Effect of Tamarind (*Tamarindus indica* L.) on the cardiometabolic health of patients living with HIV and elevated triglyceride: a dose-response double-blind, randomized exploratory trial

Supplementary methods

1. Juice Formulation

We aimed to develop sensory-acceptable functional juices from Tamarind. The juice was formulated at the Uganda Natural Chemotherapeutics Research Institute. Fresh Tamarind fruits were sourced from local farmers in Eastern Uganda. We standardized our juice formulations based on differences in fruit pulp percentages. Tamarind fruit pods were manually cracked, and the pulp was scrapped off the seed using stainless steel knives. The scrapped pulp was mashed using a blender. The mash was filtered using a food-grade muslin cloth. Four juice prototypes were formulated to contain varying proportions of Tamarind fruit pulp i.e., 10%, 20%, 30%, and 40%. Taking 1L of juice as a basis, 10% means 100g of pulp in 1L of water. The prototypes were blended with stabilizer E466, at a rate of 0.2-0.5%, preservatives E202 and E211 at a rate of 0.1-1g/L, INS 300 at a rate of 0.5g/L, INS 951 at a rate of 10g/ 100L, brown sugar at a rate of 20g/L. The juice blends were pasteurized at 92^{o}C for 15 s and hot-filled into 300 mL plastic amber bottles and crown capped and refrigerated at 4^{o}C.

2. Sensory characterization of *T. indica* L. fruit juice

2.1 Panellists

A sensory panel of untrained consumers was used to assess the acceptability and preference of the juice subsets. A convenient sample was recruited from the general population to constitute the sensory panel. Sensory evaluation studies allow the use of a small number of participants, often from the target product consumers, to represent the larger consumer population (1). This sensory study was approved by the Uganda National Council for Science and Technology (HS2923ES). Before participation, each participant gave written informed consent. Sociodemographic data and the health status of each participant were recorded.

2.2 Parameters of sensory evaluation and experimental protocol

Preference and sensory acceptability of *T. indica* fruit juice subsets were performed by use of consumer affective tests. The panel evaluated all aspects regarding; 1) Appearance (colour, and consistency), 2) Flavour (taste, and odour), and 3) Kinesthetics (texture, and viscosity) of the juice by use of human sense parameters. This evaluation determined the relationship between physicochemical properties, sensory attributes, and consumer rating of the juice. The test juice was evaluated for both acceptability and preference. This evaluation was used to determine which prototype(s) is liked more than the others. The feedback from participants was used to further optimize the sensory attributes of the selected prototypes.

Equal portions (30 mL) of the formulations were tasted at room temperature in a coded glass. Drinking water at room temperature, salt-less crackers, and expectoration tumblers were provided to enable participants to cleanse their palates in between sample evaluations to reduce carry-over effects.

2.3 Acceptability test

To assess the acceptability of the test juice product prototypes, coded samples were presented to the panel in a monadic sequential protocol. Participants were required to taste each of the four test products and evaluate them for overall liking, appearance, color, flavor, texture, and overall acceptability. A 9-point hedonic scale was used where 9 (like extremely), 8 (like very much), 7 (like moderately), 6 (like slightly), 5 (neither like nor dislike), 4 (dislike slightly), 3 (dislike moderately), 2 (dislike very much), and 1 (dislike extremely) are the extreme scores (2). The panelists were asked to select the category that most represented their perception on the 9-point scale.

2.4 Preference test

The preference test was used to select the two best product subsets from the four provided (1). A paired comparison with a complete factorial design was used (3). During independent sessions, panelists received the four (pairwise) juice prototype samples and were expected to choose two samples over the other. Participants were encouraged to choose the preferred product (forced choice). The two products that were preferred most were selected for mass production.

References

1. Lawless HT, Heymann H. Sensory evaluation of food: principles and practices: Springer Science & Business Media; 2010.
2. Wichchukit S, O'Mahony M. The 9-point hedonic scale and hedonic ranking in food science: some reappraisals and alternatives. Journal of the Science of Food and Agriculture. 2015;95(11):2167-78.
3. Scholz SW, Meissner M, Decker R. Measuring consumer preferences for complex products: A compositional approach based on paired comparisons. Journal of Marketing Research. 2010;47(4):685-98.

Supplementary methods: Phenolic characterization of the tamarind juice prototypes

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1. Materials and methods

1.1 Chemicals

Syringic acid, cinnamic acid, and quercetin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid and propyl gallate were from Acros Organics (Geel, Belgium). Apigenin, eriodictyol, naringenin, luteolin, daidzein, (+)-catechin, (−)-epicatechin, taxifolin and procyanidin B_2 were purchased from MedChemExpress (Monmouth Junction, NJ, USA). Water, acetonitrile (ACN) (both LC-MS grade), acetic acid and methanol (MeOH) were from Acros Organics.

1.2 Sample preparation

Stock solutions of all compounds were prepared in MeOH at a concentration of 10 µM. A working solution was then prepared by diluting the stock solutions in water, adjusting the concentration of each compound to the upper limit of its respective linear range, as specified in Table S-1. For the standard addition method (SAM), spiking solutions were prepared similarly at four different concentrations as specified in Table S-1. Tamarind juices (10% and 30% fruit pulp) were filtered using a 0.45 µm Chromafil RC-45/25 filter (Macherey-Nagel, Düren, Germany). The filtered juice was then diluted 10-fold in each SAM spiking solution by adding 100 µL of juice to 900 µL of each concentration. For the analysis of syringic acid, an additional 10-fold dilution with water was performed before sample preparation, resulting in a total dilution factor of 100.

Table S-1: Concentrations of working solution and standard addition samples

Compound	Working solution concentration (nM)	Standard addition method (SAM) spiking solutions			
		Conc. 1 (nM)	Conc. 2 (nM)	Conc. 3 (nM)	Conc. 4 (nM)
(+)-Catechin	200	25	50	100	200
Procyanidin B_2	200	25	50	100	200
(-)-Epicatechin	400	50	100	200	400
Taxifolin	100	12.5	25	50	100
Apigenin	50	6.25	12.5	25	50
Eriodictyol	100	12.5	25	50	100
Luteolin	50	6.25	12.5	25	50
Naringenin	100	12.5	25	50	100
Gallic acid	100	12.5	25	50	100
Syringic acid	250	31.25	62.5	125	250
Propyl Gallate	100	12.5	25	50	100
Daidzein	250	31.25	62.5	125	250
Quercetin	500	62.5	125	250	500
Cinnamic acid	3000	375	750	1500	3000

1.3 UPLC-MS/MS analysis

Analyses were performed on a Waters ACQUITY UPLC H-Class system, equipped with a quaternary pump, a column manager with an active preheater, and a sample manager with a flow-through needle. The system was coupled to a Waters Xevo TQ-S micro mass spectrometer with an orthogonal Z-spray electrospray ionization interface (Waters, Milford, MA, USA). System control was managed with MassLynx software (Waters). The compounds were separated on a ZORBAX Eclipse Plus C18 column (50 x 2.1 mm, 1.8 µm) from Agilent Technologies (Santa Clara, CA, USA).
A KrudKatcher ULTRA HPLC In-Line Filter (2.0 µm depth filter, 0.004 in internal diameter, Phenomenex, Torrance, CA, USA) was used to protect the column. The column and autosampler

temperatures were set at 40°C and 15°C, respectively. The flow rate was 0.5 mL/min, and the injection volume was 1 µL. A gradient elution program was employed with solvent A (2% acetic acid in water) and solvent B (ACN). The initial conditions (5% B) were held for 2 min, after which the percentage of B increased to 17% in 3 min, and was maintained at 17% until 7 min. From 7 to 9 min, B was increased to 30%, then to 95% B at 9.1 min and held until 10.1 min. The system was returned to the initial conditions at 10.2 min and re-equilibrated until 13 min. The Xevo TQ-S micro mass spectrometer was operated in multiple reaction monitoring (MRM) mode. Ionization parameters were individually optimized for each compound via direct infusion at 5 µL/min of the respective compound (1 µM) dissolved in MeOH, combined with a constant flow of 200 µL/min of 50% solvent A and 50% solvent B. Compound tuning was successfully achieved in negative ionization mode with a capillary voltage of -3 kV. The desolvation gas flow was set to 800 L/h, and the cone gas flow to 50 L/h. The source temperature was 120°C, and the desolvation temperature 400°C. The product ion exhibiting the highest sensitivity was selected for quantification. Details on mass transitions, cone voltages, and collision energies are provided in Table S-2. Data acquisition and peak processing were carried out using TargetLynx software (Waters). Mathematical and statistical analysis of the acquired data was performed in Microsoft Excel (Microsoft Corporation, Seattle, USA).

Table S-2: Mass transitions, cone voltages, collision energies

Compound	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)	Cone voltage (V)
(+)-Catechin	288.9	123	27	55
Procyanidin B_2	576.9	406.9	24	50
(-)-Epicatechin	288.9	203	18	30
Taxifolin	302.9	285	12	40
Apigenin	269	117	35	40
Eriodictyol	286.9	151.1	14	25
Luteolin	285.0	133	35	20
Naringenin	270.9	151	18	30
Gallic acid	168.8	125	13	40
Syringic acid	196.9	182	13	10
Propyl Gallate	211	124	24	20
Daidzein	253	224	25	20
Quercetin	300.9	151	20	20
Cinnamic acid	147	103	10	30

1.4 Method validation

Limit of quantification, linearity, calibration curves, matrix effect and precision.

Due to the unavailability of a blank matrix, calibration curves and LOQ were established in water. These calibration curves were made by serial dilution of the working solution in water (see Table S-1). The lower limit of the calibration range was set at the limit of quantification (LOQ), defined as the concentration with a signal-to-noise ratio of 10. The upper limit of quantification was determined based on accuracy, considering the percentage deviation between the measured and theoretical concentration. Each calibration curve included at least five concentrations, except for cinnamic acid, which had four. Precision was evaluated by injecting each concentration level three times, with relative standard deviations (RSD) calculated based on peak areas.

Matrix effects (ME) were assessed using standard addition curves, obtained by spiking known concentrations of the analyte (SAM spiking solutions) into the sample matrix. ME were calculated by

comparing the slopes of the standard addition curve and the standard curve in water over the same concentration range:

$$ME(\%) = \frac{Slope_{MATRIX}}{Slope_{WATER}} x\ 100$$

Quantification by Standard Addition Method (SAM)

Quantification was performed using the standard addition method to correct for matrix effects (1). Samples were analyzed both without spiking and after spiking with concentrations of the target analytes as specified in Table S-1. A standard addition curve was constructed by plotting the peak area against the added concentration. The original analyte concentration in the sample was determined by extrapolating the regression line to the x-axis, where the signal equals zero.

2. Results

2.1 UPLC-MS/MS analysis

All 14 compounds were separated on the C18 column in 10 min, as shown in Figure S-1. Some co-elution was observed between daidzein and eriodictyol, as well as between quercetin and luteolin. However, the use of MRM allowed for effective separation based on their distinct m/z transitions. The chromatogram in Figure S-1 presents all target compounds at their highest concentrations within the linear range.

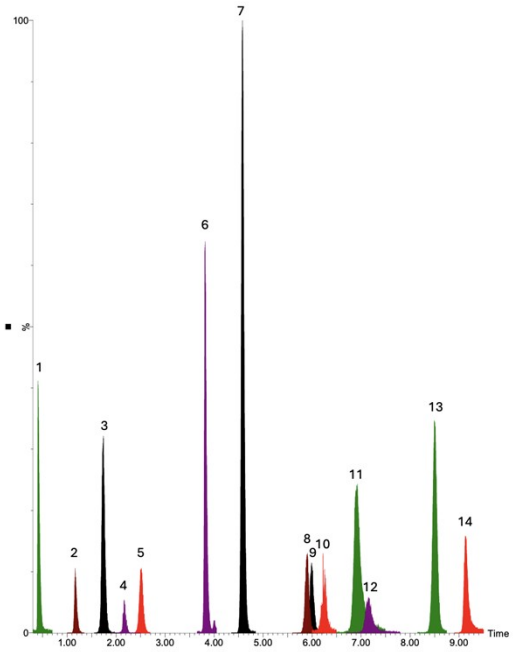

Figure S-1: MRM chromatogram of all compounds of interest. The identity of the peaks is gallic acid (1), (+)-catechin (2), procyanidin B_2 (3), syringic acid (4), (-)-epicatechin (5), taxifolin (6), propyl gallate (7), daidzein (8), eriodictyol (9), cinnamic acid (10), quercetin (11), luteolin (12), naringenin (13), and apigenin (14).

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2.2 Method validation

Limit of quantification, linearity, calibration curves, matrix effect and precision.

The calibration curves for the 14 compounds exhibited high linearity ($R^2 > 0.99$), as shown in Table S-3. RSD values obtained for triplicate injections of the same sample were always below 12% for all concentration levels of all analytes (data not shown), indicating that the method was precise. Matrix effects were assessed by comparing the slopes of the calibration curve in water with those of the standard addition curve. The %ME values generally ranged from 72% to 129%, with some outliers of 147% for quercetin. Values within 80-120% are considered acceptable, as they fall within the range of method repeatability. Therefore, quantification was performed by the standard addition method (SAM).

Table S-3: Determined calibration parameters, linear range, coefficient of determination (R^2), limit of quantification (LOQ) and matrix effect (ME).

Compound	Linear range (nM)	Curve			LOQ (nM)	ME (%)	
		a (slope)	b (offset)	R^2		10% juice	30% juice
(+)-Catechin	3-200	2674.8	-926.75	1.000	3	102	101
Procyanidin B_2	3-200	13720	-18363	1.000	3	92	94
(-)-Epicatechin	25-400	1758	-6170.9	1.000	25	92	96
Taxifolin	2-100	24141	-571.95	1.000	2	91	92
Apigenin	3-50	20482	-30052	0.996	3	112	117
Eriodictyol	6-100	9426.4	-15609	0.999	6	112	111
Luteolin	3-50	11688	-3933	1.000	3	127	129
Naringenin	3-100	26478	-10778	1.000	3	100	102
Gallic acid	2-100	17989	-1913.6	1.000	2	83	92
Syringic acid	8-250	1516.5	-2185.8	1.000	8	74	88
Propyl Gallate	0.8-100	58422	-5247.2	1.000	0.8	99	97
Daidzein	16-250	3998.5	-15036	1.000	16	103	101
Quercetin	31-500	8566.7	-252827	0.993	31	147	144
Cinnamic acid	375-3000	167	-5826.7	0.996	375	86	72

Quantification by Standard Addition Method (SAM)

A non-spiked sample was first analyzed using calibration curves in water to estimate the natural levels of the compounds. While some polyphenols were quantifiable, others were below the LOQ. Four spiking concentrations were used to construct the standard addition curves, all resulting in R^2 values above 0.99, confirming that the chosen spiking levels were not too low (2). To ensure the spiked concentrations were not excessively high, the standard addition curve was visually compared to a standard calibration curve. For syringic acid, the concentration in a tenfold diluted sample exceeded the upper limit of the calibration curve in water. Therefore, a 100-fold dilution of both juice samples was selected for its quantification. The obtained concentration levels in juice samples, adjusted for the dilution step during sample preparation, are presented in Table S-4. Most target compounds could not be quantified in both tamarind juices. However, procyanidin B_2, (-)-epicatechin, taxifolin, gallic acid, and syringic acid were quantifiable, with syringic acid being the most abundant.

Table S4: Measured concentration levels in 10% and 30% tamarind juice samples.

Compound	Concentration (nM)

	10 % juice	30 % juice
(+)-Catechin	<LOQ	<LOQ
Procyanidin B_2	2103.4	197.2
(-)-Epicatechin	1392.1	263.8
Taxifolin	172.9	350.6
Apigenin	<LOQ	<LOQ
Eriodictyol	<LOQ	<LOQ
Luteolin	<LOQ	<LOQ
Naringenin	<LOQ	<LOQ
Gallic acid	359.8	891.2
Syringic acid	4335.1	11453.2
Propyl Gallate	<LOQ	<LOQ
Daidzein	<LOQ	<LOQ
Quercetin	<LOQ	<LOQ
Cinnamic acid	<LOQ	<LOQ

3. References

1. Muchiri RN, van Breemen RB. Chemical Standardization of Milk Thistle (*Silybum marianum* L.) Extract Using UHPLC-MS/MS and the Method of Standard Addition. J Am Soc Mass Spectrom. 2024 Aug 7;35(8):1726–32.

2. Nelis M, Decraecker L, Boeckxstaens G, Augustijns P, Cabooter D. Development of a HILIC-MS/MS method for the quantification of histamine and its main metabolites in human urine samples. Talanta. 2020 Dec 1;220:121328.