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Research Article

In silico investigation of antioxidant interaction and effect of probiotic fermentation on antioxidant profiling of pearl millet-based rabadi beverage

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Abstract
Pearl millet-based food products can be used for weight control and minimize the possibility of chronic diseases. They have protein, minerals, fat, phenolic compounds, and a diminutive glycemic index. Moreover, Probiotic fermentation can bring specific additional benefits in addition to nutritional improvements. In silico analysis of the chemical-protein interaction of tannic acid and ascorbic acid of pearl millet was undertaken. Further, the role of fortification of rabadi beverage by probiotic culture was also assessed in this study at different temperatures (35, 42, and 45°C) of fermentation. In silico study has predicted the association of both tannic acid and ascorbic acid with the various human proteins responsible for the growth and development of the human immune system. In all used probiotic (*Lactobacillus rhamnosus, Lactobacillus sp.* and *Streptococcus faecalis*), *L. rhamnosus* fortified rabadi beverage at continuous increasing temperature (35, 42, 45 °C) of non-autoclaved batch showed high content of TAC (36.83 ± 5.41 µg mL^{-1}), TPC (46.1 ± 8.28 µg mL^{-1}) and TFC (29.91 ± 7.73 µg mL^{-1}); while decrease in tannins content (14.84 ± 4.64 µg mL^{-1}) as compared to control [TAC (29.32 ± 3.17 µg mL^{-1}), TPC (25.53 ± 5.75 µg mL^{-1}), TFC (21.91 ± 5.95 µg mL^{-1}), and Tannins (20.74 ± 3.43 µg mL^{-1})]. *L. rhamnosus* fortified rabadi beverage of non-autoclaved batch showed better results than *Lactobacillus sp.* and *S. faecalis* fortified rabadi beverage of both batches (autoclaved and non-autoclaved); which in turn expressed the enhanced therapeutic activity of probiotic fortified rabadi beverage.

Keywords: Antioxidant profiling, In-silico, Pearl millet, Probiotic fermentation, Total antioxidant capacity

INTRODUCTION

Millet is generally used as a source of nutrients and dietary energy in African and Asian subcontinents (Krishnan and Meera, 2018; Rani *et al.,* 2018). Pearl millet is protein-rich (Agrawal *et al.,* 2016), has high lipid content, presence of phenols, flavonoids, carotenoids have relevance for the antioxidant activity that point outs the interest of pearl millets towards human health benefits (Falcinelli *et al.,* 2018). Therefore, all age groups have consumed traditional fermented pearl millet grains in breakfast or as a refreshment food in all regions of Nigeria and sub-Saharan Africa. Pearl millet grain has been recognized to possess vital nutritive values, which include proteins (10.96 g/100g), iron (6.42 mg/100g), calcium (27.35 mg/100g), carbohydrates (61.78 g/100g), as well as numerous phenolic compounds (Longvah *et al.,* 2017). These nutrients contribute to the antioxidant and healthful benefits of the pearl millet (Akinola *et al.,* 2017; Banwo *et al.,* 2021a; Obadina *et al.,* 2017). Nutritional values, functional properties, and enhancement of health benefits

of grain substrates have been improved with controlled fermentation using selected strain (Menezes *et al.,* 2018; Okoroafor *et al.,* 2019; Yepez *et al.,* 2019; Sidari *et al.,* 2020). Several phytochemical compounds like tannins, phenolic acids, and flavonoids have high antioxidant properties in fermented food products and have been highly suggested by dietetics in daily consumption (Chandrasekara and Shahidi, 2012; Olojede *et al.,* 2020). Potent antioxidant eliminates the free radicals from the human body that prevents the cardio-cerebral syndrome, deferring decrepitude, and work as an anticancer (Noratto *et al.,* 2009), bacteriostatic (Ng *et al.,* 2019), liver-protecting agents (Callcott *et al.,* 2018), as an anti-infectent (Pe˘sic *et al.,* 2019), cholesterol-reducing agents (Liu *et al.,* 2018), immunity developer agents (Cuevas *et al.,* 2013). They play a vital role in preventing various disorders like type 2 diabetes (Xiao *et al.,* 2013; Vitale *et al.,* 2017). The previous study has been stated that probiotics-based foods and drinks have conferred the enhancement of host health when entered the live microorganism in sufficient quantity (Hill *et al.,* 2014). Probiotics have inhibited the treatment and prevention of various diseases, and since ancient times, probiotics have been used as a bio-therapeutics agent (Alard *et al.,* 2018; Hager and Ghannoum, 2017; Kerrman and Deshpande, 2014; Reid, 2017).

Rabadi beverage is widely used as a fermented beverage for human consumption. Traditionally, it is prepared with the help of pearl millet (*Pennisetum typhoideum* (L.) flour's, fermented with sour buttermilk in various concentrations. The study was undertaken to evaluate the association of the tannic acid and ascorbic acid with the various human protein as well as the functional therapeutic potentials of *Lactobacillus rhamnosus, Lactobacillus sp.,* and *Streptococcus faecalis* fortified pearl millet-based rabadi beverage through controlled fermentation.

MATERIALS AND METHODS

Materials

Pearl millet and buttermilk (mother dairy) for the standard inoculum were purchased from Mahendragarh, Haryana's local market. *L. rhamnosus* ATCC was procured from Himedia Laboratories Pvt Ltd. *Lactobacillus sp.* and *S. faecalis* were generously gifted by Dr. Gunjan Goel, Central University of Haryana Mahendragarh, Haryana, India.

Methodology

Network analysis

Interaction between proteins and chemical molecules is beneficial for the understanding of cellular as well as molecular functions of the compounds. Online network database STITCH (Search Tool for Interactions of Chemicals) was used for the chemical-protein interaction of tannic acid and ascorbic acid of pearl millet. STITCH (http://stitch.embl.de/) database is widely used in the interaction of metabolic pathways, 3-dimensional structures of molecules, experiments related to binding between protein and chemicals, and DTIs (drug-target interactions).

Milling

Pearl millet grains were collected and washed out to remove external matter. Subsequently, the grains were dried in a hot air oven at 70°C for 7 h (Ogodo *et al.,* 2018). The dried grains were ground into flour with a grinder. The whole flour was filtered through a mesh screen and put in storage such as a desiccator.

Preparation of slurry for fermentation

The pearl millet flour is assorted with distilled water and buttermilk to form a slurry for fermentation (non-autoclaved batch). However, it was subsequently autoclaved to obtain another batch (autoclaved). The slurry so obtained was cooled down to room temperature.

Inoculum preparation

L. rhamnosus, Lactobacillus sp., and *S. faecalis* cultures were activated by inoculating in De Man, Rogosa, and Sharpe agar (MRS) broth and subsequently put on incubation for a period of 48 h at 37°C. To achieve significant growth of bacterial population, sub-culturing was also carried out. The cells of the activated cultures were pelleted by centrifugation at 2500 rpm for 5 min.

Fermentation

The traditional practice of rabadi beverage fermentation is carried out in summer at room temperature, generally in the range of 40 to 50 °C in the northwest region. Therefore, the temperature for the fermentation process opted around this range. The fermentation process was performed for 12 h individually at different temperatures 35 °C, 42 °C, 45 °C. However, another setup was carried out at continuously increasing temperatures at 35 °C, 42 °C, and 45 °C with an interval of 4 hrs at each temperature. The probiotic cultures were mixed at room temperature, and subsequently, fermentation was additionally performed at 37 °C for 24 h. Before and after probiotic fermentation, the product was stored at 4 °C for further characterization.

Preparation of methanolic extracts of pearl millet

The methanolic extraction of the rabadi beverage sample was taken as per the given method of Zuo *et al.* (2002). Crush the air-dried rabadi beverage sample (5 g) and filter it through a 1 mm sieve. The methanolic extract was collected by adding 25 ml of 80% methanol and stirred with an electric stirrer at ambient temperature for 3 h. The flask contents were extracted two times with 80% methanol (20 mL), including 0.15% hy-

drochloric acid under identical conditions. The methanolic extract was filtered through Whatman No. 42 filter paper (125 mm) or 0.45 µm nylon membrane filter paper. Reduced the pressure to dry the extract at 45 °C on a rotating evaporator and stored in a -18 °C freezer until used for the additional characterization.

Determination of total antioxidant capacity

The TAC (total antioxidant capacity) was measured through the phosphomolybdenum procedure which has been stated in Prieto *et al.* (1999). Ascorbic acid (1 mg mL^{-1}) was used as a standard. 1 mL of freshly prepared transfection suspension (0.6 M sulfuric acid (H_2SO_4), 28 M sodium phosphate (Na_3PO_4), 4 mM ammonium molybdate mixture [$(NH_4)_2MoO_4$] was added to 1 mL of rabadi beverage sample extract and 1 mL of various ascorbic acid concentration (10-50 µg mL^{-1}). Incubated the mixture for 90 min at 95 °C and cooled down at ambient temperature. Absorbance at 695 nm was measured with respect to a blank (same reaction mixture without rabadi beverage sample extract). The rabadi beverage sample was expressed in ascorbic acid equivalent per gram of extract. TAC (Total antioxidant capacity) test was used to measure the antioxidant capacity of all probiotic fortified batches.

Determination of total phenols

The phenols content of rabadi beverage samples was analyzed by Folin-Ciocaltu colorimetric technique of Singleton and Rossi (1965) with some modifications, using gallic acid (1 mg mL^{-1}) as a standard. Different concentrations (20-100 µg mL -1) of the standard were taken in a test tube and made up to 6 mL with distilled water for the standard curve. After adding 0.25 mL of Folin-Ciocaltu reagent, the mixture was incubated for 3 min at 25 °C. 1 mL of saturated sodium carbonate (Na_2CO_3) and 1 mL of distilled water were added to this mixture. Further, kept the mixture in the dark for incubation for 1 h at 25 °C. The resulting blue colour absorption was measured at 720 nm against white (equivalent reaction mixture without rabadi beverage sample extract). The same procedure was used to analyze rabadi beverage samples, except that gallic acid was replaced with 1 mL of rabadi beverage sample extract. The results were expressed as gallic acid equivalents (µ mol gallic acid equivalents per gm of methanolic extract weight).

Determination of total flavonoids

The flavonoids content of rabadi beverage samples was measured by Chang *et al.* (2002) using quercetin (1 mg mL^{-1}) as standard. A sample of 1.0 mL of rabadi beverage extract and several concentrations of the standard (20-100 µg mL^{-1}) was taken in a test tube, and a volume of up to 5.0 mL was prepared using distilled water. 0.3 ml of sodium nitrite solution ($NaNO_2$) (5%, w/v) and 0.3 ml of aluminum chloride solution ($AlCl_3$) (10%, w/v) added in the mixture. The contents were mixed and kept for 6 min before adding 2 mL of sodium hydroxide solution (NaOH) (1.0 mol L^{-1}) and finally to a volume of 10 mL. After the vortex, the absorbance of the solution was analyzed with respect to the blank (the identical reaction mixture without the methanolic extract of rabadi beverage) at 510 nm. The same procedure was used to analyze the rabadi beverage samples, except to replace quercetin with 1 mL of rabadi beverage sample extract. Total flavonoids were equal to quercetin, which was calculated from the standard curve.

Determination of tannins

The tannins contents were determined according to the procedure described by Nwinuka *et al.* (2005). Tannic acid (1 mg mL^{-1}) was taken as a standard for this test. The rabadi beverage sample extract and various concentrations of the standard (20–100 µg mL^{-1}) were stirred with 0.5 mL of Folin - Ciocaltu reagent and 1.0 mL of $NaCO_3$ solution (0.5%, w/v). Total volume was made up to 5.0 mL. The absorbance of the samples was estimated at 755 nm against a blank (the same reaction mixture without rabadi beverage sample extract). The amount of tannin was analyzed through the standard curve of tannic acid.

Statistical analysis

All the experiments performed in the study were done in triplicates, and the statistical analysis was conducted using an ANOVA with a single factor test to determine the level of significance as per the method of Howell (2012).

RESULTS AND DISCUSSION

Chemical-protein network

The edges of chemical-protein interactions are specific and valuable, i.e., proteins together contribute to a shared function; it does not mean that they are physically bonded to each other. The edge confidence score determines the relevance of the protein chemical interaction if the score is 0.150 weak interaction (not significant), the score is 0.400 mean interaction (least significant), the score is 0.700 high interaction (the most significant), and the score is 0.900 highest interaction (strong interaction) between chemical and protein.

Protein- chemical network analysis of tannic acid

Protein-chemical network of tannic acid predicted nine human proteins associated with the tannic acid and the same is shown in Fig. 1. Some important characteristics were obtained through this network analysis viz. number of nodes: 9, number of edges: 3, average node degree: 0.66, the coefficient of clusters: 1, expected number of edges: 10, and protein-protein interaction

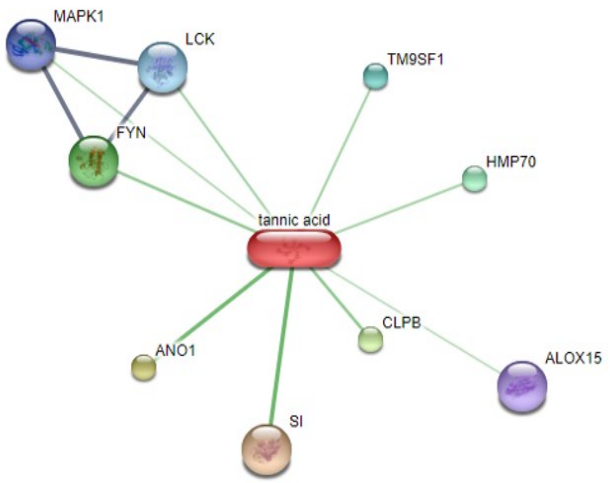

Fig. 1. *Network of tannic acid interaction with human protein by STITCH database*

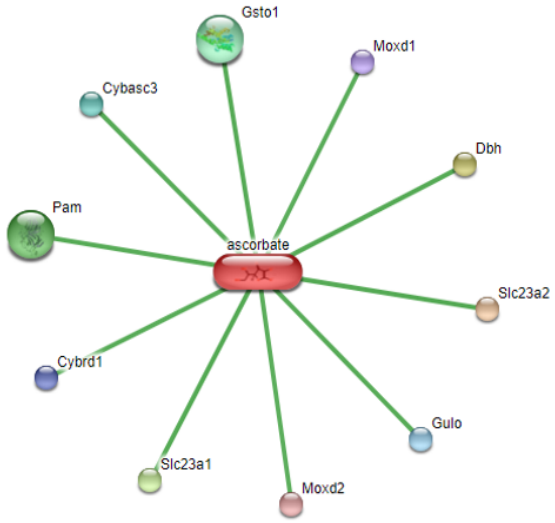

Fig. 2. *Network of ascorbic acid interaction with human protein by STITCH database*

enrichment p-value: 0.998.

Predicted functional partners of tannic acid

Chemical-protein association is an important investigation to understand the effects of chemical moieties in human health. There were nine human proteins SI, ANOI, CLPB, FYN, HMP70, TM9SF1, LCK, MAPK1, and ALOX15, directly associated with tannic acid. Table 1 shows the predicted protein partners with names and their functional activity. The ultimate goal of this In-silico work is to find the impact of tannic acid in human nutrition.

Pathways analysis of tannic acid

Kyoto encyclopedia of Gene and Genome (source: http://stitch.embl.de/.) is the database on biological systems used to analyze high-level molecular function and utilities. The STITCH Network database, based on chemical and protein association, showed that tannic acid interacts with the human protein in the default mode of STITCH database. Through the pathways analysis of tannic acid, many specific physiological functions were found responsible for osteoclast differentiation, NK (natural killer) cell facilitated cytotoxicity, T cell receptor signaling pathway, prion diseases, adherens junction, Fc epsilon RI signalling pathway, cholinergic synapse, serotonergic synapse, axon guidance and platelet activation. A detailed description of the same has been mentioned in Table 2.

Network analysis of ascorbic acid

This protein-chemical network analysis found the ten human proteins that are associated with the ascorbic acid, as shown in Fig. 2. Some essential characteristics of network analysis were obtained for the ascorbic acid, i.e. number of nodes: 10, number of edges: 0, the average degree of node: 0, the coefficient of clusters: 1, expected number of edges: 10, and protein-protein interaction enrichment p-value: 1.

Predicted functional partners of ascorbic acid

STITCH database predicted the ten human proteins viz. Slc23a2, Dbh, Slc23a1, Pam, Gsto1, Cybase3, Gulo, Cybrd1, Moxd1, and Moxd2 are directly interacting with ascorbic acid. Table 3 shows the description and length of the human protein associated with ascorbic acid.

Pathway analysis of ascorbic acid

STITCH network analysis has predicted pathways of associated protein with the help of KEGG based on chemical and protein association. The predicted gene ontology (Molecular function, Biological process and Cellular components) of associated proteins, through the KEGG pathways, were 1) Molecular function: L-ascorbate: sodium symporter activity, oxidoreductase activity, sodium-dependent L-ascorbate transmembrane transporter activity, L-ascorbic acid-binding, copper ion binding, 2) Biological process: L-ascorbic acid metabolic process, transepithelial L-ascorbic acid transport, oxidation-reduction process, small molecule metabolic process, response to transition metal nanoparticle, carboxylic acid metabolic process, single-organism metabolic process and 3) Cellular component: basal plasma membrane, an integral component of membrane, cell projection, basal part of the cell, whole membrane, apical part of the cell, transport vesicle membrane, secretory granule membrane, organelle membrane. Furthermore, details are given in Table 4.

Optimization of the fermentation process

The autoclaved batches contain a sterilized mixture of pearl millet-water-buttermilk suspensions to avoid any

Table 1. Depicting the symbol of protein, the sequence length of the protein, description of protein, and a score of the node probability based on the experimental work in STITCH database for the tannic acid.

Symbol	Length in aa	Description	Score
SI	1827	Sucrase-isomaltase (alpha-glucosidase) Plays an important role in the final stage of carbohydrate digestion, and iso-maltase activity is specific for alpha-1,4- and alpha-1,6-oligosaccharides.	0.846
ANO1	986	Anoctamin 1, calcium-activated chloride channel Calcium-activated chloride channel (CaCC) which plays a vital role in trans-epithelial anion transport and smooth muscle contraction. Acts as a major contributor to basal and stimulated chloride conductance in airway epithelial cells and play a vital role in tracheal cartilage development.	0.816
CLPB	707	ClpB caseinolytic peptidase B homolog (*E. coli*) May function as a regulatory ATPase and be related to the secretion/protein traf-ficking process.	0.627
FYN	537	FYN oncogene related to SRC, FGR, YES Non-receptor tyrosine-protein kinase plays a role in many biological processes, including cell growth and survival regulation, cell adhesion, integrin-mediated sig-naling, cytoskeletal remodeling, cell motility, immune response, and axon guidance. It is involved in the regulation of cell adhesion and motility.	0.612
HMP70	815	Transmembrane 9 superfamily member 1 Plays an essential role in autophagy	0.510
TM9SF1	606	Transmembrane 9 superfamily member 1 Plays an essential role in autophagy.	0.510
LCK	509	Lymphocyte-specific protein tyrosine kinase.	0.507
MAPK1	360	Mitogen-activated protein kinase 1 Serine/threonine kinase acts as an essential component of the MAP kinase signal transduction pathway. It participates in a signaling cascade initiated by activated KIT and KITLG/SCF. Depending on the cellular context, the MAPK/ERK cascade mediates diverse bio-logical functions such as cell growth, adhesion, survival, and differentiation through the regulation of transcription, translation, cytoskeletal rearrangements.	0.434
ALOX15	662	Arachidonate 15-lipoxygenase Converts arachidonic acid to 15S- hydro peroxy-eicosatetraenoic acid. It also acts on C-12 of arachidonate as well as on linoleic acid.	0.405

outsourced contamination. Autoclaving is reported to increase the digestibility of the autoclaved ingredient during fermentation. The autoclaved ingredient was potentially improved by different types of fermentation, i.e. natural and controlled fermentation (Sharma and Kapoor, 1996). The probiotic fortified rabadi beverage of both the batches (autoclaved and non-autoclaved) was analyzed for the antioxidant characterization.

Total antioxidant capacity (TAC)

The antioxidant contents having antioxidant potential are secondary metabolites of plants and are related to reducing the risk of chronic diseases related to oxidative stress.

As depicted in Fig. 3 and Table 5, *L. rhamnosus* fortified rabadi beverage at continuous increasing temperature (35, 42, 45 °C) of non-autoclaved batch showed maximum content of TAC (36.83 ± 5.41 µg ml^{-1}) as compared to other batches of probiotic fortified rabadi beverage as well as control fermented at various temperatures as mentioned in the methodology. Similarly, Ogunremi *et al.* (2015) showed the increased antioxidant potential of the grain-based beverage after fermentation with *Pichia kudriavzevii* OG32. The same fact has also been shown by Qian *et al*. (2012), who reported an enhancement in the antioxidant potential of *Pavlova lutheri* (microalgae) after fermentation by *Hansenula polymorpha* (*Pichia angusta*).

Total phenols, total flavonoid, and total tannins contents

Taylor and Duodu (2015) stated that phenolic compounds are considered as antinutrients because they are complex with proteins and other nutrients, thus pre-

Table 2. Depicting the pathways ID, pathways description, observed gene count, false recovery rate, matching proteins in the network (IDs), and matching proteins in the network (labels) for protein partners of tannic acid.

KEGG Pathways (Molecular function and Biological Process)						
Sr. No	**Pathways ID**	**Pathways description**	**Observed gene count**	**False recovery rate**	**Matching proteins in network (IDs)**	**Matching**
1	4380	Osteoclast differentiation	3	0.00236	ENSP00000215832,ENSP00000337825,ENSP00000346671	FYN,LCK,MAPK1
2	4650	Natural killer (NK) cell mediated cytotoxicity	3	0.00236	ENSP00000215832,ENSP00000337825,ENSP00000346671	FYN,LCK,MAPK1
3	4660	T- cell receptor signaling pathway	3	0.00236	ENSP00000215832,ENSP00000337825,ENSP00000346671	FYN,LCK,MAPK1
4	5020	Prion diseases	2	0.00964	ENSP00000215832,ENSP00000346671	FYN,MAPK1
5	4520	Adherens junction	2	0.0244	ENSP00000215832,ENSP00000346671	FYN,MAPK1
6	4664	Fc epsilon RI signaling pathway	2	0.0244	ENSP00000215832,ENSP00000346671	FYN,MAPK1
7	4725	Cholinergic synapse	2	0.0442	ENSP00000215832,ENSP00000346671	FYN,MAPK1
8	4726	Serotonergic synapse	2	0.0442	ENSP00000215832,ENSP00000293761	ALOX15,MAPK1
9	4360	Axon guidance	2	0.0471	ENSP00000215832,ENSP00000346671	FYN,MAPK1
10	4611	Platelet activation	2	0.0471	ENSP00000215832,ENSP00000346671	FYN,MAPK1
Cellular component						
1	GO.0031234	Extrinsic component of cytoplasmic side of plasma membrane	3	0.0148	ENSP00000293761,ENSP00000337825,ENSP00000346671	ALOX15,FYN,LCK
2	GO.0098552	Side of membrane	4	0.0148	ENSP00000293761,ENSP00000337825,ENSP00000346671,ENSP00000347454	ALOX15,ANO1,FYN,LCK
3	GO.0009898	Cytoplasmic side of plasma membrane	3	0.0158	ENSP00000293761,ENSP00000337825,ENSP00000346671	ALOX15,FYN,LCK
4	GO.0098805	Whole membrane	6	0.0158	ENSP00000215832,ENSP00000261789,ENSP00000264382,ENSP00000337825,ENSP00000347454,ENSP00000433967	ANO1,HMP70,LCK,MAPK1,SI,TM9SF1
5	GO.0000421	Autophagosome membrane	2	0.0162	ENSP00000261789,ENSP00000433967	HMP70,TM9SF1

Table 3. Depicting the symbol of protein, the sequence length of the protein, description of protein, and score of the node probability based on the experimental work in STITCH database for the ascorbic acid.

Symbol	Length in aa	Description	Score
Slc23a2	647	Solute carrier family 23 member 2 Sodium/ascorbate cotransporter. Mediates electrogenic uptake of vitamin C, with a stoichiometry of 2 Na (+) for each ascorbate.	0.999
Dbh	621	Dopamine beta-hydroxylase Conversion of dopamine to noradrenaline.	0.997
Slc23a1	604	Solute carrier family 23 member 1 Sodium/ascorbate cotransporter. Mediates electrogenic uptake of vitamin C, with a stoichiometry of 2 Na (+) for each ascorbate.	0.995
Pam	977	Peptidyl-glycine alpha-amidating monooxygenase Peptidylglycine alpha-hydroxylating monooxygenase Peptidyl-alpha-hydroxyglycine alpha-amidating lyase Bifunctional enzyme that catalyzes 2 sequential steps in C-terminal alpha-amidation of peptides. C-terminal amidation of peptides such as neuropeptides is essential for full biological activity.	0.981
Gsto1	241	Glutathione S-transferase omega-1 Exhibits glutathione-dependent thiol transferase and dehydroascorbate reductase activities. Has S-(phenacyl) glutathione reductase activity and glutathione S-transferase activity. Participates in the biotransformation of inorganic arsenic and reduces monomethylarsonic acid (MMA) and dimethylarsonic acid.	0.981
Cybasc3	256	Cytochrome b ascorbate-dependent protein 3 Ferric-chelate reductase that reduces Fe (3+) to Fe (2+) before its transport from the endosome to the cytoplasm. Probably uses ascorbate as electron donor.	0.977
Gulo	440	L-gulonolactone oxidase Oxidizes L-gulono-1,4-lactone to hydrogen peroxide and L-xylo-hexulonolactone, which spontaneously isomerizes to L- ascorbate	0.976
Cybrd1	286	Cytochrome b reductase 1 Ferric-chelate reductase that reduces Fe (3+) to Fe (2+). Present at the brush border of duodenal enterocytes, probably reducing dietary Fe (3+), thereby facilitating its transport into the mucosal cells. Uses ascorbate as electron donor. It may be involved in extracellular ascorbate recycling in erythrocyte membranes. It may also act as a ferrireductase in airway epithelial cells.	0.969
Moxd1	613	Monooxygenase, DBH-like 1; Protein Moxd1	0.957
Moxd2	619	Monooxygenase, DBH-like 2 precursor	0.957

Table 4. Depicting the pathways ID, pathways description, observed gene count, false recovery rate, matching proteins in the network (IDs), and matching proteins in the network (labels) for protein partners of ascorbic acid.

Biological process						
Sr. No	**Pathways ID**	**Pathways description**	**Observed gene count**	**False recovery rate**	**Matching proteins in network(IDs)**	**Matching**
1	GO.0019852	L-ascorbic acid metabolic process	3	5.23E-05	ENSRNOP00000016851,ENSRNOP00000022703,ENSRNOP00000028885	Gsto1,Gulo,Slc23a2
2	GO.0070904	Transepithelial L-ascorbic acid transport	2	0.000794	ENSRNOP00000027048,ENSRNOP00000028885	Slc23a1,Slc23a2
3	GO.0019853	L-ascorbic acid biosynthetic process	2	0.00286	ENSRNOP00000016851,ENSRNOP00000022703	Gsto1,Gulo
4	GO.0055114	Oxidation-reduction process	5	0.0034	ENSRNOP00000012942,ENSRNOP00000016851,ENSRNOP00000028094,ENSRNOP00000046774,ENSRNOP00000057915	Cybasc3,Cybrd1,Dbh,Gsto1,Pam
5	GO.0044281	Small molecule metabolic process	5	0.0197	ENSRNOP00000016851,ENSRNOP00000022703,ENSRNOP00000028885,ENSRNOP00000046774,ENSRNOP00000057915	Dbh,Gsto1,Gulo,Pam,Slc23a2
6	GO.1990267	Response to transition metal nanoparticle	3	0.0295	ENSRNOP00000012942,ENSRNOP00000046774,ENSRNOP00000057915	Cybrd1,Dbh,Pam
7	GO.0019752	Carboxylic acid metabolic process	4	0.0367	ENSRNOP00000016851,ENSRNOP00000022703,ENSRNOP00000028885,ENSRNOP00000046774	Gsto1,Gulo,Pam,Slc23a2
8	GO.0044710	Single-organism metabolic process	6	0.0367	ENSRNOP00000012942,ENSRNOP00000016851,ENSRNOP00000028094,ENSRNOP00000028885,ENSRNOP00000046774,ENSRNOP00000057915	Cybasc3,Cybrd1,Dbh,Gsto1,Pam,Slc23a2
Molecular function						
1	GO.0008520	L-ascorbate:sodium symporter activity	2	0.000233	ENSRNOP00000027048,ENSRNOP00000028885	Slc23a1,Slc23a2
2	GO.0016715	Oxido-reductase activity,	2	0.000233	ENSRNOP00000046774,ENSRNOP00000057915	Dbh,Pam

Contd……..

3	GO.0070890	Sodium-dependent L-ascorbate transmembrane transporter activity	2	0.000233	ENSRNOP00000027048,ENSRNOP00000028885	Slc23a1,Slc23a2
4	GO.0016491	Oxidoreductase activity	5	0.000651	ENSRNOP00000012942,ENSRNOP00000016851,ENSRNOP00000028094,ENSRNOP00000046774,ENSRNOP00000057915	Cybasc3,Cybrd1,Dbh,Gsto1,Pam
5	GO.0031418	L-ascorbic acid binding	2	0.0146	ENSRNOP00000046774,ENSRNOP00000057915	Dbh,Pam
6	GO.0005507	Copper ion bind-	2	0.046	ENSRNOP00000046774,ENSRNOP00000057915	Dbh,Pam
Cellular Component						
1	9925	Basal plasma	2	0.0176	ENSRNOP00000027048,ENSRNOP00000028885	Slc23a1,Slc23a2
2	16021	Integral component of membrane	7	0.0176	ENSRNOP00000012942,ENSRNOP00000022703,ENSRNOP00000027048,ENSRNOP00000028094,ENSRNOP00000028885,ENSRNOP00000046774,ENSRNOP00000057915	Cybasc3,Cybrd1,Dbh,Gulo,Pam,Slc23a1,Slc23a2
3	42995	Cell projection	5	0.0201	ENSRNOP00000012942,ENSRNOP00000016851,ENSRNOP00000027048,ENSRNOP00000046774,ENSRNOP00000057915	Cybrd1,Dbh,Gsto1,Pam,Slc23a1
4	45178	Basal part of cell	2	0.0201	ENSRNOP00000027048,ENSRNOP00000028885	Slc23a1,Slc23a2
5	98805	Whole membrane	5	0.0201	ENSRNOP00000012942,ENSRNOP00000027048,ENSRNOP00000028094,ENSRNOP00000046774,ENSRNOP00000057915	Cybasc3,Cybrd1,Dbh,Pam,Slc23a1
6	45177	Apical part of cell	3	0.0269	ENSRNOP00000027048,ENSRNOP00000028885,ENSRNOP00000057915	Dbh,Slc23a1,Slc23a2
7	30658	Transport vesicle membrane	2	0.0283	ENSRNOP00000046774,ENSRNOP00000057915	Dbh,Pam
8	30667	Secretory granule membrane	2	0.0317	ENSRNOP00000046774,ENSRNOP00000057915	Dbh,Pam
9	31090	Organelle membrane	5	0.0327	ENSRNOP00000016851,ENSRNOP00000022703,ENSRNOP00000028094,ENSRNOP00000046774,ENSRNOP00000057915	Cybasc3,Dbh,Gsto1,Gulo,Pam
10	16020	Membrane	7	0.0488	ENSRNOP00000012942,ENSRNOP00000016851,ENSRNOP00000022703,ENSRNOP00000027048,ENSRNOP00000028094,ENSRNOP00000046774,ENSRNOP00000057915	Cybasc3,Cybrd1,Dbh,Gsto1,Gulo,Pam,Slc23a1

Table 5. Antioxidant parameters (µg mL^{-1}) of probiotic fortified pearl millet-based rabadi beverage (non-autoclaved and autoclaved batches) fermented at different temperatures.

PROBIOTIC	TEMPERATURE (°C)	NON-AUTOCLAVED BATCHES				AUTOCLAVED BATCHES			
		TAC (µg mL^{-1})	PHENOLS (µg mL^{-1})	FLAVONOIDS (µg mL^{-1})	TANNIC ACID (µg mL^{-1})	TAC (µg mL^{-1})	PHENOLS (µg mL^{-1})	FLAVONOIDS (µg mL^{-1})	TANNIC ACID (µg mL^{-1})
L. rhamnosus	35	31 ± 4	28.38 ± 6.67	24.25 ± 8.87	19.38 ± 3.66	32.58 ± 5.31	28.84 ± 5.33	24 ± 6	28.3 ± 2.86
	42	4.87 ± 1.5	27.61 ± 4.89	19.66 ± 4.50	17.76 ± 4.21	30.83 ± 2.91	27.69 ± 7.08	16 ± 4	31.76 ± 6.57
	45	23.62 ± 5.7	24 ± 5	16 ± 4	15 ± 4	29.83 ± 6.70	37.3 ± 5.05	27.83 ± 6.37	26.12 ± 4.44
	35, 42, 45	36.83 ± 5.41	46.1 ± 8.28	29.91 ± 7.73	14.84 ± 4.64	21.75 ± 5.09	30.84 ± 5.06	14.41 ± 3.64	24.69 ± 5.6
Lactobacillus sp.	35	29.25 ± 3.54	31.3 ± 4.68	29.66 ± 6.35	19.38 ± 3.79	30.25 ± 5.12	36.53 ± 3.76	29 ± 4	23.38 ± 2.05
	42	4.08 ± 1.06	30.3 ± 5.9	24.16 ± 6.03	21.46 ± 5.12	35.04 ± 5.94	20.38 ± 4.90	25.83 ± 5.12	22.84 ± 4.01
	45	20.04 ± 4.05	23.23 ± 4.93	17.41 ± 4.04	18.07 ± 1.99	20.79 ± 2.05	45.23 ± 5.08	17 ± 4	28.84 ± 6.13
	35, 42, 45	17.5 ± 5.87	40.46 ± 7.14	21.58 ± 4.90	16 ± 4	20.79 ± 4.07	25.15 ± 5.98	19.34 ± 5.90	19.07 ± 4.10
Streptococcus faecalis	35	26 ± 2	37.69 ± 5.69	28.33 ± 7.42	18.76 ± 2.18	29.04 ± 4.98	13.46 ± 2.76	29.49 ± 6.43	24.84 ± 4.29
	42	4.2 ± 1.05	22.07 ± 4.03	18.58 ± 5.55	21.84 ± 6.34	28.62 ± 3.20	21.38 ± 5.09	25.83 ± 6.20	23.69 ± 5.52
	45	26.83 ± 5.10	23.38 ± 5.86	26.41 ± 6.86	21 ± 3	21.79 ± 2.95	27.84 ± 6.07	27.91 ± 5.40	27.15 ± 4.02
	35, 42, 45	25.37 ± 8.20	43.38 ± 5.90	16.58 ± 6.16	16.23 ± 6.43	22.62 ± 6.18	23.38 ± 4.93	18.25 ± 4.06	23.84 ± 5.97
Control	4	29.32 ±3.17	25.53 ± 5.75	21.91 ± 5.95	20.74 ± 3.43	32.99 ± 4.39	27.03 ± 4.04	20.81 ± 5.63	25.4 ± 3.21

TAC: Total Antioxidant Capacity; Values are mean ± SD of three independent experiments.

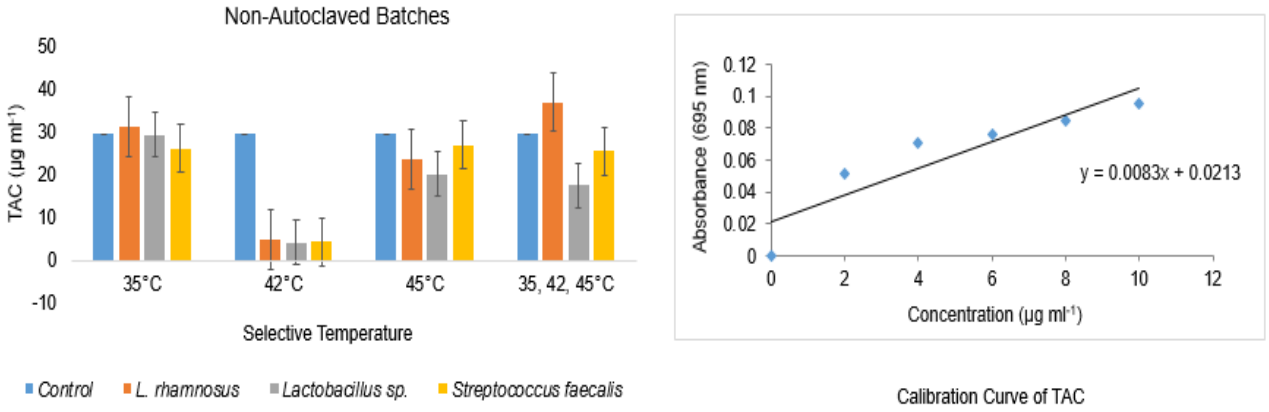

Fig. 3. *TAC (µg ml^{-1}) of pearl millet based probiotic fortified rabadi beverage fermented (non-autoclaved batch) at selective temperature*

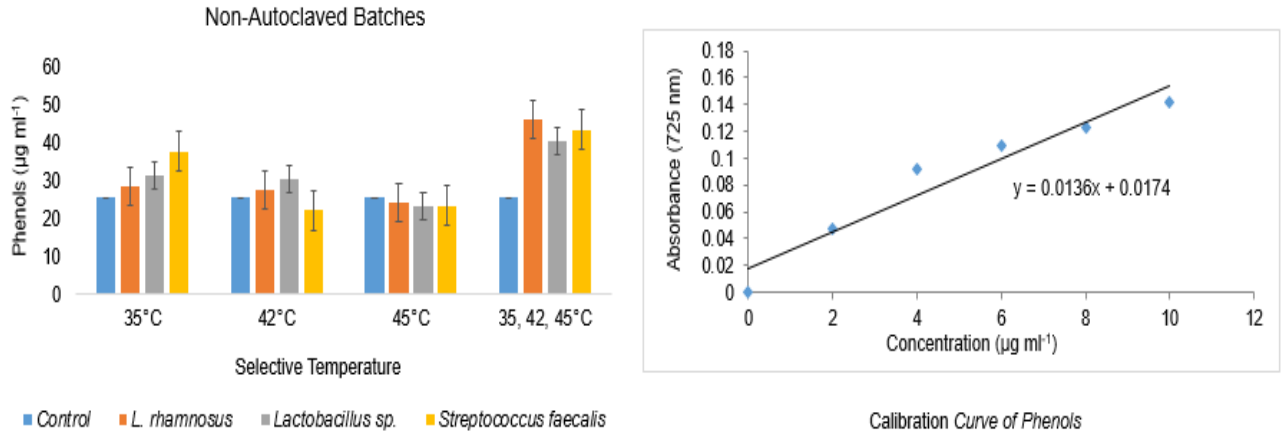

Fig. 4. *Phenols (µg ml^{-1}) of pearl millet based probiotic fortified rabadi beverage fermented (non-autoclaved batch) at selective temperature*

venting the bio-accessibility of nutrients. However, several studies have reported that these phenolic contents have health enhancing characteristics, including reducing heart disease and the inhibition of additional degenerative diseases (Dhankher and Chauhan 1987; Nambiar *et al.*, 2011; Taylor and Duodu, 2015).

As depicted in Fig. 4 and 5, *L. rhamnosus* fortified rabadi beverage at 35, 42, 45 °C of non-autoclaved batch showed significantly high content of TPC (46.1 ± 8.28 µg mL^{-1}) and TFC (29.91 ± 7.73 µg Ml^{-1}) as compared to control (25.53 ± 5.75 µg mL^{-1} and 21.91 ± 5.95 µg mL^{-1}, respectively) and other batches of *Lactobacillus* sp. and *S. faecalis* fortified rabadi beverage (Table 5). A similar report by Banwo *et al.* (2021a) showed the fermentation of millet and sorghum fortified with *L. fermentum* KL4, *Lb. plantarum* MOBL1, *Candida tropicalis* OBY6, and *C. tropicalis* MKY resulted in enhancement of total phenolics and flavonoid in the experimental outcome. The obtained outcomes agreed with the results reported by Salar *et al.* (2012). They showed that the solid-state fermentation increased the total phenolic content (free phenol) and antioxidant potential of corn to the growth rates of 20.05% and 36.73%, respectively. Dlamini *et al*. (2007) also reported that the submerged fermentation also ensured significant results on the total phenolic content (free phenol) and antioxidant potential of sorghum (sludge and gruel).

Taylor and Duodu (2015) reported that these enhanced values were undoubtedly due to the discharge of bound phenol content from the millet's cell walls throughout the experimental procedure, which subsequently led to enhanced clearance of phenolic content. The mechanism of action for fermentation promotes the bioavailability of phenols in cereal grains. Matrix of cell wall has broken due to degrading enzymes in both microbes and grains and enhance the availability of bound and conjugated phenolics to enzymatic hydrolysis reaction (Akinola *et al*., 2017; Obadina *et al.,* 2017; Aryal *et al*., 2019). During millets' fermentation processes, the synthesis or enzymatic modification of several bioactive components occurred, as evident in the study of Banwo *et al*. (2021b).

As depicted in Fig. 6, *L. rhamnosus* fortified rabadi beverage at 35, 42, 45 °C of non-autoclaved batch

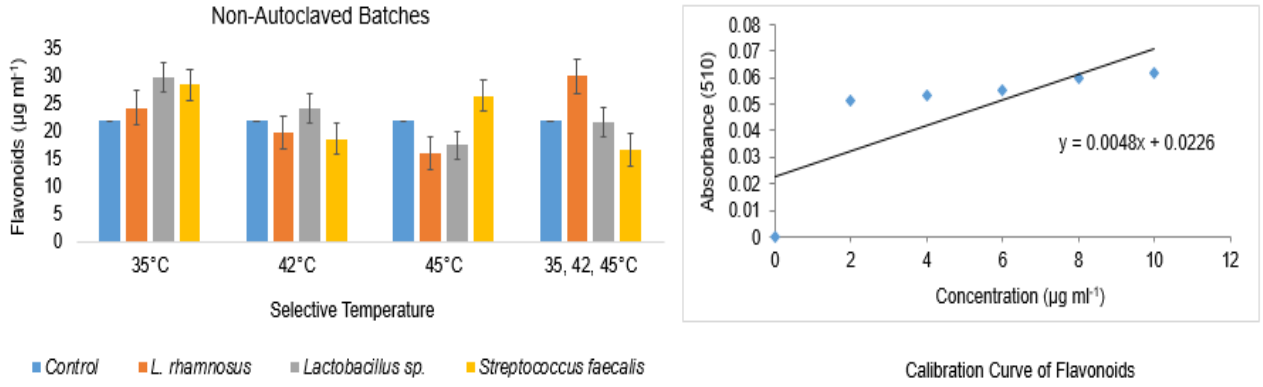

Fig. 5. *Flavonoids (µg ml^{-1}) of pearl millet based probiotic fortified rabadi beverage fermented (non-autoclaved batch) at selective temperature*

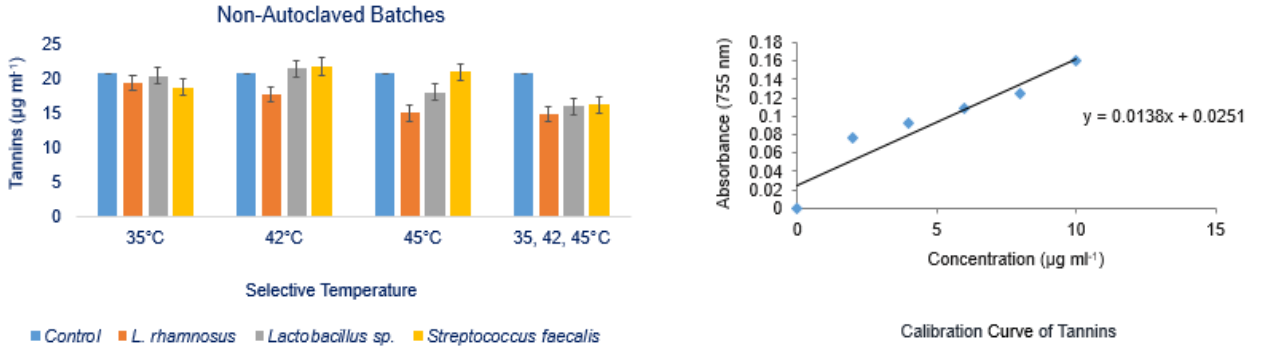

Fig. 6. *Tannins (µg ml^{-1}) of pearl millet based probiotic fortified rabadi beverage fermented (non-autoclaved batch) at selective temperature*

showed less tannins content (14.84 ± 4.64 µg mL^{-1}) as compared to control, i.e., 20.74 ± 3.43 µg mL^{-1}. Agboola and Ojo (2018) reported that the reducing tannic acid content (42.00 mg/kg to 24.03 mg/kg) during fermentation with *Saccharomyces cerevisiae* and *L. plantarum* increases the mineral composition of millet-based fermented foods. Similarly, Srivastava *et al.* (2021) reported that during fermentation, reduction of the tannins present in the barnyard millet was measured from 2.07-0.006 mg, in the range of tannic acid equivalent per gram. The bond that has been made between tannins and cotyledon endosperm is the issue for decrement of tannins content, which is unusual through the regular method because of the insolubility of tannins in the solvent or action of bacterial phenoloxidase.

Conclusion

The In silico study has proven the tremendous therapeutic potential of tannic acid and ascorbic acid in pearl millet. The study demonstrated that tannic acid and ascorbic acid were good antioxidants because they had interaction of such human protein that plays a key role in natural killer cell-mediated cytotoxicity, osteoclast differentiation, responsible to prion diseases, and platelet activation. In various high-level research, the chemical protein interaction has been widely used for biochemical reactions, enzyme nomenclature, disease-related network variations, human diseases, and drugs. *L. rhamnosus* fortified pearl millet-based rabadi beverage (non-autoclaved batch) through controlled fermentation showed the functional therapeutic potentials for developing functional food.

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Conflict of interest

The authors declare that they have no conflict of interest.

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