jharkhand Rai University, Ranchi, Ulatu - 834010, Jharkhand, India

Abstract

Background: *Stevia rebaudiana* Bertoni leaves give similar satiety as that of sucrose. These have the additional benefit of diminished calorie intake and cause a reduction in body weight. Bananas have vital nutrients and high potassium content. Tomato is a rich source of water-soluble antioxidants. **Aim:** Stevia leaves, banana fruits and tomato fruits are processed in search of an extract equipped with antioxidant activity mainly due to water-soluble constituents. **Methods:** Ripe and unripe fruit extracts were mixed in various ratios and screened for DPPH inhibition. The dual component fruit extracts possessing the highest free radical scavenging (banana extract consisting of ripe banana extract: unripe banana extract in a ratio of 2:1 while tomato extract consisted of ripe tomato extract: unripe tomato extract in a ratio of 1:2) were further intermingled with stevia extract. **Results:** The triple component extract consisting of stevia extract: tomato extract: and banana extract in a ratio of 7:3:3 exhibited the highest total phenolic content, total flavonoid content and superior DPPH inhibition activity. The composition of the aqueous extract was stevia leaves: ripe banana fruit: unripe banana fruit: ripe tomato fruit: unripe tomato fruit in the ratios of 7: 2: 1: 1: 2. **Conclusion:** The mingling of aqueous extracts of stevia, ripe/unripe banana and ripe/unripe tomato may be a suitable strategy in free radical-mediated disorders such as diabetes. This paves the path for further phytochemical and pharmacological screening for stevia leaves, banana fruits and tomato fruits-based prophylactic/therapeutic options.

Major Findings: The intermingling of the water-soluble secondary metabolites from *Stevia rebaudiana* Bertoni leaves, *Musa acuminata* Colla. fruits and *Solanum lycopersicum* L. fruits result in enhanced antioxidant activity.

Keywords: Antioxidant, Banana, Polyphenols, Stevia, Tomato

1. Introduction

Fruit intake decreases mortality from senescence-related disorders relatively due to the antioxidant compounds[1,2]. Their aqueous extracts are most tolerable[3] and antioxidant activity is related to total phenolic compounds and flavonoids. Low sucrose content resent in reduced concentration in unripe as well as ripe tomato fruits[4]. This is due to sucrose breakdown into glucose and fructose by invertase[5].

*Author for correspondence

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Organic acids in tomato[6] impart acidity and ripening decreases the acidity due to low concentration of organic acids[7]. *Musa acuminata* Colla. fruits also have a variety of water-soluble components such as phenolics[8], flavonoids, quercetin, kampferol[9], ferulic acid. Phenolic and flavonols promote the antioxidant activity of banana[10] and dopamine[11] contributes significantly to the antioxidant activity. *Stevia rebaudiana* Bertoni leaves exhibit high antioxidant activity with the additional advantage of low-calorie[12] load. Its rebaudioside A elicits a cleaner sweetness and is highly soluble in hot water due to its more polar nature. Stevia is an added advantage of thermal as well as chemical stability and absence of toxic nature[13].

Aqueous extracts of tomato fruits, banana fruits and stevia leaves extract show a high presence of water-soluble secondary metabolites[14]. Polyphenols present in the fruits have an edge because saccharides increase their assimilation. Saccharides particularly monosaccharides couple to polyphenols as hydrogen ligands to hydroxyl groups of polyphenols[15]. Synergism between stevioside and polyphenols is known to alter anthocyanin content[16].

pH is known to play a sturdy role in microbial contamination. The titratable acidity of tomatoes has large fluctuations. Higher titratable acidity discourages fungal infection. Total sugars directly relate to pH and titratable acidity. Thus plants with high sugars have elevated free organic acids and reduced pH while plants with low sugars[17]. Safety regulations ensure that tomato products are acidified to pH 4.6 or below. This prevents the growth of microorganisms such as *Clostridium botulinum*[18].

Tomatine is a mixture of two glycoalkaloids, α-tomatine and dehydrotomatine and exhibits toxicity against humans[19]. Unripe tomato fruits have a high concentration of tomatine which is reduced to about 5 mg/kg in ripe tomato fruits[20]. The aqueous extracts are rich in polyphenols[21] and a high concentration of biologically active components might increase the toxicity of stevia extracts[22]. Thus, toxicity study in natural products is mandatory due to the chances of detrimental interactions between constituents.

Antioxidant compounds are known to act synergistically[23]. The above literature encouraged us the fortify the water-soluble ingredients of stevia leaves and banana and tomato fruits to enhance the antioxidant potential of the formulation.

2. Materials and Methods

2.1 Chemicals

2,2-diphenyl-1-picrylhydrazyl, methanol, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, aluminium chloride, potassium acetate, quercetin was purchased from Sigma Aldrich (India).

2.2 Plant Materials

Stevia rebaudiana Bertoni leaves (family: *Asteraceae*), *Musa acuminata* Colla. fruits (family: *Musaceae*) and *Solanum lycopersicum* L. fruits (family: *Solanaceae*) were purchased from the local market of Lucknow, India and subjected to authentication (IU/PHAR/HRB/20/03, IU/PHAR/HRB/20/01, IU/PHAR/HRB/20/02) at Department of Pharmacy, Faculty of Pharmacy, Integral University, Lucknow, India.

3. Preparation of Aqueous Extracts

3.1 Stevia Leaves Extract (SE)

Air-dried stevia leaves were ground into a coarse powder. 4.5 g powder and 77.5 mL water were added to the soxhlet assembly and subjected to three cycles of extraction. Copper is an integral part of the antioxidant enzymes Cu, Zn-SOD and ceruloplasmin[24]. To add traces of copper, the obtained extract was stored in a copper vessel for 5 hours for further use[25].

3.2 Ripe Banana Fruit Extract (RB)

Mesocarp of the ripe banana fruits were sliced into thin strips, air dried in the shade[26] and reduced to a coarse powder. 5 g of powder was taken in a soxhlet assembly containing 100 mL water and subjected to three cycles of extraction.

3.3 Unripe Banana Fruit Extract (UB)

Unripe banana fruits were sliced into thin strips, air-dried in the shade and reduced to coarse powder. 5 g of powder was added to 150 mL water and subjected to an aqueous microwaved extraction process at 450 watts for 15 minutes to obtain the extract.

3.4 Ripe Tomato Fruit Extract (RT)

Ripe tomato fruits were sliced into thin strips, air-dried in the shade and reduced to a coarse powder. 5 g of powder was added to a soxhlet assembly containing

100 mL water and subjected to three successive extraction cycles[27].

3.5 Unripe Tomato Fruit Extract (UT)

Unripe tomato fruits were sliced into thin strips; air-dried in the shade and reduced to a coarse powder. 5 g of powder and 150 mL of water were subjected to an aqueous microwaved extraction process at 450 watts for 15 minutes to obtain the extract.

4. *In Vitro* Assay of Fruit Extracts

Tomato and banana extracts were rationalized individually by mixing extracts of unripe and unripe fruits and screening for free radical foraging as per the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Different combinations of ripe and unripe fruit extracts (5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5) were subjected to *in vitro* antioxidant activity.

4.1 DPPH Assay

DPPH (0.0050 g) was dissolved in methanol (100 mL) to generate a DPPH stock solution (50 µg/mL). Working solutions were prepared at the time of the experiment by dilution of DPPH stock solution with methanol to obtain the absorbance at 517 nm below one[28]. Extract stock solution (100 µg/mL) was prepared in methanol and serially diluted with methanol to obtain test solution (50, 40, 30, 20, 10 µg/mL). Three mL of DPPH solution was taken in a quartz cuvette, and absorbance was recorded at 517 nm. 1 mL of extract solution was added to the DPPH solution, mixed rapidly, and kept in the dark for incubation at 20 ± 2 ºC for 60 minutes and absorbance recorded at 517 nm by UV-Visible spectrophotometer. The assay was performed in triplicate using ripe fruit extract and unripe fruit extracts as references. The percentage of radical scavenging activity is equal to [(Control absorbance – Sample absorbance) / Control absorbance] X 100. Per cent

Table 1. Free radical scavenging of mixtures of aqueous extracts of ripe and unripe fruits (dual component)

Reference extracts	Test extracts	IC_{50}	Unripe fruit extract as reference		Ripe fruit extract as reference	
			Change in IC_{50}	p-value (significance level)	Change in IC_{50}	p-value (significance level)
IC_{50}: Unripe tomato (46.9597 ± 0.2368) Ripe tomato (54ºº6438 ± 0.254517)	Ripe tomato extract + Unripe tomato extract (5:1) [RT5:UT1]	52.0687 ± 0.6073	-5.109	0.001431 (Very significant)	2.575	0.017386 (Very Significant)
	Ripe tomato extract + Unripe tomato extract (4:1) [RT4:UT1]	53.9489 ± 0.0655	-6.989	0.000009 (Highly significant)	0.695	0.057334 (Non-significant)
	Ripe tomato extract + Unripe tomato extract (3:1) [RT3:UT1]	53.5198 ± 0.6454	-6.560	0.000673 (Highly significant)	1.124	0.180514 (Non-significant)
	Ripe tomato extract + Unripe tomato extract (2:1) [RT2:UT1]	50.9597 ± 0.2368	-4.000	0.000281 (Highly significant)	3.684	0.000449 (Highly significant)
	Ripe tomato extract + Unripe tomato extract (1:1) [RT1:UT1]	45.8102 ± 0.3930	1.149	0.066386 (Nonsignificant)	8.834	0.000046 (Highly significant)
	Ripe tomato extract + Unripe tomato extract (1:2) [RT1:UT2]	44.3811 ± 0.1566	2.579	0.000815 (Highly significant)	10.26	0.000004 (Highly significant)
	Ripe tomato extract + Unripe tomato extract (1:3) [RT1:UT3]	45.3338 ± 0.1722	1.625	0.005158 (Significant)	9.309	0.000007 (Highly significant)
	Ripe tomato extract + Unripe tomato extract (1:4) [RT1:UT4]	47.2869 ± 0.8551	-0.327	0.730947 (Nonsignificant)	7.357	0.001180 (Very significant)
	Ripe tomato extract + Unripe tomato extract (1:5) [RT1:UT5]	54.3902 ± 0.1366	-7.431	0.000011 (Highly significant)	0.2536	0.429545 (Non-significant)

(*Continued*)

Table 1. Continued...

IC_{50}: Unripe banana (57.23333 ±0.466036) Ripe banana (62ª94667 ± 1.589647)	Ripe banana extract + Unripe banana extract (5:1) [RB5:UB1]	56.7745 ± 0.0777	0.4588	0.386494 (Nonsignificant)	4.172	0.058711 (Non-significant)
	Ripe banana extract + Unripe banana extract (4:1) [RB4:UB1]	53.6341 ± 0.3716	3.599	0.003792 (Highly significant)	7.313	0.010995 (Very significant)
	Ripe banana extract + Unripe banana extract (3:1) [RB3:UB1]	53.5145 ± 0.4711	3.719	0.004954 (Highly significant)	7.432	0.010968 (Very significant)
	Ripe banana extract + Unripe banana extract (2:1) [RB2:UB1]	52.4836 ± 0.5040	4.750	0.002289 (Highly significant)	8.463	0.007106 (Highly significant)
	Ripe banana extract + Unripe banana extract (1:1) [RB1:UB1]	54.2473 ± 0.3406	2.986	0.006638 (Highly significant)	6.699	0.014603 (Very significant)
	Ripe banana extract + Unripe banana extract (1:2) [RB1:UB2]	56.2160 ± 0.4333	1.017	0.185141 (Nonsignificant)	4.731	0.045416 (Significant)
	Ripe banana extract + Unripe banana extract (1:3) [RB1:UB3]	59.9057 ± 0.1914	-2.672	0.006069 (Highly significant)	1.041	0.551061 (Non-significant)
	Ripe banana extract + Unripe banana extract (1:4) [RB1:UB4]	60.2353 ± 0.2608	-3.002	0.004924 (Highly significant)	0.7113	0.681632 (Non-significant)
	Ripe banana extract + Unripe banana extract (1:5) [RB1:UB5]	60.0302 ± 0.1525	-2.797	0.004670 (Highly significant)	0.9165	0.596758 (Non-significant)

scavenging activity was graphed with concentration and IC_{50} value (extract concentration providing fifty percentage radicals scavenging activity) recorded from a graph using linear regression analysis.

5. Intermingling of Tomato and Banana Extracts with Stevia Extract

The highest free radical scavenging banana extract (ripe banana extract and unripe banana extract in a ratio of 2:1 extract) and tomato extract (ripe tomato extract and unripe tomato extract in a ratio of 1:2) were further intermingled with stevia extract. Acid contents in foods may be high (pH < 3.7), moderate like tomato (3.7 < pH < 4.5) or low like banana and stevia (pH > 4.5)[29]. We mixed the aqueous extracts of stevia leaves, tomato fruits and banana fruits in different ratios to obtain tricomponent extracts having desired pH of 4.60. The three extracts (tomato, stevia and banana) were mixed as a three-way factorial design (independent variables: tomato extract in a ratio of 1 – 3, banana extract in a ratio of 1 – 3 and stevia extract in a ratio of 1 – 10) and one observed value of pH of final extract (dependent variable). Stevia extract was mixed in ratio of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 (SE1, SE2, SE3, SE4, SE5, SE6, SE7, SE8, SE9, SE10 respectively); Tomato extract mixed in ratio of 1, 2, 3 (TE1, TE2, TE3 respectively) and the banana extract was mixed in the ratio of 1, 2, 3 (BE1, BE2, BE3 respectively).

6. Analysis of Triple Component Aqueous Extract (Stevia, Tomato and Banana)

The obtained triple component extracts having a pH of 4.6 were subjected to total phenolic and total flavonoid determinations along with screening for DPPH inhibition.

6.1 Total Phenolic Content by Folin-Ciocalteu Assay

500 microliter of aqueous extract solution (1000 µg/mL), 3.7 millilitres of water, 250 microliters of Folin-Ciocalteu reagent and 500 microliters of sodium carbonate (5%) mixed and placed in the dark at 20 ± 2 °C for 1 hour. The absorbance of the sample was noted at 760 nm in comparison to the reagent blank. A gallic acid (0 – 100 µg/mL) standard curve was utilized to derive the total phenolic content of the sample calculated as equivalents of gallic acid.

Table 2. pH of the tricomponent extract prepared by mixing different ratios of aqueous extracts of stevia, tomato and banana

Tomato extract	Banana extract	Stevia extract									
		SE1	SE2	SE3	SE4	SE5	SE6	SE7	SE8	SE9	SE10
TE1	BE1	4.543 ± 0.003	4.587 ± 0.003	4.617 ± 0.003	4.637 ± 0.003	4.650 ± 0.000	4.667 ± 0.003	4.673 ± 0.003	4.667 ± 0.003	4.677 ± 0.003	4.680 ± 0.000
TE1	BE2	4.737 ± 0.003	4.743 ± 0.003	4.737 ± 0.003	4.743 ± 0.003	4.737 ± 0.003	4.743 ± 0.003	4.740 ± 0.000	4.733 ± 0.003	4.730 ± 0.000	4.723 ± 0.003
TE1	BE3	4.857 ± 0.003	4.833 ± 0.003	4.827 ± 0.003	4.803 ± 0.003	4.803 ± 0.003	4.807 ± 0.003	4.790 ± 0.000	4.783 ± 0.003	4.780 ± 0.006	4.783 ± 0.003
TE2	BE1	4.303 ± 0.003	4.373 ± 0.003	4.400 ± 0.035	4.477 ± 0.003	4.513 ± 0.003	4.537 ± 0.003	4.563 ± 0.003	4.567 ± 0.003	4.587 ± 0.003	4.600 ± 0.000
TE2	BE2	4.513 ± 0.003	4.537 ± 0.003	4.573 ± 0.003	4.590 ± 0.000	4.610 ± 0.000	4.620 ± 0.000	4.630 ± 0.000	4.633 ± 0.003	4.637 ± 0.003	4.653 ± 0.003
TE2	BE3	4.650 ± 0.000	4.657 ± 0.003	4.667 ± 0.003	4.670 ± 0.000	4.677 ± 0.003	4.680 ± 0.000	4.683 ± 0.003	4.687 ± 0.003	4.687 ± 0.003	4.703 ± 0.003
TE3	BE1	4.160 ± 0.006	4.243 ± 0.007	4.323 ± 0.003	4.373 ± 0.003	4.413 ± 0.003	4.443 ± 0.003	4.470 ± 0.000	4.493 ± 0.003	4.513 ± 0.003	4.523 ± 0.003
TE3	BE2	4.353 ± 0.003	4.400 ± 0.000	4.447 ± 0.003	4.483 ± 0.003	4.500 ± 0.000	4.523 ± 0.003	4.540 ± 0.000	4.553 ± 0.003	4.570 ± 0.000	4.583 ± 0.003
TE3	BE3	4.490 ± 0.000	4.512 ± 1.330	4.543 ± 0.003	4.567 ± 0.003	4.577 ± 0.003	4.587 ± 0.003	4.600 ± 0.000	4.613 ± 0.003	4.623 ± 0.003	4.633 ± 0.003

Table 3. Total phenolic content of the triple component aqueous extract

Total phenolic content (mg GAE/g dry weight)			p value (Level of significance
Reference extract	Test extract	Δ	
Stevia (18.7167 ± 0.3135)	Extract A (21.6800 ± 0.3245)	2.963	0.002782 (Very significant)
Banana (14.7000 ± 0.3027)		6.980	0.000095 (Highly significant)
Tomato (26.7833 ± 0.4028)		-5.103	0.000592 (Highly significant)
Stevia (18.7167 ± 0.3135)	Extract B (30.0400 ± 0.3002)	11.32	0.000013 (Highly significant)
Banana (14.7000 ± 0.3027)		15.34	0.000004 (Highly significant)
Tomato (26.7833 ± 0.4028)		3.257	0.002918 (Very significant)

6.2 Total Flavonoid Content

500 µl aqueous extract solution (1000 µg/mL), 1.5 µl methanol, 0.1 µl aluminium chloride (10 %), 1.0 µl potassium acetate and 2.8 µl water mixed and incubated in dark at 20 ± 2 °C for 30 minutes. The absorbance was measured at 415 nm against a reagent blank. A quercetin (0 – 200 µg/mL) standard graph was utilized to derive the total flavonoid content of the sample as equivalents of quercetin.

6.3 *In Vitro* Assay of The Triple Component Aqueous Extract by DPPH Assay

DPPH assay for the extract was done using ascorbic acid and stevia extract as reference.

7. Results

7.1 Dual Component Extract

Tomato extracts of unripe and ripe fruits were found to have IC_{50} in the range of 44.38 - 54.39 µg/mL as compared to unripe tomatoes (46.95 µg/mL) and ripe tomatoes (54.64 µg/mL). Extract consisting of ripe

Table 4. Total flavonoid content of the triple component aqueous extract

Total flavonoid content (mg QE/g dry weight)			p-value (Level of significance
Reference extract	Test extract	Δ	
Stevia (10.060 ± 0.3381)	Extract A (12.9833 ± 0.2431)	2.923	0.002170 (Very significant)
76Banana (7.9367 ± 0.2742)		5.047	0.000161 (Highly significant)
Tomato (16.8333 ± 0.2695)		-3.850	0.000448 (Highly significant)
Stevia (10.060 ± 0.3381)	Extract B (18.9233 ± 0.2469)	8.863	0.000029 (Highly significant)
Banana (7.9367 ± 0.2742)		10.99	0.000008 (Highly significant)
Tomato (16.8333 ± 0.2695)		2.090	0.004632 (Very significant)

Table 5. DPPH assay of the triple component aqueous extract

$I_{C5}0$			p-value (Level of significance
Reference	Test extract	Δ	
Ascorbic acid (29.9597 ± 0.5383)	Triple component aqueous extract A (41.5569 ± 0.3662)	11.60	0.000058 (Highly significant)
Stevia extract (52.3509 ± 0.9422)		-10.79	0.000436 (Highly significant)
Ascorbic acid (29.9597 ± 0.5383)	Triple component aqueous extract B (35.8426 ± 0.4471)	5.883	0.001096 (Very significant)
Stevia extract (52.3509 ± 0.9422)		-16.51	0.000093 (Highly significant)

tomato extract and unripe tomato extract in ratio of 1:2 was the most active (IC_{50} = 44.38 µg/mL). Banana extracts of unripe and ripe fruits exhibited IC_{50} in the range of 52.48 - 60.24 µg/mL as compared to unripe banana (57.23 µg/mL) and ripe banana (62.95 µg/mL). Extract consisting of ripe banana extract and unripe banana extract in proportion of 2:1 was the most active (IC_{50} = 52.48 µg/mL) (Table 1).

7.2 Tricomponent Extract

The three extracts (stevia extract: pH = 4.73, banana extract: pH 5.34 and tomato extract: pH = 3.56) were mixed as per three-way factorial design. We obtained two triple components (stevia, banana, tomato) extracted mixture with a target pH of 4.6. They consisted of stevia extract: tomato extract: and banana extract in ratios of 10:2:1 (A) and 7:3:3 (B) (Table 2).

7.3 Screening of Triple Component Extracts

The total phenolic content of the triple component extracts was determined as mg gallic acid equivalent per gram weight of dry extract and compared with the extracts of stevia (18.72), banana (14.70), and tomato (26.78). Triple component extract consisting of stevia extract: tomato extract: and banana extract in ratio of 7:3:3 (B) and 10:2:1 (A) exhibited phenolic content of 30.04 and 21.68 respectively (Table 3). The total flavonoid content of the triple component extracts was determined as mg quercetin per gram weight of dry extract and compared with extracts of stevia (10.06), tomato (16.83) and banana (7.94). Triple component extracts 7:3:3 (B) and 10:2:1 (A) demonstrated flavonoid content as 18.92 and 12.98 respectively (Table 4). The DPPH inhibition assay (IC_{50}) of the triple component extracts was determined using ascorbic acid (29.95) and stevia extract (52.35) as references. Triple component extract 7:3:3 (B) and 10:2:1 (A) exhibited IC_{50} of 35.84 and 41.56 respectively (Table 5).

8. Discussion

Aqueous extraction is known to ensure high recovery of polyphenols[30,31], glycoside[32,33] and protocatechuic acid[34]. Phenols such as caffeic acid and catechin appear trendy in aqueous solvents compared to nonaqueous solvents. Phenolic compounds often exhibit different stabilities in various solvents. They may benefit from better stabilisation in aqueous environments due to interactions with water molecules, which can affect their chemical reactivity and degradation. Caffeic Acid: In water, caffeic acid can remain more stable because the aqueous environment helps to stabilize the phenolic

structure and prevent unwanted reactions. Catechin: Catechin's stability in water may be attributed to similar interactions with water that protect it from degradation compared to less polar solvents. These shreds of evidence prompted us to aqueous extractions of stevia leaves, unripe tomato fruits, ripe tomato fruits, unripe banana fruits and ripe banana fruits. Thus a solvent has established safety[35,36] and superiority in extracting soluble polyphenols[37] was used for the extraction.

Ripe and unripe fruit extracts were mixed in various ratios and screened for DPPH inhibition. These mixtures exhibited enhanced free radical scavenging as compared to individual extracts of unripe fruits and ripe fruits. Antioxidant activities of banana extracts having higher ratios of ripe banana were more as compared to extracts having higher ratios of unripe banana (Figure 1) while the reverse pattern was seen in tomato extracts (Figure 2). The altering antioxidant activity of mixed extracts of ripe and unripe fruits may be a result of variations in constitutions.

The high concentration of acids in unripe tomatoes may have contributed to the antioxidant activity of the extracts by promoting metal ion chelation[38]. Thus activity variation in the dual component extract may be correlated to a great diversity[39] in phenolic compound content[40,41] and total flavonoid content[42] in ripe and unripe fruits.

The enhanced free radical scavenging of the mixed (unripe and ripe fruit) extracts may be attributed to antioxidant network-mediated synergistic effects[43], glycosides[44] and chelation of pro-oxidative metal ions like Fe^{2+} and Cu^{2+} [45]. The dual component fruit extracts possessing the highest free radical scavenging (banana extract consisting of ripe banana extract: unripe banana extract in a ratio of 2:1 while tomato extract consisted of ripe tomato extract: unripe tomato extract in a ratio of 1:2) were further intermingled with stevia extract. The reported antioxidant activity[46,47] and low-calorie profile of stevia leaves encouraged us for its addition to fruit extracts. We hoped for further enhancement of antioxidant activity with an additional benefit from the low-calorie load of the developed extract.

The pH of aqueous extracts of stevia leaves and banana fruits have been previously reported to follow a range between 5.8[48]-5.95[49] and 4.50-5.97[50] respectively while aqueous extract of tomato has a pH array of

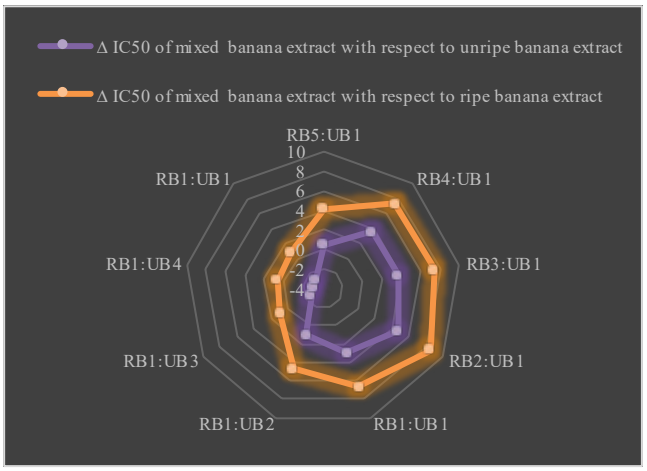


Figure 1. IC_{50} of mixed banana extracts in comparison to individual extracts of unripe/ripe banana.

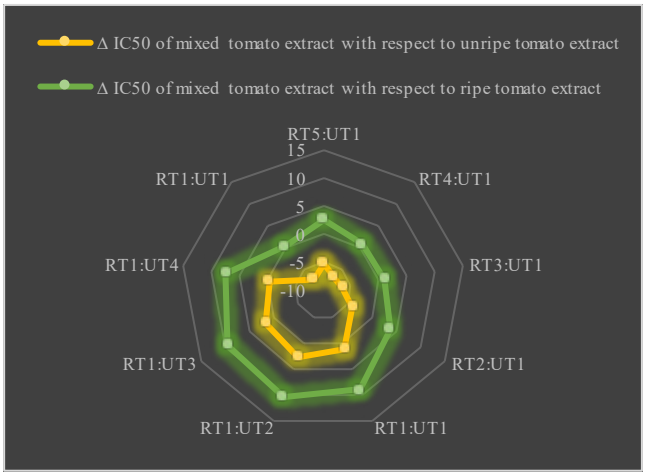


Figure 2. IC_{50} of mixed tomato extracts in comparison to individual extracts of unripe/ripe tomato.

3.6-4.6[51]. The same pH profiles are observed in the current study. Banana and stevia extracts were more basic as compared to tomato extracts. High-pH products such as stevia and bananas provide a good medium for the growth and survival of microorganisms[52]. In the current study, the possibility of microorganism growth in banana and stevia extracts was cut off by mixing these two extracts with the tomato extract in different ratios. The fact that mixing banana and stevia extracts with tomato extract inhibited microorganism growth is a significant indicator. Tomato extract contains various bioactive compounds, including lycopene, which are known for their antimicrobial properties. This suggests that the antimicrobial components in tomato extract

might be contributing to the preservation of the mixed extracts. Inhibition of microbial growth: If the tomato extract effectively prevents microbial growth in the mixture, it could imply that the antimicrobial properties are enhancing the stability of the extracts. This can potentially extend the shelf life of the banana and stevia extracts when combined with tomato extract. The stability of the extracts over time may be influenced by the interactions between the components of the extracts. Antioxidant properties: Both banana and stevia extracts have antioxidant properties. Tomato extract, rich in lycopene and other antioxidants, might contribute to the overall antioxidant capacity of the mixture. Enhanced antioxidant activity can help in preserving the quality of the extracts by preventing oxidative degradation. pH levels: The pH of the mixture can affect the stability of the extracts. Tomato extract is acidic, which might lower the pH of the mixture. Lower pH levels can inhibit the growth of certain microorganisms and slow down the degradation of some compounds, potentially contributing to the stability and shelf life of the extracts.

8.1 Shelf-life Implications

Our target was to obtain tricomponent extract having a pH of 4.60 without the addition of an external acidifier. The pH of the resultant extracts is juggling the pH of the stevia extract, banana extract and tomato extract (Figure 3). The intermingling of stevia extract: tomato extract: and banana extract in ratios of 10:2:1 and 7:3:3 resulted in a pH of 4.6. Tomato extract decreased the pH of banana and stevia extracts while a pH lower than 4.6 was avoided. This increases the acceptability of the developed extract even in patients having acid-related gastric problems. A higher ratio of stevia extract is being used in the current study so that the tricomponent extract may act as a low-calorie sweetener due to the low glycemic load of the extract.

The triple component extract consisting of stevia extract: tomato extract: and banana extract in a ratio of 7:3:3 exhibited higher total phenolic content, total flavonoid content and superior DPPH inhibition activity as compared to extract having triple components in a ratio of 10:2:1 (A). Our results are analogues to the previous studies supporting that antioxidant activity is directly correlated with total phenolic content and total flavonoid content[53,54]. Pharmacodynamics and pharmacokinetic interactions between ingredients[55] may also have contributed to synergism[56]. The enhanced activity of the extract supported gulcin[57] findings that interactions between constituents result in effects that are not the obligatory properties of the individual constituents. Its banana extract consists of ripe banana: unripe banana in the proportion of 2:1

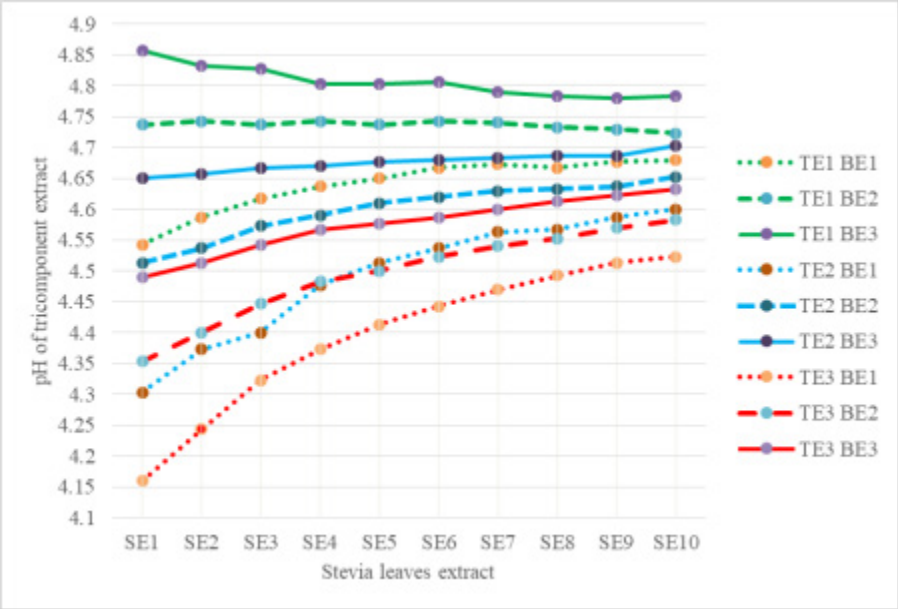


Figure 3. pH of the tricomponent extracts.

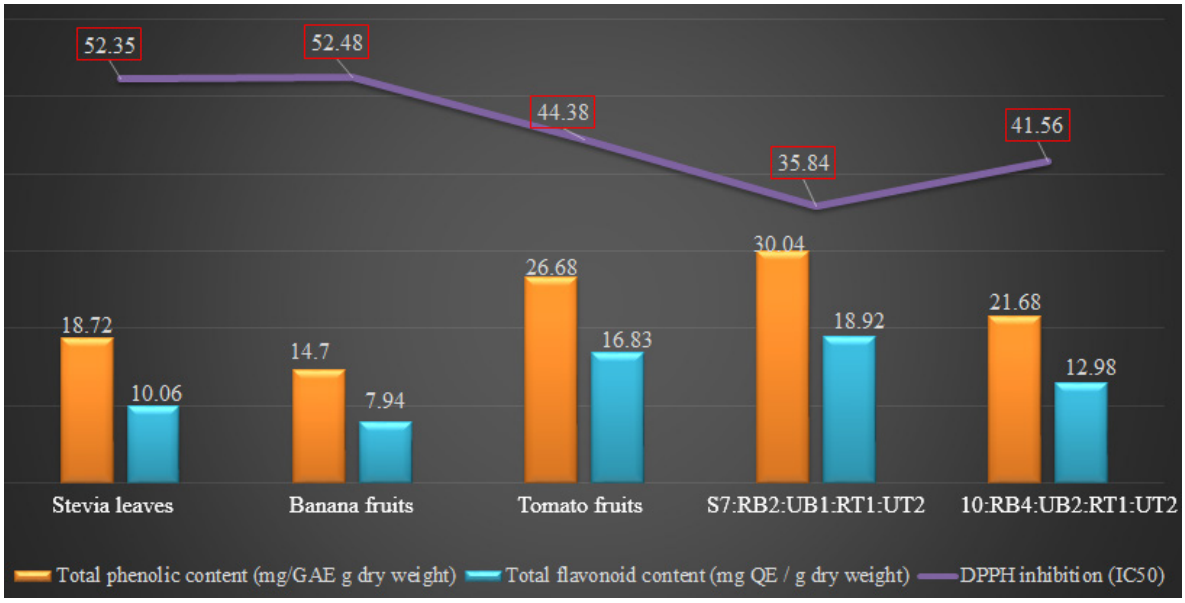


Figure 4. Total phenolic content, total flavonoid content and DPPH inhibition by tricomponent aqueous extracts.

while tomato extract comprises ripe tomato: unripe tomato in the ratio of 1:2. Thus composition of the extract B (7:3:3) can be summarized as the extracts of stevia: ripe banana: unripe banana: ripe tomato: unripe tomato in the ratio of 7: 2: 1: 1: 2. (S7:RB2:B1:RT1:UT2) (Figure 4).

9. Conclusion

The intermingling of the water-soluble secondary metabolites from *Stevia rebaudiana* Bertoni leaves, *Musa acuminata* Colla. fruits and *Solanum lycopersicum* L. fruits result in enhanced antioxidant activity. Acute toxicity screening reveals that oral administration of the extract is non-toxic[58]. Plant-derived water-soluble compounds demonstrate a plethora of beneficial biological activities. Catechin increases LDL resistance to oxidation, quercetin promotes overall cardiovascular health. Phenolic compounds lower blood sugar levels by inhibiting α-amylase. Flavonoids prevent the oxidation of antioxidants like ascorbic acid by undergoing privileged oxidation.

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11. Author Contribution

Shom Prakash Kushwaha: Experimental work; Sujeet Kumar Gupta: Supervision of work; Munendra Mohan Varshney: Drafting of research findings; Deepanshu Sharma: Drafting of research findings; Irfan Aziz: Drafting of research findings; Hemendra Mishra: Drafting of research findings; Syed Misbahul Hasan: Drafting of research findings; Kuldeep Singh: Drafting of research findings; Arun Kumar: Drafting of research findings; Sushil Kumar: Supervision of work. All authors have read and agreed to the published version of the manuscript.

12. References

1. Chen Z, Bertin R, Froldi G. EC50 estimation of antioxidant activity in DPPH assay using several statistical programs. Food Chem. 2013; 138(1):414-420. https://doi.org/10.1016/j.foodchem.2012.11.001. PMID: 23265506.
2. Kumar A. *Carissa carandas* Linn. (Karonda): An exotic minor plant fruit with immense value in the nutraceutical and pharmaceutical industries. Asian J Biomed Pharm Sci. 2018.

3. Gaweł-Bęben K, Bujak T, Nizioł-Łukaszewska Z, Antosiewicz B, Jakubczyk A, Karaś M, *et al*. *Stevia rebaudiana* Bert. leaf extracts as a multifunctional source of natural antioxidants. Molecules. 2015; 20(4):5468-5486. https://doi.org/10.3390/molecules20045468. PMID: 25826787. PMCID: PMC6272195.
4. Paolo D, Bianchi G, Scalzo RL, Morelli CF, Rabuffetti M, Speranza G. The chemistry behind tomato quality. Nat Prod Commun. 2018; 13(9):1934578X1801300927. https://doi.org/10.1177/1934578X1801300927
5. Richardson DL, Davies HV, Ross HA, Mackay GR. Invertase activity and its relation to hexose accumulation in potato tubers. J Exp Bot. 1990; 41(1):95-99. https://doi.org/10.1093/jxb/41.1.95
6. Beckles DM. Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum* L.) fruit. Postharvest Biol Technol. 2012; 63(1):129-140. https://doi.org/10.1016/j.postharvbio.2011.05.016
7. Islam MS, Matsui T, Yoshida Y. Effect of carbon dioxide enrichment on physicochemical and enzymatic changes in tomato fruits at various stages of maturity. Sci Hortic (Amsterdam). 1996; 65(2):137-149. https://doi.org/10.1016/0304-4238(95)00867-5
8. Arif M, Zaman K, Fareed S. Pharmacognostical studies and evaluation of total phenolic contents of trunk bark of *Spondias mangifera* Willd. Nat Prod Res. 2009; 8(2): March-April.
9. Kevers C, Falkowski M, Tabart J, Defraigne JO, Dommes J, Pincemail J. Evolution of antioxidant capacity during storage of selected fruits and vegetables. J Agric Food Chem. 2007; 55(21):8596-8603. https://doi.org/10.1021/jf071736j. PMID: 17880151.
10. Singh B, Singh JP, Kaur A, Singh N. Bioactive compounds in banana and their associated health benefits—a review. Food Chem. 2016; 206:1-11. https://doi.org/10.1016/j.foodchem.2016.03.033. PMID: 27041291.
11. Someya S, Yoshiki Y, Okubo K. Antioxidant compounds from bananas (*Musa Cavendish*). Food Chem. 2002; 79(3):351-354. https://doi.org/10.1016/S0308-8146(02)00186-3
12. Puri M, Sharma D, Tiwari AK. Downstream processing of stevioside and its potential applications. Biotechnol Adv. 2011; 29(6):781-791. https://doi.org/10.1016/j.biotechadv.2011.06.006. PMID: 21726623.
13. Parker KJ. Alternatives to sugar. Nature. 1978; 271(5645):493-495. https://doi.org/10.1038/271493a0. PMID: 340963.
14. Perez GRM, Zavala SMA, Perez GS, Perez GC. Antidiabetic effect of compounds isolated from plants. Phytomedicine. 1998; 5(1):55-75. https://doi.org/10.1016/S0944-7113(98)80060-3. PMID: 23195700.
15. Cieślik E, Gręda A, Adamus W. Contents of polyphenols in fruit and vegetables. Food Chem. 2006; 94(1):135-142. https://doi.org/10.1016/j.foodchem.2004.11.015
16. Kaushik R, Narayanan P, Vasudevan V, Muthukumaran G, Usha A. Nutrient composition of cultivated stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts. J Food Sci Technol. 2010; 47(1):27-33. https://doi.org/10.1007/s13197-010-0011-7. PMID: 23572597. PMCID: PMC3550978.
17. Getinet H, Seyoum T, Woldetsadik K. The effect of cultivar, maturity stage, and storage environment on the quality of tomatoes. J Food Eng. 2008; 87(4):467-478. https://doi.org/10.1016/j.jfoodeng.2007.12.031
18. Heflebower R, Washburn C. The influence of different tomato varieties on acidity as it relates to home canning. J Extension. 2010; 48. https://doi.org/10.34068/joe.48.06.21
19. Milner SE, Brunton NP, Jones PW, O'Brien NM, Collins SG, Maguire AR. Bioactivities of glycoalkaloids and their aglycones from *Solanum* species. J Agric Food Chem. 2011; 59(8):3454-3484. https://doi.org/10.1021/jf200439q. PMID: 21401040.
20. Friedman M. Chemistry and anticarcinogenic mechanisms of glycoalkaloids produced by eggplants, potatoes, and tomatoes. J Agric Food Chem. 2015; 63(13):3323-3337. https://doi.org/10.1021/acs.jafc.5b00818. PMID: 25821990.
21. Kim IS, Yang M, Lee OH, Kang SN. The antioxidant activity and the bioactive compound content of *Stevia rebaudiana* water extracts. LWT-Food Sci Technol. 2011; 44(5):1328-1332. https://doi.org/10.1016/j.lwt.2010.12.003
22. Bast A, Haenen GR. The toxicity of antioxidants and their metabolites. Environ Toxicol Pharmacol. 2002; 11(3-4):251-258. https://doi.org/10.1016/S1382-6689(01)00118-1. PMID: 21782609.
23. Qusti S, Abo-khatwa AN, Lahwa MA. Screening of antioxidant activity and phenolic content of selected food items cited in the Holy Quran [Internet]. undefined. Available from: /paper/SCREENING-OF-ANTIOXIDANT-ACTIVITY-AND-PHENOLIC-OF-Qusti-Abo-khatwa/b6eba4f56247f0b0656e99d7a424873d973151af [Accessed 2021 Jan 26].
24. Johnson MA, Fischer JG, Kays SE. Is copper an antioxidant nutrient? Crit Rev Food Sci Nutr. 1992; 32(1):1-31. https://doi.org/10.1080/10408399209527578. PMID: 1290583.
25. Jiddimani SR, Tonni SS, Vedantam G. Quality assessment of water stored in vessels made of different materials. J Ayurveda. 2021; 15(4):287. https://doi.org/10.4103/joa.joa_51_21
26. Jannat K, Morshed MZ, Akter S, Rahmatullah M. Improved oral glucose tolerance with ripe fruit peels of *Musa seminifera lour.* Arch Nat Med Chem. 2018.
27. Guiné RPF, Gonçalves F, Lerat C, Idrissi TE, Rodrigo E, Gonçalves PMRC, *et al*. Extraction of phenolic compounds with antioxidant activity from beetroot (*Beta vulgaris* L.). Curr Nutr Food Sci. [Internet]. Available from: https://www.eurekaselect.com/153065/article [Accessed 2020 Sep 27].

28. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 2011; 48(4):412-422. https://doi.org/10.1007/s13197-011-0251-1. PMID: 23572765. PMCID: PMC3551182.
29. Barrett DM, Somogyi LP, Ramaswamy H. Processing fruits: science and technology. 2nd ed. Routledge and CRC Press. Available from: https://www.routledge.com/Processing-Fruits-Science-and-Technology-Second-Edition/Barrett-Somogyi-Ramaswamy/p/book/9780849314780 [Accessed 2021 Jan 29].
30. Kim IS, Yang M, Lee OH, Kang SN. The antioxidant activity and the bioactive compound content of *Stevia rebaudiana* water extracts. LWT-Food Sci Technol. 2011; 44:1328-3132. https://doi.org/10.1016/j.lwt.2010.12.003
31. Kim JH, Sung NY, Kwon SK, Jung PM, Choi JI, Yoon YH, *et al*. Antioxidant activity of stevia leaf extracts prepared by various extraction methods. J Korean Soc Food Sci Nutr. 2010; 39:313-318. https://doi.org/10.3746/jkfn.2010.39.2.313
32. Zaidan UH, Mohamad Zen NI, Amran NA, Shamsi S, Gani SSA. Biochemical evaluation of phenolic compounds and steviol glycoside from *Stevia rebaudiana* extracts associated with *in vitro* antidiabetic potential. Biocatal Agric Biotechnol. 2019; 18:101049. https://doi.org/10.1016/j.bcab.2019.101049
33. Ceunen S, Geuns JMC. Steviol glycosides: chemical diversity, metabolism, and function. J Nat Prod. 2013; 76(6):1201-1228. https://doi.org/10.1021/np400203b. PMID: 23713723.
34. Muanda FN, Soulimani R, Diop B, Dicko A. Study on chemical composition and biological activities of essential oil and extracts from *Stevia rebaudiana* Bertoni leaves. LWT-Food Sci Technol. 2011; 44(9):1865-1872. https://doi.org/10.1016/j.lwt.2010.12.002
35. Bhebhe M, Füller TN, Chipurura B, Muchuweti M. Effect of solvent type on total phenolic content and free radical scavenging activity of black tea and herbal infusions. Food Anal Methods. 2016; 9(4):1060-1067. https://doi.org/10.1007/s12161-015-0270-z
36. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010; 15(10):7313-73152. https://doi.org/10.3390/molecules 15107313. PMID: 20966876. PMCID: PMC6259146.
37. Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, *et al*. Techniques for extraction of bioactive compounds from plant materials: a review. J Food Eng. 2013; 117(4):426-436. https://doi.org/10.1016/j.jfoodeng.2013.01.014
38. Hernández M, Rodríguez-Rodríguez EM, Díaz C. Analysis of organic acid content in cultivars of tomato harvested in Tenerife. Eur Food Res Technol. 2008; 226:423-435. https://doi.org/10.1007/s00217-006-0553-0
39. Sulaiman SF, Yusoff NAM, Eldeen IM, Seow EM, Sajak AAB, Supriatno, *et al*. Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (*Musa* sp.). J Food Compos Anal. 2011; 24(1):1-10. https://doi.org/10.1016/j.jfca.2010.04.005
40. Borges CV, Amorim VB, Ramlov F, Ledo CA, Donato M, Maraschin M, *et al*. Characterisation of metabolic profile of banana genotypes, aiming at biofortified *Musa* spp. cultivars. Food Chem. 2014; 145:496-504. https://doi.org/10.1016/j.foodchem.2013.08.041. PMID: 24128506.
41. Mattos LA, Amorim EP, Amorim VB, Cohen KO, Ledo CA, Silva SO. Agronomical and molecular characterization of banana germplasm. *Pesqui Agropecu Bras*. 2010; 45(2):146-154. https://doi.org/10.1590/S0100-204X2010000200005
42. Slimestad R, Fossen T, Verheul MJ. The flavonoids of tomatoes. J Agric Food Chem. 2008; 56(7):2436-2441. https://doi.org/10.1021/jf073434n. PMID: 18318499.
43. Gupta E, Purwar S, Sundaram S, Rai GK. Nutritional and therapeutic values of *Stevia rebaudiana*: a review. 2013; 7:3343-3353.
44. Criado MN, Barba FJ, Frígola A, Rodrigo D. Effect of *Stevia rebaudiana* on oxidative enzyme activity and its correlation with antioxidant capacity and bioactive compounds. Food Bioprocess Technol. 2014; 7(5):1518-1525. https://doi.org/10.1007/s11947-013-1208-3
45. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology. 2011; 283(2-3):65-87. https://doi.org/10.1016/j.tox.2011.03.001. PMID: 21414382.
46. Shukla S, Mehta A, Mehta P, Bajpai VK. Antioxidant ability and total phenolic content of aqueous leaf extract of *Stevia rebaudiana* Bert. Exp Toxicol Pathol. 2012; 64(7-8):807-811. https://doi.org/10.1016/j.etp.2011.02.002. PMID: 21377849.
47. Bae H, Jayaprakasha GK, Crosby K, Jifon JL, Patil BS. Influence of extraction solvents on antioxidant activity and the content of bioactive compounds in non-pungent peppers. Plant Foods Hum Nutr. 2012; 67(2):120-128. https://doi.org/10.1007/s11130-012-0290-4. PMID: 22569831.
48. Abou-Arab AE, Abou-Arab AA, Abu-Salem MF. Physico-chemical assessment of natural sweeteners steviosides produced from *Stevia rebaudiana* Bertoni plant.
49. Chughtai MFJ, Pasha I, Zahoor T, Khaliq A, Ahsan S, Wu Z, *et al*. Nutritional and therapeutic perspectives of *Stevia rebaudiana* as an emerging sweetener; a way forward for sweetener industry. CyTA J Food. 2020; 18(1):164-177. https://doi.org/10.1080/19476337.2020.1721562
50. Kookal S, Thimmaiah A. Nutritional composition of staple food bananas of three cultivars in India. Am J Plant Sci. 2018; 9:2480-9243. https://doi.org/10.4236/ajps.2018.912179
51. Khandaker M, Hossain ABMS, Sani W, Saifuddin M. Effect of stages of maturity and ripening conditions on

the biochemical characteristics of tomato. Am J Biochem Biotechnol. 2008; 4:336-344. https://doi.org/10.3844/ajbbsp.2008.336.344

52. Tribst AAL, Sant'Ana AS, de Massaguer PR. Microbiological quality and safety of fruit juices—past, present and future perspectives. Crit Rev Microbiol. 2009; 35(4):310-339. https://doi.org/10.3109/10408410903241428. PMID: 19863382.
53. Alothman M, Bhat R, Karim AA. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem. 2009; 115(3):785-788. https://doi.org/10.1016/j.foodchem.2008.12.005
54. González-Montelongo R, Lobo MG, González M. The effect of extraction temperature, time and number of steps on the antioxidant capacity of methanolic banana peel extracts. Sep Purif Technol. 2010; 71(3):347-355. https://doi.org/10.1016/j.seppur.2009.12.022
55. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: concept of Ayurveda. Pharmacogn Rev. 2014; 8(16):73-80. https://doi.org/10.4103/0973-7847.134229. PMID: 25125878. PMCID: PMC4127824.
56. Karole S, Shrivastava S, Thomas S, Soni B, Khan S, Dubey J, *et al.* Polyherbal formulation concept for synergic action: a review. J Drug Deliv Ther. 2019; 9(1-s):453-466. https://doi.org/10.22270/jddt.v9i1-s.2339
57. Gülçin İ. Antioxidant activity of food constituents: an overview. Arch Toxicol. 2012; 86(3):345-391. https://doi.org/10.1007/s00204-011-0774-2. PMID: 22102161.
58. Ugwah-Oguejiofor CJ, Okoli CO, Ugwah MO, Umaru ML, Ogbulie CS, Mshelia HE, *et al.* Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N.E. Brown in mice and rats. Heliyon. 2019; 5(1):e01179. https://doi.org/10.1016/j.heliyon.2019.e01179. PMID: 30775575. PMCID: PMC6356088.