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Toxicological evaluation of *Asparagus racemosus* – based low-alcohol nutraceutical beverage: acute and subacute safety assessment in mice†

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Asparagus racemosus, commonly known as Shatavari, is a traditional Ayurvedic medicinal plant widely recognized for its broad spectrum of health-promoting properties, including antioxidant, anti-inflammatory, and adaptogenic activities. In recent years, fermentation has emerged as a powerful tool to enhance the bioavailability, therapeutic efficacy, and functional potential of such medicinal herbs. This study focuses on the development and toxicological evaluation of a Shatavari-based Low Alcohol Nutra-Beverage (SLANB), which is formulated through a controlled fermentation process to retain and possibly enhance its bioactive components. To ensure the safe consumption of SLANB as a functional food and potential therapeutic product, a comprehensive toxicological assessment was conducted. Metabolite profiling was performed using Liquid Chromatography-Mass Spectrometry (LC-MS), which led to the identification of ten major bioactive compounds that contribute to SLANB's health benefits. Additionally, Gas Chromatography-Mass Spectrometry (GC-MS) was employed to characterize volatile compounds, including key aroma constituents and ethanol content, present in the beverage after 28 days of fermentation. For safety evaluation, both acute and subacute toxicity studies were performed using Swiss albino mice as the animal model. In the acute toxicity study, SLANB was administered orally at a dose of 1.5 ml per 100 grams of body weight. The animals were closely observed for 7 days to monitor any signs of toxicity, behavioral changes, or mortality. No adverse effects or fatalities were observed during this period. Furthermore, a 28 days subacute toxicity study was carried out in which mice were divided into groups and administered SLANB orally at three different dose levels. Throughout the study period, the animals were monitored for changes in physiological parameters, including body weight, food and water intake, organ weight, and behavioral patterns. No abnormalities or signs of toxicity were noted in any of the treated groups, indicating that SLANB is well-tolerated. The absence of toxicological effects in both acute and subacute studies confirms the safety of SLANB for consumption. These findings strongly support its application as a safe nutraceutical beverage with potential utility in functional food and medicinal formulations. This research contributes valuable insights into the development of plant-based fermented functional beverages with health-promoting properties.

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Sustainability spotlight

The developed Shatavari-based low-alcoholic Nutra-beverage (SLANB) promotes sustainable health solutions by utilizing *Asparagus racemosus*, a medicinal plant known for its antioxidant, anti-inflammatory, and adaptogenic properties. Fermentation significantly enhanced the bioavailability and functional potential of its key bioactive compounds. LC-MS analysis confirmed the presence of 10 distinct bioactive metabolites essential for health benefits, while GC-MS profiling revealed natural aroma and ethanol compounds after 28 days of fermentation. Toxicological evaluations demonstrated proven safety: an acute toxicity study using a 1.5 ml/100 g BW dose in mice showed no mortality or adverse effects over 14 days, and a 28 days subacute study across three dose levels confirmed no physiological toxicity. This eco-friendly innovation supports the development of a safe, functional nutraceutical beverage and validates SLANB as a non-toxic, plant-based formulation with potential applications in medicinal and wellness-oriented food systems.

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1 Introduction

Indian traditional herbs have increasingly garnered international interest due to their rich ethnopharmacological heritage and widespread use in traditional medicine systems such as Ayurveda, Siddha, and Unani. This global recognition is largely attributed to their diverse therapeutic potentials and centuries-long historical application.[1] One such herb is Shatavari (*Asparagus racemosus* Willd), a climbing perennial plant belonging to the Asparagaceae family. It is widely distributed across tropical and subtropical regions, including Asia, Africa, and Australia, with its origins traced to the Himalayan regions of Nepal and India.[2] In Indian traditional medicine, especially in the northern and southern states, the air-dried roots of Shatavari have been extensively utilized as a general health tonic and for the management of various reproductive and gastrointestinal disorders.

Shatavari is known for its inclusion in nutrient-enriched functional beverages due to its wealth of secondary metabolites, including steroidal saponins, flavonoids, polyphenols, and alkaloids, which are associated with numerous health benefits such as antioxidant, anti-inflammatory, adaptogenic, and immunomodulatory effects.[3,4] The conversion of Shatavari roots into shelf-stable and value-added formulations not only prolongs their usability but also enhances the economic returns for producers and industries focused on functional and nutraceutical food products. One of the sustainable and efficient approaches to achieving this transformation is bioprocessing through fermentation, which has been increasingly recognized for its ability to improve nutritional quality, safety, and organoleptic properties of food products.[5] Fermentation facilitates the breakdown of complex phytochemicals into bioavailable forms, potentially enhancing the pharmacological efficacy of herbal components.

Numerous studies have highlighted the pharmacological significance of Shatavari, attributing to it a broad spectrum of therapeutic properties including aphrodisiac, galactagogue, diuretic, antispasmodic, and adaptogenic effects.[6] These therapeutic potentials have led to its inclusion in more than 64 traditional Ayurvedic formulations such as Shatavari Kalpa, Phalaghrita, and Vishnu Taila, which are employed for the management of reproductive, digestive, and nervous system disorders in both men and women.

Artificial Neural Network (ANN) coupled with Genetic Algorithm (GA) was successfully utilized to model and optimize fermentation parameters for the development of a low-alcohol Shatavari-based Nutra-beverage. The ANN-GA model effectively predicted the fermentation outcomes, including ethanol yield and antioxidant activity, thus providing a computationally robust tool for process optimization. Specifically, the model predicted and experimental outcomes aligned closely, with ethanol yield reaching 3.21 g L^{-1} and antioxidant activity recorded at 421.47 μg L^{-1}, indicating enhanced functional properties during fermentation.[7] This demonstrates the potential of ANN-GA in improving biotechnological applications in the food and nutraceutical sectors.[8]

Despite the promising pharmacological attributes of Shatavari, safety concerns associated with herbal and alternative medicines have gained attention, particularly with growing evidence of toxicity and adverse effects in some Ayurvedic formulations. Such safety risks are often linked to contamination, adulteration, or incorrect dosage, emphasizing the importance of rigorous toxicological evaluation.[9,10] Currently, limited data exist regarding the acute and sub-acute toxicity of Shatavari-based nutraceutical beverages, thereby necessitating systematic *in vivo* studies to evaluate their safety profiles before commercialization. Ensuring the toxicological safety of these preparations is critical for consumer health and regulatory compliance.

To address these concerns, our study includes acute and sub-acute toxicity assessments in animal models, alongside sensory evaluation protocols to determine consumer acceptance of the developed product.

2 Material and methods

2.1 Chemicals used

Biochemical Diagnostic kits such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), total and high-density lipoprotein (HDL) cholesterol, triglycerides and creatinine were obtained from Agappe (India). E-Merck India Ltd supplied other Guaranteed Reagent (GR) chemicals utilized in the experiment.

2.2 Animals

Animal studies were conducted with the approval from CPCSEA, Government of India through the Institutional Animal Ethics Committee under protocol (CIMAP/IAEC/2020–23/23). The study was conducted on Swiss albino mice, weighing 20–25 g. The animals were kept at 25 ± 2 °C with controlled humidity and a 12 hours light/dark cycle. Standard mice feed and water were provided ad libitum.

2.3 Preparation of shatavari-based beverage

Fresh Shatavari roots (*Asparagus racemosus*) were procured from the CSIR–Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. The roots were thoroughly washed, chopped, and dried in a hot air oven for five days, then ground into powder. A slurry was prepared by mixing 500 g of root powder with 1 L of distilled water. Enzymatic hydrolysis was carried out by adding α-amylase (25 U g^{-1}) and incubating at high temperature for 1 hour, followed by saccharification using glucoamylase (78 U g^{-1}) at 50 °C for 2 hours with continuous stirring.[11]

For fermentation, *Saccharomyces cerevisiae* NCIM 2428 was cultured in YEPD medium and incubated at 150 rpm for 24 hours. The activated inoculum was transferred to sterile Shatavari juice (pH 3.4) in 3-L Erlenmeyer flasks. Sucrose was added to increase sugar content to 190 g L^{-1} (as glucose), and fermentation parameters such as temperature (28–35 °C), pH (4–6), and inoculum concentration (1–5%, v/v) were optimized according to experimental design.[12]

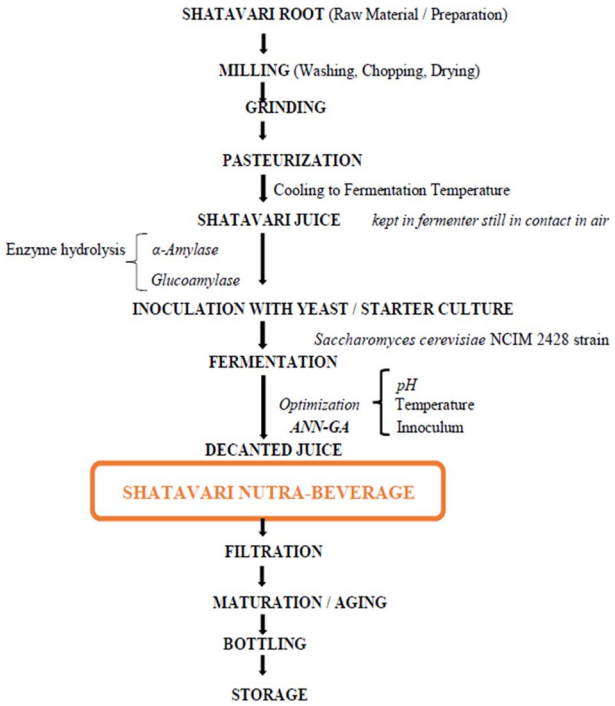

Fig. 1 Preparation process of Shatavari-based low-alcohol Nutra-beverage.

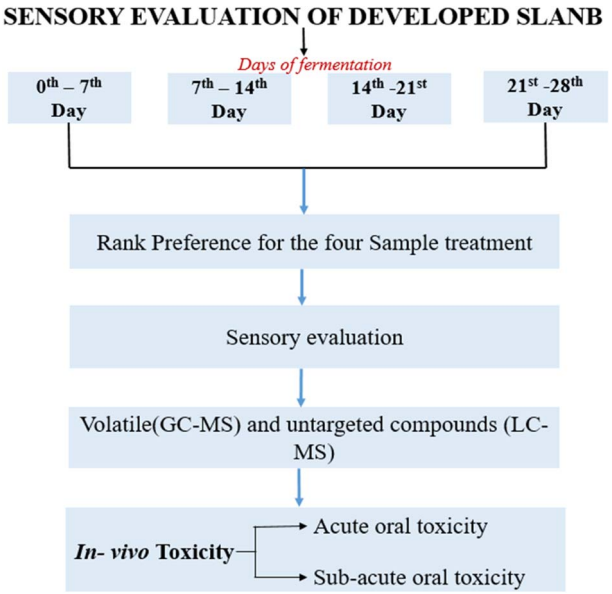

Fig. 2 Flowchart of sensory evaluation and chemical profiling of Shatavari-based low-alcoholic Nutra-beverage (SLANB) across fermentation stages, including preference ranking, GC-MS/LC-MS analysis, and *in vivo* toxicity assessment.

Fermentation was carried out under sterile conditions in a 3-L BioFlo/CelliGen 115 bioreactor with a working volume of 2 L and agitation set at 150 rpm. Upon completion, the fermented product was centrifuged at 4000 rpm for 10 minutes, and the resulting supernatant was stored at 20 °C in the dark for 90 days.[13] The preparation process of the Shatavari-based low-alcohol Nutra-beverage is illustrated in Fig. 1.

2.4 Sensory evaluation

2.4.1 Hedonic scale. The acceptance test for the Shatavari-based Low-Alcohol Nutra-Beverage (SLANB) was conducted using a random selection of 20 individuals in a laboratory setting, with each participant providing feedback *via* a structured form. Sensory evaluation was performed in dedicated sensory panel rooms using hedonic taste sheets. The beverages were tested at room temperature, with each sample consisting of 10 ml of the respective beverage type. Key sensory attributes—including texture, aroma, taste, color, flavor, and aftertaste—along with overall acceptance, were assessed using a structured 9-point hedonic scale (1 = extremely dislike to 9 = extremely like).

Participants were advised to cleanse their palates with water between samples to ensure accurate evaluation and to clean their glasses. The sensory evaluation followed the method described.[14] The overall experimental flow diagram of SLANB sample is depicted in Fig. 2, outlining the stages from formulation to consumer feedback. The hedonic scale test was conducted prior to the safety evaluation of the beverages and spanned from day 0 to day 28 of the experiment. Once the samples receiving the highest sensory scores—based on consumer preferences for taste and odor—were identified, the corresponding fermentation day was selected for further analysis. A toxicity study was subsequently performed on the beverage sample from this optimal day to evaluate its safety.

2.5 Volatile compounds by GC-MS

The volatile compounds in Nutra-beverage samples were analyzed using GC-MS following a procedure described.[15] In a 20 ml glass vial, 2 g of the sample and 10 ml of the internal standard (4-methyl-2-pentanol at 80.2 g ml^{-1} in methanol) were combined. Peak detection and integration of peak area were performed using TraceFinder 4.1 (Thermo Scientific) with the total ion chromatogram (TIC). The analysis was conducted using an Agilent 5977B EI/CI MSD with a column oven temperature of 80 °C, injection temperature of 260 °C, split injection mode, linear velocity flow control mode, pressure of 81.9 kPa, total flow of 16.3 ml min^{-1}, and column flow of 1.21 ml min^{-1}. The hold time was 2 min at 80 °C and 18 min at 280 °C.

2.6 Untargeted compounds and high resolution LC-ESI/MS (LC-MS)

The validated sample analysis was confirmed using the LC-ESI/MS (LC-MS) Waters Alliance e2695/HPLC-TQD Mass Spectrometer instrument. This analysis was conducted at the Central Drug Research Institute (CDRI), Lucknow, as detailed.[16] The chromatographic conditions were as follows: Waters Alliance 2695 HPLC pump initial conditions, solvents: Degasser normal stroke volume 130.0. Final flow setting being held at the end of run A% 0.0H_2O, B% 5.0 CAN, C% 0.0 MEOH, D% 95.0 0.1% FA. Flow (ml min^{-1}) 0.600, flow ramp 1.00, stop time (mins) 40.0,

column temperature (°C) 30.0, column temperature limit (°C) 5.0, min pressure (Bar) 0.0, max pressure (Bar) 300.0, pre-column volume (μl) 0.00, column position no change pre-column volume 0.00. Waters Alliance 2695 Autosampler initial conditions: needle depth (mm) 0.00, sample temperature (°C) 20.0, sample temperature limit (°C) 5.0, purge loop volumes 0.00, sample run injection parameter, injection volume (μl) – 2.00.

2.7 *In vivo* acute oral toxicity

Acute oral toxicity of nutraceutical beverage was conducted according to the Organization for Economic Cooperation and Development (OECD) test guideline no. 423.[17] Briefly, 12 mice were taken and divided into two groups comprising 6 mice (3 males and 3 females) in each group. Animals were acclimatized for 7 days prior to experimentation. Group 1 mice were given sterile water and group 2 mice were given Nutraceutical Beverage (single dose at 1.5 ml/100 g of body weight). On 7th day, mice were euthanized and blood was collected from retro orbital plexus for haematological parameters and serum was isolated to study biochemical parameters. Change in body weight, weight of vital organs like liver, spleen, kidney, heart, and lungs were also recorded.[18]

2.8 *In vivo* sub-acute oral toxicity

Sub-acute oral toxicity of Nutra-Beverage was conducted according to the Organization for Economic Co-operation and Development (OECD) test guideline no. 407.[19] Briefly, 24 mice were taken and divided into four groups comprising 6 mice (3 males and 3 females) in each group. Animals were acclimatized for 7 days prior to experimentation. Group 1 mice were given sterile water and mice in group 2, 3 and 4 were given different volumes (at 0.375 ml, 0.75 ml and 1.5 ml/100 g body weight for 28 days) of Nutra-Beverage (7 days a week for 28 days). On day 28th, mice were euthanized by cervical dislocation and blood was collected for haematological parameters and serum was isolated to study biochemical parameters. Change in body weight, weights of vital organs like liver, spleen, kidney, heart, and lungs were also recorded.

2.9 Statistically analysis

The statistical analyses were conducted using analysis of variance (ANOVA) to assess the data. For the *in vivo* experiment, a two-way ANOVA was employed to evaluate the effects of multiple factors. Windows-based GraphPad Prism 6.04 was used for the analysis.

3 Result and discussion

3.1 Sensory evaluation of shatavari based low alcohol nutra-beverage

Hedonic data analysis shows the frequency with which tasters identified each attribute in the samples during sensory evaluation, resulting in the contingency in Table 1. The section provides data on the sensory acceptability of the developed product among the target group of consumers. Hedonic analysis's tasters' ratings for each fermented product's texture, fragrance, taste, color, flavor, and aftertaste are shown in Table 1.

The texture, aroma, taste, colour, flavour, and aftertaste of four distinct samples were evaluated, and their ratings are presented in Table 1. The results revealed that Sample 1 had a texture rating of 6, making it moderately likeable, while Sample 2 received a slightly higher rating of 7. Sample 3 achieved the highest texture rating of 8, indicating it was the most preferred among all samples. In contrast, Sample 4 received a lower rating of 4, suggesting it was the least liked.

In terms of aroma, Sample 3 was again the most favoured. Regarding taste, Sample 3 received the highest overall score of 16, making it the most satisfying in terms of flavour. Although Samples 1, 2, and 4 were acceptable, they did not surpass Sample 3 in any category.

The flavour and aftertaste of Sample 3 (14th–21st day of fermentation) were also well-received, though no other sample matched its high ratings. Similarly, in terms of colour, Sample 3 was considered the best overall. Fig. 3 presents a radar chart illustrating the sensory acceptability of the samples from the 0th to the 28th day of fermentation.

3.2 Dynamic of volatile compounds (GC-MS) during fermentation

The GC-MS method enabled the identification of components in the Shatavari-based low-alcohol Nutra-beverage with a confidence level exceeding 72%. These included three alcohols (phenylethyl alcohol, propargyl alcohol, and 1-butanol), one ketone (acetoin), one fatty acid (2-hydroxyoctanoic acid), four carboxylic acids (ethyl acetate, succinic acid, oxalic acid, and

Table 1 Summary of sensory attributes and their rating by consumers

	Summary of sensory			
Attributes	Sample 1 (0th–7th day)	Sample 2 (7th–14th day)	Sample 3 (14th–21st day)	Sample 4 (21st–28th day)
Texture	6	7	8	4
Aroma	9	10	11	9
Taste	13	15	16	10
Color	15	17	18	18
Flavor	10	14	15	13
Aftertaste	14	15	16	12

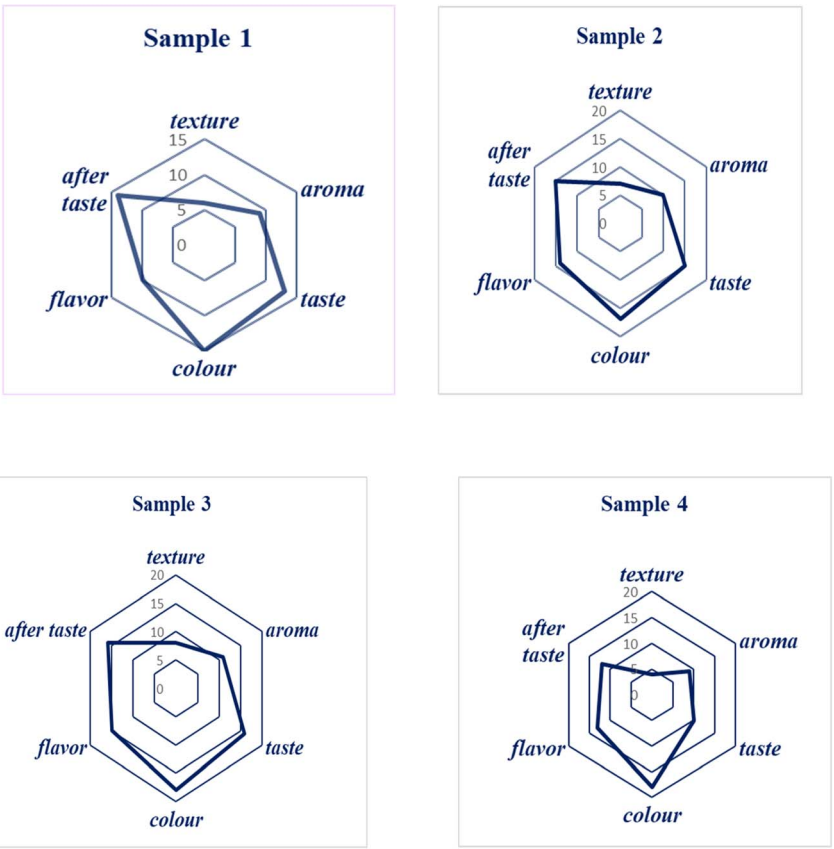

Fig. 3 Sensory acceptability of 0th–28th days of fermentation, 0th–7th day (Sample 1); 7th–14th day (Sample 2); 14th–21st day (Sample 3); and 21st–28th day (sample 4).

Table 2 Volatile compounds identified in Shatavari original juice and low alcohol Shatavari-based fermented beverage by GC-MS

	Fermentation days					
Compounds	Retention time	0th day	7th day	14th day	21st day	28th day
Ether group						
Ethyl ether	4.33	2.21	7.03	1.23	0.33	nd
Alkane						
Pentane	5.41	9.05	9.05	nd	nd	nd
Hexane	5.90	nd	nd	10.69	4.77	nd
Carboxylic group						
Ethyl acetate	7.36	nd	nd	nd	31.26	27.05
Succinic acid	4.34	nd	nd	5.48	7.54	6.08
Oxalic acid	5.07	nd	nd	0.95	0.28	nd
Acetic acid	7.02	nd	nd	0.04	0.07	nd
Fatty acid						
2-Hydroxyoctanoic acid	4.23	nd	nd	nd	31.91	23.05
Ketones						
Acetoin	12.36	nd	2.09	2.19	2.39	nd
Alcohols						
Phenylethyl alcohol	31.65	nd	0.50	nd	nd	Nd
Propargyl alcohol	14.52	nd	nd	0.85	nd	0.10
1-Butanol	12.59	nd	2.58	nd	32.61	Nd

acetic acid), two alkanes (pentane and hexane), and one ether (ethyl ether), as presented in Table 2.

During the fermentation process, the composition of volatile compounds varied over different days. Alcoholic compounds were notably detected on the 7th day of fermentation. Phenylethyl alcohol appeared with a peak area of 0.50 and a retention time of 31.65 minutes, but was not detected on subsequent days. Similarly, propargyl alcohol was detected on the 7th day with a peak area of 0.85 and a retention time of 14.52 minutes. 1-Butanol was observed at a retention time of 12.59 minutes on both the 7th and 21st days, with peak areas of 2.58 and 32.61, respectively. The ketone compound acetoin was identified with a retention time of 12.36 minutes. The corresponding chromatogram peaks are shown in ESI Fig. S1.†

GC-MS analysis revealed that the number and relative abundance of volatile compounds were influenced by storage duration. In the freshly fermented Shatavari beverage (Day 7, D7), 11 volatile components were identified, comprising a total area percentage of 21.25%. During storage at 32 °C, the number of volatiles varied: 12 components on Day 14 (D14), 10 on Day 21 (D21), and 8 on Day 28 (D28). Interestingly, the total area percentage of volatile compounds increased to 20.57%, 111.16%, and 56.28% on D14, D21, and D28, respectively.

These findings suggest that volatile compounds peak around the 14th day of fermentation, contributing to enhanced aroma. After the 21st day, the levels begin to decline, likely due to the release of CO_2, which may reduce the concentration of aroma-active compounds. The fruity and floral aroma in fermented products such as wine is typically associated with esters, which are synthesized through esterification of alcohols with fatty acids, or *via* alcohol acetyltransferase-mediated reactions involving acetyl-CoA and higher alcohols.[20]

While the fermented Shatavari beverage contained fewer esters than the fresh beverage, ester content increased after 7–14 days at 32 °C compared to day 0. Alcohols in the beverage are primarily derived from alcoholic fermentation, amino acid metabolism, and the oxidation of linolenic acid degradation products,[21] all of which contribute desirable aromas.[22] The alcohols detected during storage included phenylethyl alcohol, propargyl alcohol, and 1-butanol (Table 2). The highest total alcohol area percentage (32.61%) was observed on the 21st day, while lower values were recorded on the 7th and 14th days, at 3.18% and 0.85%, respectively. The higher alcohol content in stored fermented Shatavari beverages, compared to freshly fermented ones, could be attributed to continued yeast activity at elevated temperatures, which promotes the conversion of residual sugars into alcohol. These alcohols impart fruity, floral, and characteristic alcoholic aromas to the beverage.

Acids and ketones serve as both primary metabolites and precursors to aromatic compounds. Secondary metabolites such as esters and lactones are formed through the interaction of volatiles—including alcohols and fatty acids—during fermentation.[23] Although present in small amounts, these additional volatile compounds may play a significant role in the aroma and flavor profile of the fermented Shatavari beverage.

Table 3 Untargeted compounds in fermented Shatavari beverage[a]

Compounds name	Formula	*m/z*	Retention time/relative abundance (%)					Pharmacological importance/literature references
			0th day	7th day	14th day	21st day	28th day	
Chalconaringenin 2′-rhamnosyl-(1->4)-glucoside	$C_{27}H_{32}O_{14}$	580.2	nd	nd	10.89 (30%)	nd	nd	Antioxidant
Shatavarin I	$C_{51}H_{86}O_{23}$	1066.5	nd	11.13 (25%)	10.69 (80%)	10.70 (40%)	10.70 (40%)	Antioxidant (3)
Shatavarin VIII	$C_{50}H_{82}O_{22}$	1034.5	nd	13.83 (73%)	13.85 (87%)	nd	13.84 (72%)	Antioxidant (3)
Shatavarin V	$C_{45}H_{74}O_{17}$	886.4	nd	17.32 (75%)	17.32 (80%)	17.32 (85%)	17.32 (80%)	Immunomodulatory (6)
Shatavarin IX	$C_{45}H_{74}O_{18}$	902.4	17.28 (37%)	17.28 (40%)	17.28 (45%)	17.28 (45%)	17.28 (32%)	Immunomodulatory (6)

[a] nd = not detected; *m/z* = mass-to-charge ratio; retention time given in minutes; relative abundance presented in percentage (%); pharmacological importance reported as antioxidant or immunomodulatory based on existing literature; detected compounds were tentatively identified using LC-MS/MS.

3.3 Untargeted compounds detected during fermentation using LC-ESI/MS (LC-MS) Waters Alliance e2695/HPLC-TQD mass spectrometer

The Shatavari nutraceutical beverage was utilized for a safety evaluation study. To ensure its quality, reproducible LC-ESI/MS (LC-MS) analysis was conducted using reverse-phase separation with a methanol–water mobile phase (detailed methodology is provided in the Experimental section). Partial untargeted compounds identified in the fermented Shatavari beverage are listed in Table 3. Phenolic compounds such as gallic acid and caffeic acid, which readily react with oxygen, function as antioxidants. In fermented foods, the growth and reproduction of microbial colonies are closely associated with fermentation duration. As fermentation progresses, colony development typically follows four phases: lag phase, logarithmic (exponential) phase, stationary phase, and stable (decline) phase. Chromatographic peaks observed from day 0 to day 28 of fermentation confirmed the presence of major compounds, as illustrated in ESI Fig. S2.†

The composition of the colony will change accordingly,[24] leading to changes in mycelium color, colony vitality, texture characteristics of the product, sensory acceptance, and product quality. The longer the fermentation time, the darker the color of the FAR fermented Shatavari beverage, due to the oxidation of polyphenol and flavonoid compounds, resulting in color browning. Other factors, such as light and temperature, might contribute to the degradation of phenolic compounds.[25] When these substances are oxidized, they form brown pigments that may eventually precipitate.[26]

The five signature compounds were identified by LC-ESI/MS (LC-MS) analysis using Waters Alliance e2695/HPLC-TQD Mass spectrometer instrument, namely Chalconaringenin 2′-rhamnosyl-(1->4)-glucoside, Shatavarin I, Shatavarin VIII, Shatavarin V, Shatavarin IX, Asparoside B, Quercetin, Lysyl-Asparagine compounds. To further investigate the effect of fermentation, representative metabolites were selected for relative abundance analysis in this study. The data from Table 3 regarding the relative abundance of specific compounds in the Shatavari-based nutra-beverage are refined as a Shatavarin VIII is observed to be present in relatively high abundance within the nutra-beverage formulation. Both Shatavarin I and Shatavarin V are found in equal abundance levels, suggesting a balanced presence of these saponins. Asparoside B emerges as the most abundant compound, highlighting its significant contribution to the beverage's composition. Conversely, the compound Chalconaringenin 2′-rhamnosyl-(1->4)-glucoside is noted for its low abundance in the formulation, all of which are shown in Fig. 4.

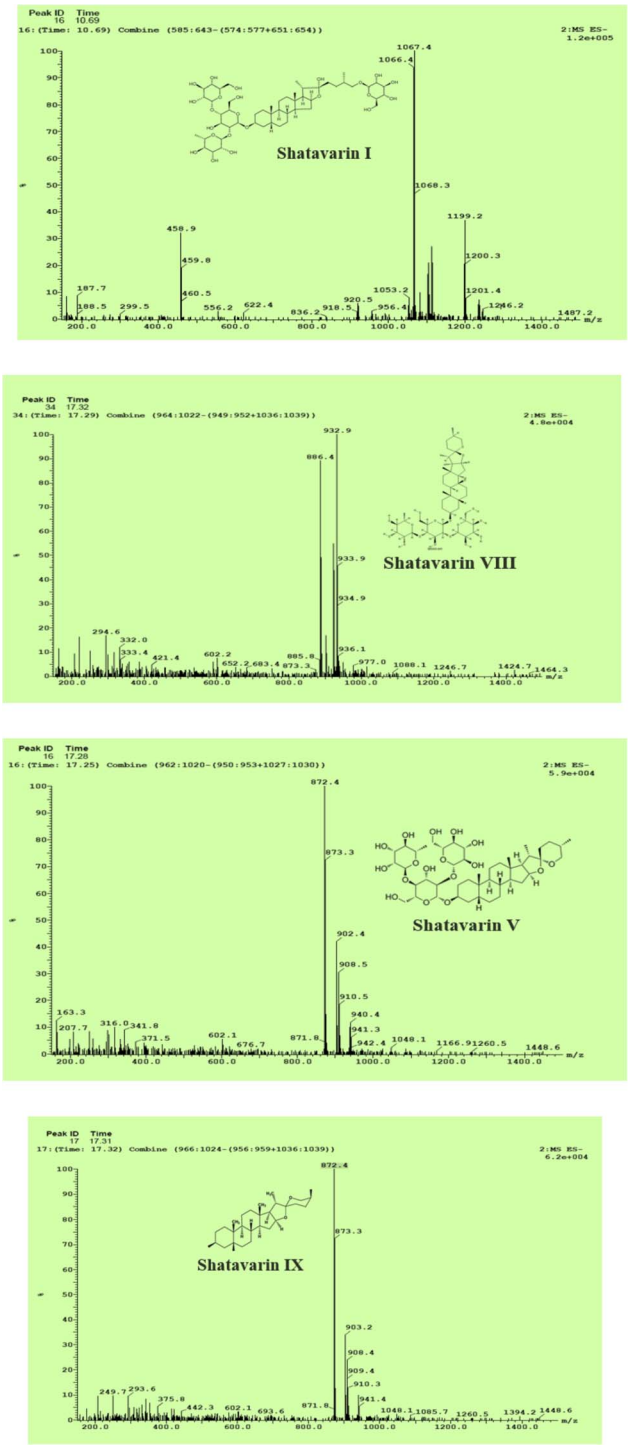

Fig. 4 Untargeted compounds identified in the product on the 14th day of fermentation showing relative abundance and validation.

3.4 Acute oral toxicity in mice

Mice treated with nutraceutical beverages (at 1.5 ml/100 g of body weight) showed no observable changes when compared with control. No instances of illness or fatality were recorded over the entire study duration. The experimental and control groups had comparable weight alterations (Table 4).

Moreover, no significant difference was observed in the mean values of absolute and relative weights of essential organs, including the kidney, liver, lung, heart, and spleen, in the treatment group compared to the control group (see Fig. 5).

Nutraceutical beverage at a single dose of 1.5 ml/100 g body weight did not induce any significant change in haematological parameters [RBC count, WBC count and Haemoglobin (Hb)] in

Table 4 Effect of Nutra-beverage as a single acute oral dose on haemogram and serum biochemical parameters in mice (Mean ± SEM; $n = 6$)[a]

Parameters	Control	Nutrabeverage
Change in bd wt (g)	3.79 ± 0.56	3.89 ± 0.42
Haemoglobin (g dl^{-1})	17.92 ± 0.75	18.28 ± 1.31
RBC (million/mm^3)	6.02 ± 0.08	6.11 ± 0.13
WBC (×1000/mm^3)	6.81 ± 0.23	6.21 ± 0.85
SGOT (U l^{-1})	51.38 ± 2.05	49.73 ± 2.10
SGPT (U l^{-1})	30.05 ± 2.89	28.11 ± 2.76
ALP (U l^{-1})	102.82 ± 16.58	111.07 ± 13.08
Creatinine (mg dl^{-1})	0.80 ± 0.37	0.51 ± 0.23
Triglyceride (mg dl^{-1})	122.07 ± 4.01	121.14 ± 6.38
Cholesterol (mg dl^{-1})	161.90 ± 4.74	158.18 ± 6.30
Bilirubin (mg dl^{-1})	0.36 ± 0.03	0.30 ± 0.04

[a] Values are expressed as mean ± standard deviation ($n = 6$). SGOT: serum glutamate oxaloacetate transaminase; SGPT: serum glutamate pyruvate transaminase; ALP: alkaline phosphatase; RBC: red blood cells; WBC: white blood cells. No significant difference ($p > 0.05$) was observed between control and Nutra-beverage groups.

either of the sex studied (Table 4). Creatinine, SGOT, SGPT, total cholesterol, triglycerides, bilirubin, and alkaline phosphatase did not vary significantly either.

3.5 Sub-acute oral toxicity in mice

In sub-acute oral toxicity studies, there was no significant difference in change in body weight in group 2 and 3, while change in body weight in group 4 was found to be decreased when compared with control that implies the high antioxidant nature of nutraceutical that helps in reducing the body weight (Table 5).

Moreover, the absolute and relative organ weights of kidney, lung, heart, liver, and spleen in group 2, 3, and 4 was comparable to the control group (see Fig. 6). Additionally, the haematological parameters including RBC count, WBC count and Haemoglobin (Hb) also remained within normal ranges across all three dosage of 0.375 ml, 0.75 ml and 1.5 ml/100 g body weight. Furthermore, there was no significant changes were observed in biochemical parameters such as serum creatinine, SGOT, SGPT, total cholesterol, triglycerides, bilirubin, and Alkaline phosphatase (ALP).

3.6 In-life observation

The animals were checked daily for mortality and any signs of illness. A general clinical examination was conducted before and after the start of the experiment, including observations of changes in the skin, mucous membranes, eyes, and any secretions or excretions. Additionally, responses such as tearing,

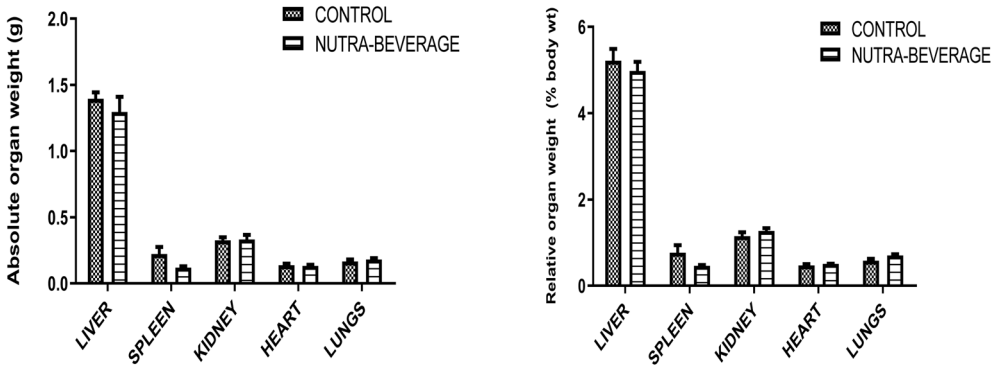

Fig. 5 Effect of Nutra-beverage as a single acute oral dose at 1.5 ml/100 g body weight on absolute and relative organ weight in mice ($n = 6$).

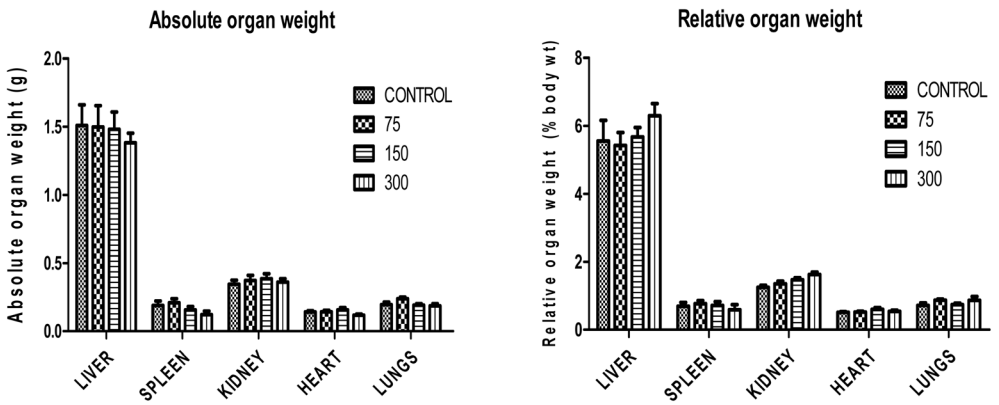

Fig. 6 Effect of Nutra-beverage as a single acute oral dose at 0.375 ml, 0.75 ml, and 1.5 ml g^{-1} body weight on absolute and relative organ weight in mice ($n = 6$).

Table 5 Effect of Nutra-beverage as a sub-acute oral dose on haemogram and serum biochemical parameters in mice (mean ± SEM; $n = 6$)[a]

Parameters	Control	75	150	300
Body weight (g)	27.34 ± 1.04	27.41 ± 1.32	26.41 ± 2.23	22.14 ± 0.95
Change in bd wt (g)	5.50 ± 1.52	6.81 ± 0.64	7.56 ± 1.39	−2.12 ± 0.57
Haemoglobin (g dl^{-1})	16.57 ± 0.59	16.22 ± 0.40	17.17 ± 0.44	19.3 ± 0.32
RBC (million/mm^3)	7.05 ± 0.14	7.02 ± 0.39	7.11 ± 0.05	6.84 ± 0.17
WBC (×1000/mm^3)	6.66 ± 0.19	6.20 ± 0.20	6.23 ± 0.16	6.63 ± 0.26
SGOT (U l^{-1})	34.51 ± 2.72	39.46 ± 2.17	38.97 ± 2.37	21.72 ± 1.30
SGPT (U l^{-1})	22.98 ± 4.74	22.01 ± 1.45	29.67 ± 2.91	23.17 ± 3.27
ALP (U l^{-1})	82.50 ± 9.67	84.03 ± 3.01	89.99 ± 6.31	72.88 ± 2.46
Creatinine (mg dl^{-1})	0.72 ± 0.24	0.56 ± 0.10	0.67 ± 0.16	0.72 ± 0.12
Triglyceride (mg dl^{-1})	161.90 ± 8.42	165.95 ± 15.89	163.81 ± 3.02	184.29 ± 4.67
Cholesterol (mg dl^{-1})	127.23 ± 7.90	119.39 ± 4.07	117.65 ± 2.65	134.42 ± 7.31
Bilirubin (mg dl^{-1})	0.14 ± 0.01	0.13 ± 0.01	0.20 ± 0.03	0.23 ± 0.02

[a] Values are expressed as mean ± standard deviation (SD), $n = 6$ animals per group. Control: received distilled water only. 75, 150, 300: received SLANB at doses of 75, 150, and 300 mg kg^{-1} body weight, respectively, for 28 days. No significant differences ($p > 0.05$) observed in most parameters 115 among treated groups compared to control, indicating no adverse effects. Negative change in body weight observed only at 300 mg kg^{-1}.

raised hair, and respiratory patterns were noted. Changes in gait, posture, and response to handling were also observed.[27]

3.7 Hematological and biochemical analysis

Neubauer's chamber was utilized to measure the total count of red blood cells (RBC) and white blood cells (WBC). Serum biochemical markers, including creatinine, SGOT, SGPT, total cholesterol, triglycerides, bilirubin, and alkaline phosphatase, were assessed utilizing kits from Agappe (India). The outcomes of the hedonic analysis consist of the evaluators' scores for each fermented product for texture, scent, taste, color, flavor, and aftertaste. The test substance, a nutraceutical beverage, was administered orally to Swiss albino mice in acute (1.5 ml/100 g body weight as a single dose) and sub-acute doses of 0.375 ml, 0.75 ml, and 1.5 ml/100 g body weight once daily for 28 days to investigate oral toxicity, enabling data extrapolation to human patients.[28]

No one died or got sick throughout the experiment. At 0.375 ml and 0.75 ml/100 g of BW, acute and sub-acute testing showed no significant changes in body weight, however group 4 (1.5 ml/100 g of BW) showed a reduction compared to control. This impact could be due to the high antioxidant nature of nutraceutical beverage that helps in losing body weight. No alterations were noted in the assessment of vital organ weights, both absolutely and relatively. The hematological parameters exhibited negligible alterations. SGOT, serum creatinine, total cholesterol, triglycerides, bilirubin, and ALP all showed no significant increases (Table 5). This is the first report on the safety profile of chemically characterized Nutraceutical beverage, an herbal formulation of much use in Ayurvedic Medicine. A parallel investigation with *Bacopa monnieri* leaf enriched extract at acute and sub-acute levels in Sprague-Dawley rats found that 500 mg kg^{-1} body weight for 14 days and 85, 210, and 500 mg kg^{-1} for 90 days were well tolerated.

However, there is no report on the toxicity profile of Nutraceutical beverage. We have used Nutraceutical beverage, which is chemically defined and chemical constituents for the safety profiling. Nutraceutical beverage did not affect morbidity and mortality in our investigation, including body weight, important organ weight, and main biochemical indicators. Our data indicate that a single oral dose of Nutraceutical beverage at 1.5 ml g^{-1} of body weight is well tolerated in both male and female Swiss albino mice, as well as doses of 0.375 ml, 0.75 ml, and 1.5 ml g^{-1} of body weight administered once daily for 28 days.

3.8 Statistical analysis

The evaluation of relative organ weight showed no significant differences between the control and beverage-treated groups, indicating that the beverage consumption did not affect the proportional weight of key organs. This finding suggests that the beverage does not induce adverse effects on organ size relative to body weight, supporting its safety for consumption all shown in ESI Tables T6–T8.†

Similarly, the analysis of absolute organ weight demonstrated no significant variations between the control and beverage groups. This further confirms that the beverage does not interfere with the normal growth or development of individual organs, underscoring its non-toxic nature in terms of organ-specific impacts.

In terms of body weight, no significant differences were observed between the control and beverage groups, highlighting that the beverage had no detrimental effect on overall body growth or weight regulation. This outcome suggests that the beverage is metabolically neutral, with no apparent influence on weight gain or loss during the study period.

Overall, these findings collectively indicate the safety of the beverage, as evidenced by the absence of adverse effects on relative and absolute organ weights as well as body weight in comparison to the control group. This provides a strong basis for considering the beverage as a safe functional product for further development and consumption.

4 Conclusion

Shatavari has a variety of pharmacological effects and holds promise as both a functional food and a medicinal agent. Fermentation is typically used for making Shatavari drinks. Shatavari-based low-alcohol nutraceutical drinks (SLANB) have not been tested for toxicity. In this study, we evaluated the sensory, volatile/untargeted compounds and toxicological characteristics of SLANB for the first time. SLANB was administered over a 4 weeks (28 days) period, and its texture, aroma, taste, colour, flavour, and aftertaste were evaluated by 20 panellists using a 9-point hedonic scale. Sample 3 received the highest rating, indicating that it was the most well-liked product among the samples tested. It was also discovered that volatile and untargeted compounds present in Shatavari root were identified during shelf life analysis. This study sheds light on the chemical and biochemical properties of SLANB and its fermentation process. The toxicological properties of SLANB were evaluated in group 2 mice at a dosage of 1.5 ml/100 g body weight, and no fatalities or toxicity were observed in the acute study, indicating that SLANB may be regarded as non-toxic at the administered dose. In the 28 days sub-acute toxicity study, mice were fed orally with SLANB at different doses showed no adverse effects on mortality, body weight, relative and absolute organ weights, or haematology. SLANB had no harmful effects in both sexes in the sub-acute toxicity investigation. These findings indicate safe SLANB usage as a functional food and medicine.

Ethical statement

Animal experiments were conducted under the protocol (CIMAP/IAEC/2020–23/22) approved by Institutional Animal Ethics Committee of CSIR-CIMAP, Lucknow, India.

Data availability

Data will be made available on reasonable request.

Author contributions

Divya Choudhary: conceptualization, methodology, data curation, formal analysis, writing – original draft, writing – review & editing. Satyanarayan Naik: supervision, writing – review & editing, resources, project administration. Vidushi Tyagi and Anirban Pal: conceptualization, supervision, writing – review & editing, resources, experiment, and project administration. Hariprasad P.: supervision.

Conflicts of interest

The authors declare no conflict of interest.

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References

1 A. Shaito, D. T. B. Thuan, H. T. Phu, T. H. D. Nguyen, H. Hasan, S. Halabi, *et al.*, Herbal medicine for cardiovascular diseases: Efficacy, mechanisms, and safety, *Front. Pharmacol*, 2020, **11**, 422, DOI: 10.3389/fphar.2020.00422.

2 S. Alok, S. K. Jain, A. Verma, M. Kumar, A. Mahor and M. Sabharwal, Plant profile, phytochemistry and pharmacology of Asparagus racemosus (Shatavari): A review, *Asian Pac. J. Trop. Dis.*, 2013, **3**(3), 242–251, DOI: 10.1016/S2222-1808(13)60049-0.

3 R. A. Kamble, S. C. Choudhary and R. K. Bhardwaj, Pharmacological activities of Asparagus racemosus (Shatavari): A Review, *Pharmacogn. Rev.*, 2008, **2**(3), 105–113.

4 R. Pandey and R. S. Gupta, Screening of antioxidant activity of different extracts of Asparagus racemosus, *J. Med. Plants Res.*, 2005, **5**(13), 2713–2716.

5 C. Voidarou, A. Tzora, M. Antoniadou, K. Fotou, E. Tzora, I. Skoufos and E. Bezirtzoglou, Fermented foods as functional foods and the impact of bioprocessing on their functionality, *Microorganisms*, 2021, **9**(1), 74, DOI: 10.3390/microorganisms9010074.

6 R. K. Goyal, J. Singh and H. Lal, Asparagus racemosus—An update, *Indian J. Med. Sci.*, 2003, **57**(9), 408–414.

7 D. Choudhary, S. N. Naik and P. Hariprasad, A novel approach for the optimization of Shatavari (Asparagus racemosus) plant-based low alcohol using Saccharomyces cerevisiae NCIM 2428 in conjunction with ANN and GA, *J. Food Sci. Technol.*, 2025, DOI: 10.1007/s13197-025-06275-2.

8 T. Dey, S. Pal and M. Ghosh, Artificial neural network–genetic algorithm-based optimization for the development of low-alcohol functional beverage, *J. Food Process Eng.*, 2021, **44**(2), e13586, DOI: 10.1111/jfpe.13586.

9 E. Ernst, Harmless herbs? A review of the recent literature, *Am. J. Med.*, 1998, **104**(2), 170–178, DOI: 10.1016/S0002-9343(97)00356-1.

10 S. K. Pal and Y. Shukla, Herbal medicine: Current status and the future, *Asian Pac. J. Cancer Prev.*, 2003, **4**(4), 281–288.

11 A. Ahmad, S. Srivastava and M. Srivastava, Enzymatic hydrolysis and fermentation of cassava starch to ethanol, *Afr. J. Biotechnol.*, 2011, **10**(66), 14962–14969.

12 H. Peng, *et al.*, Optimization of fermentation conditions using Saccharomyces cerevisiae for production of functional beverages, *Bioresour. Technol.*, 2009, **100**(19), 4480–4486.

13 D. R. Rivera, *et al.*, Stability of antioxidant activity in fermented plant-based beverages during storage, *Food Chem.*, 2013, **138**(2–3), 1489–1495.

14 M. Kitamura, M. Okazaki, Y. Osaki and K. Oishi, Evaluation method of sensory test for taste and texture in beverages, *J.*

Food Sci. Technol., 2016, **53**(3), 1505–1512, DOI: **10.1007/s13197-015-2084-3**.

15 Y. Wang, Y. Liu, Y. Zhang, X. Li, Y. Zhao and J. Sun, Analysis of volatile compounds in fermented functional beverages using gas chromatography–mass spectrometry, *Food Chem.*, 2022, **376**, 131870, DOI: **10.1016/j.foodchem.2021.131870**.

16 S. Rawat, R. K. Verma, N. Singh, F. Khan., 2022.

17 M. Jonsson, C. Johansson, H. Tjälve and C. Lindqvist, Acute Oral Toxicity Testing in Rodents According to OECD Guideline 423: A Practical Approach, *Regul. Toxicol. Pharmacol.*, 2013, **66**(1), 60–67, DOI: **10.1016/j.yrtph.2012.12.008**.

18 S. K. Jaganathan, M. Mandal and M. V. Vellayappan, Impact of phenolic compounds on the immune system, *Food Chem.*, 2012, **136**(3–4), 302–312, DOI: **10.1016/j.foodchem.2012.08.098**.

19 T. Higashihara, M. Shibutani, K. Y. Lee, H. Sato, H. Takagi, K. Mitsumori, *et al.*, Sub-acute oral toxicity study of a synthetic food dye, Allura Red AC in F344 rats, *Toxicology*, 2007, **239**(1–2), 104–110, DOI: **10.1016/j.tox.2007.06.089**.

20 W. Fan, Y. Xu and Y. Zhang, Characterization of aroma compounds of Chinese yellow rice wine by gas chromatography–mass spectrometry and sensory evaluation, *J. Inst. Brew.*, 2006, **112**(2), 137–143, DOI: **10.1002/j.2050-0416.2006.tb00717.x**.

21 P. A. Girardi, B. M. Silva, J. C. F. Moreira and D. O. de Souza, Wine aroma compounds derived from amino acid metabolism, *Braz. J. Food Technol.*, 2010, **13**(4), 262–270.

22 J. Zhu, M. Liu and Y. Liu, Recent developments in the flavor chemistry of alcoholic beverages, *Crit. Rev. Food Sci. Nutr.*, 2016, **56**(Suppl 1), S48–S67, DOI: **10.1080/10408398.2013.787969**.

23 H. Z. Mo, H. Zhang and Y. Q. Xu, Traditional fermented functional foods for improving intestinal health, *Crit. Rev. Food Sci. Nutr.*, 2009, **49**(6), 555–567, DOI: **10.1080/10408390903001707**.

24 J. L. Willems, *Development of Authenticity Methods for Apple and Pear Juices [dissertation]. Saskatoon (Canada)*, University of Saskatchewan, 2017, **https://harvest.usask.ca/handle/10388/8189**.

25 V. Cheynier, Phenolic compounds: From plants to foods, *Phytochem. Rev.*, 2012, **11**(2), 153–177, DOI: **10.1007/s11101-012-9242-8**.

26 L. F. Casassa, R. C. Larsen and J. F. Harbertson, Effects of vineyard and winemaking practices impacting berry size on evolution of phenolics during winemaking, *Am. J. Enol. Vitic.*, 2016, **67**(3), 257–268, DOI: **10.5344/ajev.2016.15108**.

27 J. J. Allan, *et al.*, Safety evaluation of a standardized phytochemical composition extracted from Bacopa monnieri in Sprague–Dawley rats, *Food Chem. Toxicol.*, 2007, **45**(10), 1928–1937.

28 G. E. Paget and J. M. Barnes. Toxicity tests, in *Evaluation of Drug Activities: Pharmacometrics*, ed. Lawrence D. R. and Bacharach A. L., Academic Press, London, 1964. p. 140–161.