Research Article **Open Access**

The potential health benefits of a novel synbiotic yogurt fortified with purple-leaf tea in modulation of gut microbiota

Mucheru Patrick[1], Chege Peter[1], Muchiri Mary[2*]

[1]School of Applied Human Sciences, Department of Food, Nutrition and Dietetics, Kenyatta University, P.O BOX 43844-00100, Nairobi, Kenya; [2]Department of Food Science and Nutrition/Tea Institute, Karatina University, BOX 1957, Karatina, Kenya.

***Corresponding Author:** Muchiri Mary, [2]Department of Food Science and Nutrition/Tea Institute, Karatina University, BOX 1957, Karatina, Kenya.

Submission Date: February 23rd, 2024; **Acceptance Date**: March 26th, 2024; **Publication Date:** April 1st, 2024

Please cite this article as: Mucheru P, Chege P and Muchiri M.. The potential health benefits of a novel synbiotic yogurt fortified with purple-leaf tea in modulation of gut microbiota. *Bioactive Compounds in Health and Disease* 2024; 7(4): 170-184. DOI: https://doi.org/10.31989/bchd.v7i3.1323

ABSTRACT

Background: The use of synbiotic functional yogurt is a trending dietary approach of positively modulating the gut and alleviating dysbiosis. These yogurts are fermented using probiotic live microorganisms that confer health benefits on the host. Purple-leaf tea (*Camellia sinensis)* which contains prebiotic polyphenolic compounds, has all the health benefits of tea in addition to high content of flavonoid bioactive anthocyanin compounds.

Object ve: The goal of the current study was to investigate the effect of a novel symbioti yogurt fermented using probiotic microbes and fortified with purple leaf tea puree in modulating gut bacteria profile using an *in vivo* animal experiment with white male Wilstar rats.

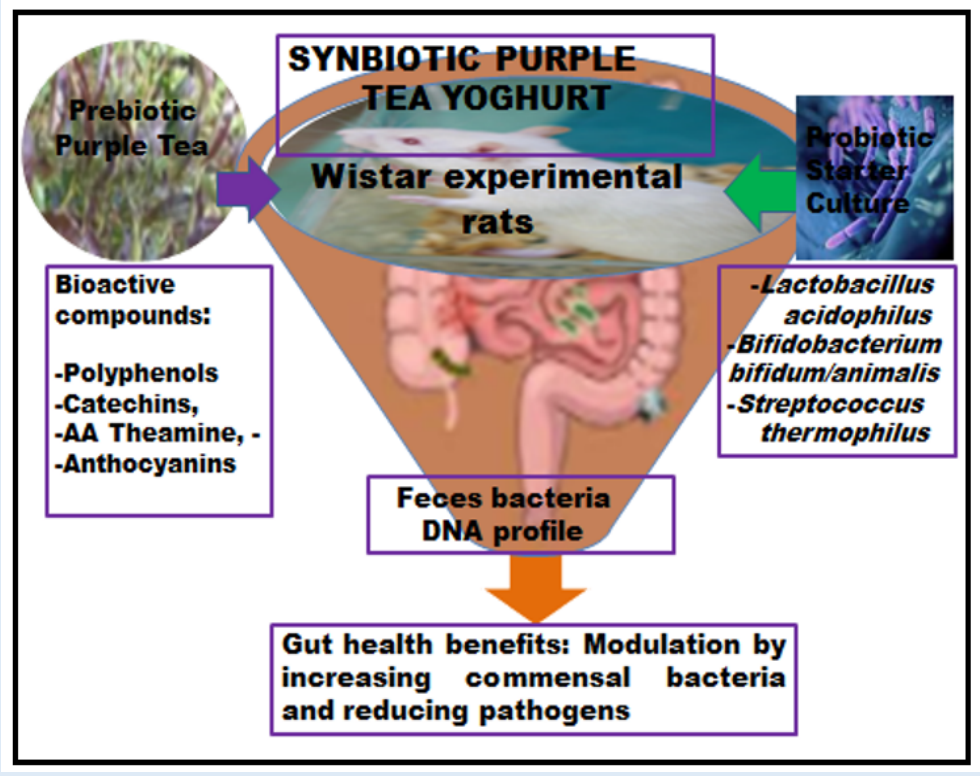
Methods: An *in vivo* animal study was used to investigate the effect of synbiotic yogurt (PYPT) produced by fermentation of milk with probiotic starter culture ABT 5 (*Lactobacillus acidophilus* LA-5*, Bifidobacterium bifidum* BB-12 *and Streptococcus thermophiles)* and fortified with purple-leaf tea puree. A total of 16 Wistar rats were intraperitoneally injected with 8 doses of 4mg/g of monosodium glutamate (MSG) to induce obesity. In a randomized control design, 8 of the experimental control rats were fed with normal standard rat feed, while 8 intervention rats were fed with the

formulated yogurt for a period of 28 days. The gut health bacteria biomarkers were determined from the rats' fecal matter by extraction of DNA using the ZymoBIOMIC kit protocol and profiled by the polymerase chain reaction (qPCR) molecular technique, of 16S rRNA. The data of the bacteria taxonomic classifications and abundance was processed and interpreted using DADA2 package and Quantitative Insight into Microbial Ecology 2 (Qiime2).

Results: No statistical differences in diversity of bacterial phyla were reported, but the composition of Actinobacteria increased in the intervention group and decreased in the control, while Proteobacteria decreased drastically in the intervention rats. Notably, the population of beneficial Lactobacillales and Bifidobacteriales in the intervention cohort increased significantly, while there was a reduction of bacteria with species with potential of pathogenic activity.

Conclusions: Fortification of probiotic yogurt with purple tea with prebiotic polyphenols increased the population of beneficial gut modulating bacteria while reducing the pathogens. Therefore**,** the study demonstrates the constructive collaboration of probiotic microbes and the purple tea bioactive compounds in the novel synbiotic yogurt in improving the healthful gut commensal bacteria. Future research can profile more commensal bacteria and analyze gut metabolites such as butyrate as well as conducting human clinical trials.

Keywords: Gut bacteria modulation, Synbiotic yogurt, Probiotics, Prebiotics, Purple-leaf tea, Fortification, Functional foods



INTRODUCTION

The human gastrointestinal (GI) tract is inhabited by a variety of microbiota which influences digestion, immunity, detoxification and other physiological activities. The microbiota includes bacteria, viruses, unicellular eukaryotes and archaea [1-4] that start the colonization of the gut at birth, but change during infancy depending on the delivery method, maternal flora, and mode of breastfeeding. Thereafter, an infant develops a similar pattern of gut microbiome composition to that of an adult at the age of about 3 years. Other intrinsic and extrinsic factors affecting the development of the gut microbiota include environmental factors, host genome, age and lifestyle factors such as disease, diet and antibiotic exposure [5-6].

The microbial community and diversity in the human gut is a significant determinant of a person's gut health. The dominant bacterial phyla of a healthy gut include: Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Verrucomicrobia and Fusobacteria [7-8]. Dysbiosis, a disruption of the microbiome in the gut, can lead to negative changes in gut composition by lowering bacterial diversity, changing the Firmicutes to Bacteroidetes (F/B) ratio. It can also increase gut permeability, leading to gut inflammation. Imbalance in the gut microbiota has also been linked to gastrointestinal disorders such as irritable bowel syndrome, cardiovascular disease, type 2 diabetes, inflammatory bowel disease and obesity [9-12].

Functional foods, are natural or processed foods that contain biologically active compounds which provide clinically proven health benefits. These biomolecules have been proven to be useful for human health using specific biomarkers that reduce the risk of chronic or viral diseases or manage their symptoms [13-14]. The Functional Food Center has proposed a new system by which these functional food products can be classified, (A, B, or C), that can better communicate the prospects of symptom management and risk reduction on the part of food items [15]. With the increasing demand for functional foods, stemming from the growing health consciousness among consumers and their preference for products that provide more than just basic nutrition, there is need for clear steps for development and commercialization of functional food [16- 17]. Probiotics, living microorganisms with health benefits to the host when taken in adequate amounts have been used to develop different types of functional foods [18]. For example, the oral administration of synbiotic or probiotic foods or supplements is being used in restoration of dysbiotic microbiota and prevention of obesity related IBD. Probiotics work through different mechanisms such as: (a) immunomodulation; (b) competition of nutrients and adhesion site with the pathogens; (c) anti-microbial properties and (d) improvement of epithelial barrier function [19-20]. Although the optimal dose of different single or mixed probiotics is unclear, the recommended dose for example of *Lactobacillus rhamnosus* GG is 1 to 2 x 10^{10} CFU/day [21].

Dairy products such as yogurt, fermented milk and cheese have been used as vector for probiotic delivery in humans. There is an increasing demand for non-dairy probiotic foods such as fruit/ vegetable juices, soy and certain cereal products due to vegetarianism, lactose intolerance, dairy allergies, as well as food consumers' interest in low cholesterol foods [22]. However, dairy products remain the major vehicle in delivering probiotics. Numerous studies demonstrate that fermented milk is effective in modulating inflammatory responses and the gut microbiota composition [23- 24].

All processed teas (*Camellia sinensis*) green, yellow, white or black teas as well as the herbal teas are loaded with healthful bioactive compounds in the form of polyphenols (catechins and epicatechins), theaflavins,

flavanol glycosides, amino acid-L-theanine, caffeine and theobromine [25]. These compounds contain a diverse group of health benefits for humans that include antioxidants that scavenge free radicals, anti-inflammatory and anti-microbial properties, prevention of diet induced obesity by modulation of lipid metabolism [26- 27] and positive modulation of gut microbiota composition [28- 29]. The purple-leaf tea cultivar has all the health benefits of most teas in addition to the flavonoid bioactive compound, anthocyanins with scientifically proven benefits of strong antioxidant activity, reduced risk of cardiovascular disease, and type 2 diabetes, improved weight maintenance and neuroprotection [30]. The Kenyan purple- cultivar, TRFK 306/1 has been researched and conferred to have extra health benefits such as higher free radical scavenging properties, improving vision, cholesterol & blood sugar regulation, anti-inflammatory, antifungal [31-32] and neuroprotective effects on brain [33- 34]. Moreover, this tea has lower caffeine content, no iron inhibiting tannins, more theanine, the amino acid known for the relaxing effect on brain and anti-metastatic blood pressure reducing properties [35-37]. Purple orthodox tea does not undergo oxidation (aeration) during processing as is the case with CCT black tea. This makes it have the appealing sensory attributes a purple coloration and a rich elegant floral or fruit aroma, with a sweet and mellow taste [38-39].

The current study therefore hypothesized that a probiotic yogurt formulated by fortification with a novel purple-leaf tea (PYPT) puree has bioactive compounds and probiotic microbes with synbiotic health beneficial effects of positively modulating the diversity and composition of gut bacteria microbiota.

MATERIALS AND METHODS

Novel probiotic purple tea yogurt (PYPT): A novel probiotic yogurt (PYPT) was formulated and produced by the fermentation of pasteurized milk with probiotic starter culture from CHR Hansen Limited, ABT-5: (*Lactobacillus acidophilus LA-5, Bifidobacterium bifidum BB-12 and Streptococcus thermophilus*). Subsequently, the fortification of the yogurt was done using a formulated and patented purple tea puree with hibiscus natural food colorant and permitted food grade stabilizers at the Foods and Tea Workshop of Karatina University, Kenya. The finished product was stored under 4^{o}C refrigerated temperature.

***In vivo* Wistar rats' experiment:** An animal model experiment using white male Wistar rats was used to provide the insight of host-diet (PYPT) health implications on the rats' gut microbiota. Sixteen (16) male Wistar rats were housed in sanitized rat cages in a controlled temperature environment of 21 - 28°C at the Small Animal Facility for Research and Innovation (SAFARI) of Jomo Kenyatta University, Kenya. They were administered with 0.1 mL 1% ivermectin to eliminate any parasite infestation. Subsequently, they were all fed on standard commercial pellets for 6 weeks and 8 doses of 4mg/g of body weight of mono-sodium glutamate for 21 days to induce obesity. Some of the fecal matter of these rats was collected and stored in sterile bags at -20^{o}C. This sample constituted the baseline microbial count. In a randomized control trial design, the 16 rats were assigned to two study groups. One of the groups was the control which continued to feed on the commercial rat pellets while the other 8, the intervention group, were fed on 3 ml of the formulated purple tea probiotic yogurt (PYPT) daily for 28 days.

- Baseline group: 16 Rats that were fed standard commercial pellets and mono-sodium glutamate for 21 days
- End-line Control group: 8 Rats that fed on commercial rat pellets for 28 days

- End-line Intervention group: 8 Rats that fed on the formulated purple tea probiotic yogurt (PYPT) for 28 days

All procedures and protocols involving the use of rats in the experiments were followed to the rules and regulations authorized by the Kenyatta University Institutional Animal Care and Use committee (KU-IACUC).

Fecal matter collection and microbial profiling by molecular technique: Fecal matter was collected from the rats after 21 days for the baseline, and after 28 days of feeding for the experimental group (intervention rats), and from rats fed solely commercial feed for the control group. The three fecal samples were stored in a tight, sterile, and sealed container in a freezer at -20^{0}C. This fecal matter was used to establish the microbial composition and diversity by DNA extraction using ZymoBIOMICs DNA Kits protocol. The samples were later sent to the Inqaba Biotech Limited for Polymerase chain reaction (PCR) amplification and sequencing of the 16S rRNA using PacBio sequencing technology. This technology target-specific PCR primers tailed with M13 forward (/5AmMC6/GTAAAACGACGGCCAGT) and reverse primers (/5AmMC6/CAGGAAACAGCTATGAC). The resulting PCR product was used as input into a second-round PCR reaction using barcoded M13 primers producing an asymmetrically barcoded PCR product for SMRTbell library construction and sequenced (in multiplex) on the PacBio® Sequel® System / Sequel II and Sequel IIe Systems (Sequel Systems). With asymmetric barcoding, the different barcode sequences were used on the forward (F) and reverse (R) PCR primers. After PCR amplification and sequencing, PacBio 16s/ITS1F analysis was done using DADA2 and qiime2 and an output table containing amplicon sequence variants (ASV) which are comparable to Operational Taxonomic Units (OTU's) in qiime1. The generated. FASTQ files and filtered feature table ASV were further analyzed by identifying the bacterial community, number and diversity in various taxonomic classifications.

ETHICAL CLEARANCE

The researchers ensured procedures, facility and the practices followed were in recommendation with the animal care and use program. Additionally, the animals were treated with high levels of scientific and ethical standards. Authority to conduct the study was sought from Karatina University and Kenyatta University Graduate School. Ethical clearance was also sought from Kenyatta University Institutional Animal Care and Use Committee (KU-IACUC). Permission to conduct the main research project was granted by the Kenya National Commission for Science, Technology and Innovation (NACOSTI), License no. NACOSTI/P/22/15557.

STATISTICAL ANALYSIS

The effect of the experimental yogurt (PYPT), on gut health was determined from taxonomic classifications and changes of bacteria profile of the rat DNA fecal matter. Insight into the bacteria profile was analyzed by Quantitative Insights into Microbial Ecology (Qiime2) and interpreted using DADA2 software package. The generated data was subjected to statistical differences tests at p- values of 0.05 or less using GenStat version 13 software.

RESULTS

The gut microbial community composition, number and diversity from the Wistar rats' fecal material DNA was used to determine the effect of consumption of the purple tea probiotic yogurt (PYPT) on the experimental rats and its implication to gut health.

Gut microbial community at Phylum taxon level of Wistar rats' fecal bacteria: The current study found (Figure 1) 11 different phyla levels of bacteria taxon at the baseline level, with the total number of Bacteroidetes bacteria as the most dominant (61.38 %) followed by the Firmicutes (29.36%), Actinobacteria (0.73%) and Proteobacteria (1.03%). This relates closely to a healthy human gut bacteria distribution profile at the phyla level [7-8]. At the end of the 28 experimental days, the distribution of Bacteroidetes reduced both in control cohort rats that were fed on commercial rat feed only (47.82 %) and the intervention cohort fed with the purple probiotic yogurt (48.65%). Whereas the Firmicutes increased to 41.81 % and 44% for the control and the intervention group respectively, the Actinobacteria reduced in both the experimental cohort rats, while the Proteobacteria reduced only in the PYPT fed rats (0.0327 %). The current study found no significant statistical difference in the total number of the different bacteria phyla taxon of the baseline rats (11), the intervention group (9), and the control group (14).

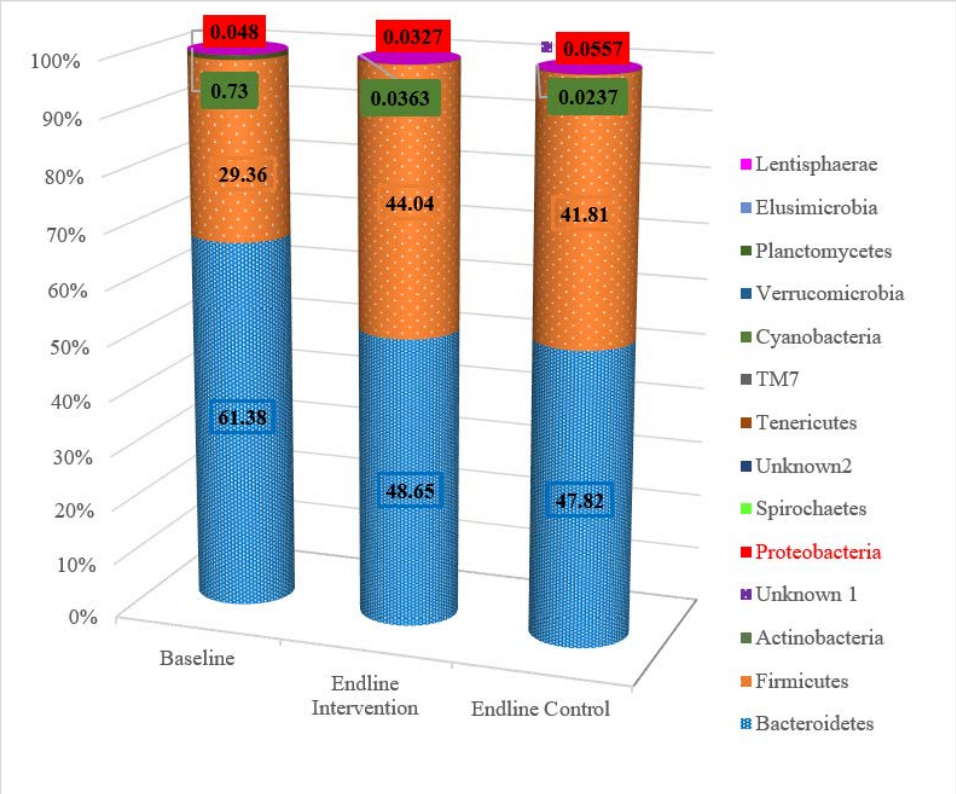


Figure 1. Percentage (%) microbial community and diversity of the main bacteria at phylum taxonomy level

Fecal gut microbial community and diversity at the genus level of classification: At the genus taxa level (Figure 2), the total number of bacteria increased from the baseline level (10950) to (11661) and (16392) in control and intervention cohort rats respectively, with no significant difference in diversity (38 ± 1). *Prevotella* genus was the most abundant irrespective of the type of feed administered, with the baseline level group of rats at (45%), which reduced in the control group (35.4%) but with no significant difference from the intervention group (35.2%). However, *Lactobacillus* which was at 5 % at the baseline level increased both in the control group

(15%), and with significant difference to the intervention group (23%). Likewise, the beneficial *Bifidobacterium* which was negligible at the baseline level (0.02%), increased significantly in the intervention (1.0 %), and with significant difference to the control cohort (0.03%). There were also several unclassified bacteria at the baseline level (36.9%) which increased in the control group (38%) but decreased in the intervention group (35%) with no significant difference.

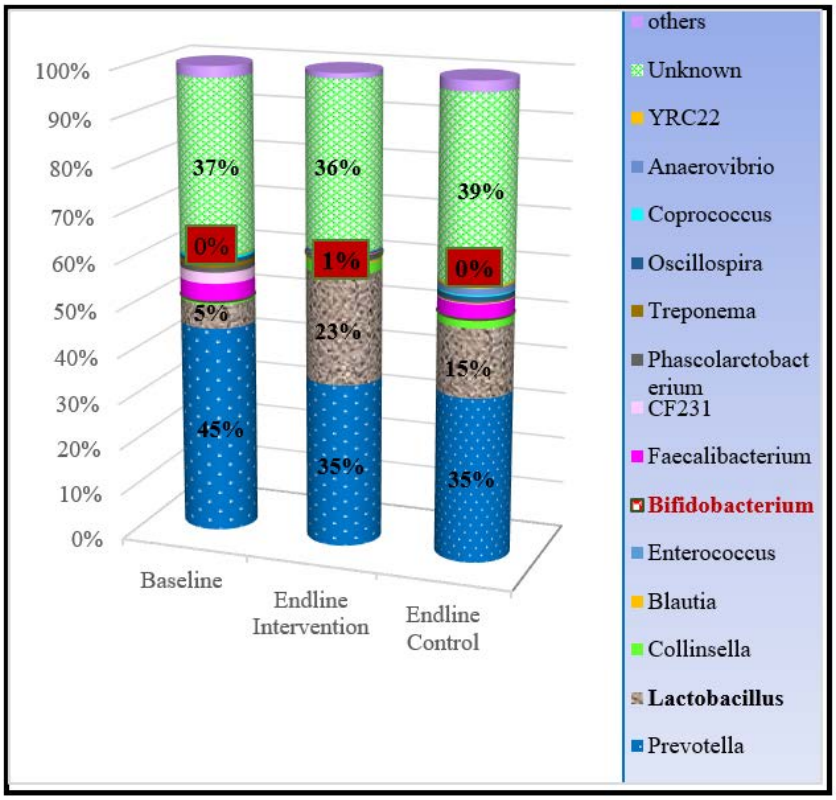


Figure 2: Some microbial community and diversity at the genus taxonomy

Fecal gut microbial community and diversity at the species taxon level: At the species level (Figure 3), the recorded diversity of bacteria did not differ significantly from the baseline level (49) to that of the experimental feed rats at 45 and 51 for the intervention and control rats, respectively. Notably, the intervention group of rats had the highest total number of bacteria (16,405), followed by control rats (11,653) while the baseline level was lowest (10,952). At baseline level, an unclassified bacterium was the most abundant (27.9%), followed by the most abundant which increased from (0.07% and 0.08%) respectively from the baseline level, to (0.84% and 0.54%) at the intervention group rats and (1.96% and *Provotella copri* (23.4%). However, at the end of the experimental feeding period, the *P. copri* was the most abundant in the intervention group (30.44%), while in the control cohort, the unclassified bacteria dominated (33.95 %), followed by *P. copri* (13.5 %). Worth noting is the relative increase in the abundance of an unclassified *Lactobacillus* which was (3.65%) at the baseline but increased to (6.48%) in the control group and with a high margin level (11.87%) in the intervention group. Of the classified *Lactobacillus, L. reuteri* and *L. helveticus* were 0.19%) at the control group rats. As expected, *Bifidobacterium animalis*, one of the starter culture bacteria that was used in the production of the

experimental probiotic yogurt was exceptionally low at the baseline level (0.02%) but increased significantly in the intervention group of the rats (0.47%) and remained low in the control cohort (0.03 %). There were unclassified *Streptococcus* bacteria which decreased from 0.09 % at the baseline level to 0.02 % in the intervention rats but remained the same at the control group of rats (0.09 %). *Faecalibacterium prausnitzii,* a naturally common human gut bacteria decreased significantly from baseline level (3.89 %) to 0.4% in the intervention group of rats but remained the same in the control cohort (3.16%).

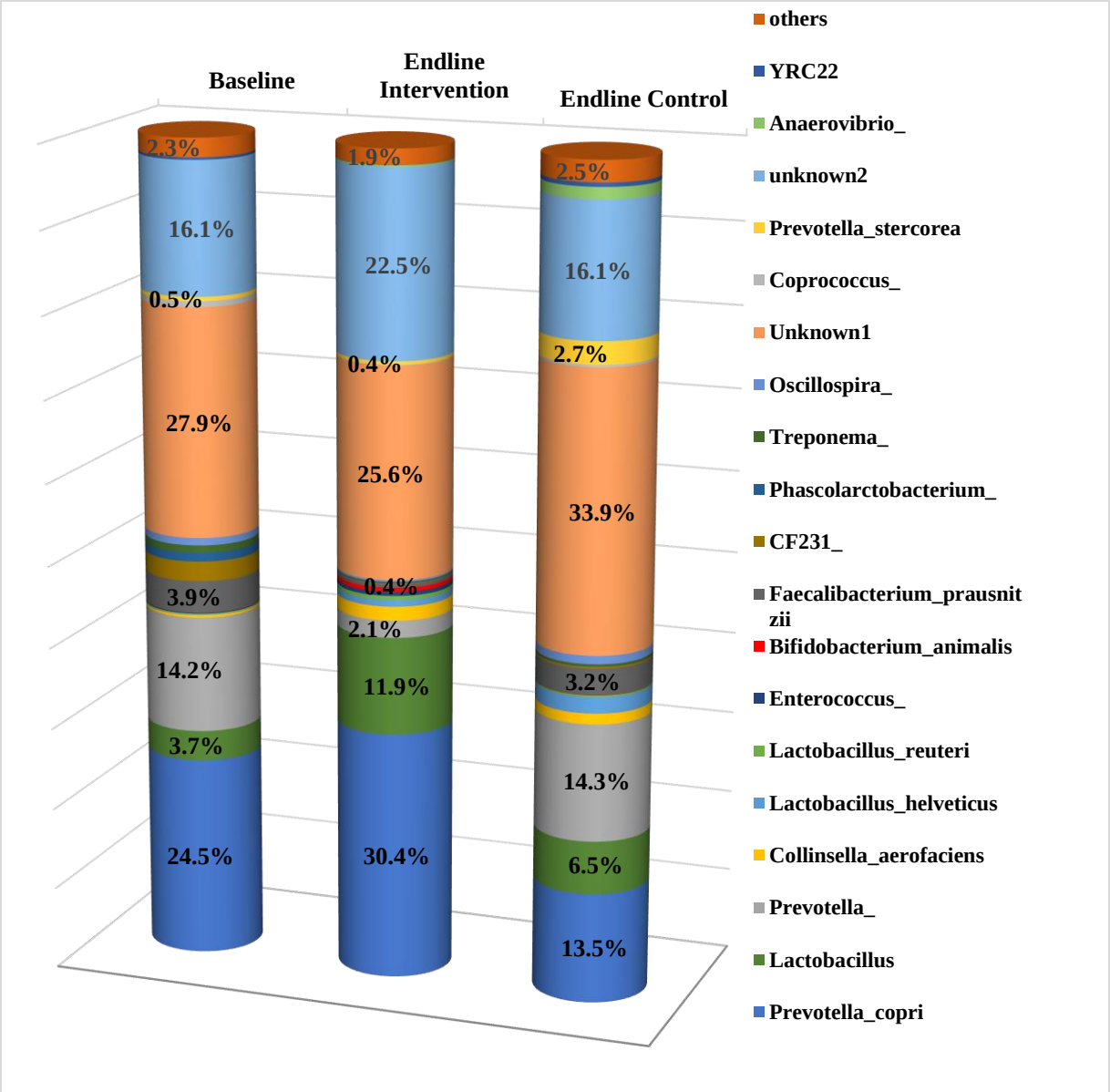


Figure 3: Some of the microbial community and diversity at species taxonomy

Profile of beneficial commensal bacteria: The relative abundance of the gut bacteria which is known to offer positive healthy modulation in the gut, increased in the cohort group of rats fed with the intervention PYPT yogurt compared to the control group of rats (Figure 4).

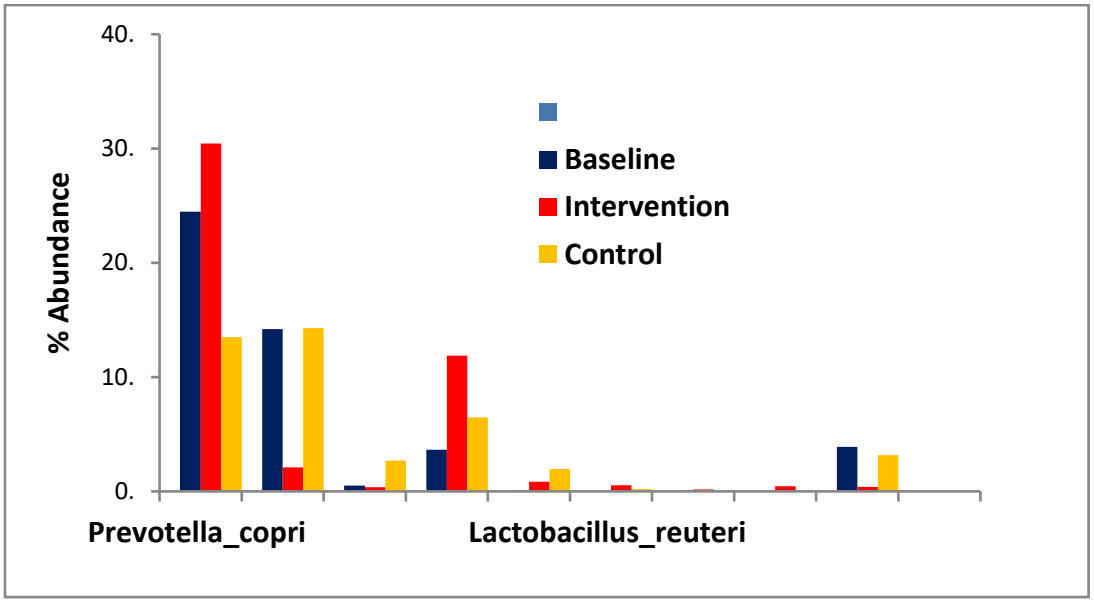


Figure 4: Profile of beneficial commensal bacteria

Potential pathogens: Although the profile of pathogenic bacteria in the current study could not be well classified at the species level due to the low number, at the genus level, *Staphylococcus, Helicobacter, Mycoplasma, and Aerococcus* were found to be the most prevalent (Table 1). Worth noting is the *Staphylococcus* genera count which reduced from 29 at baseline to zero (not detected) in the intervention cohort rats, though significantly different at 24 in the control group.

Table 1. Potential pathogenic bacteria at species level (count and % abundance)

	Baseline Count	%	Intervention Count	%	Control Count	%
Clostridium_	2	0.02	0	0	4	0.03
Staphylococcus sciuri	2	0.02	0	0	11	0.09
Staphylococcus_	26	0.24	0	0	12	0.1
Helicobacter apodemus	2	0.02	0	0	4	0.03
Helicobacter rodentium	7	0.06	2	0.01	5	0.04
Mycoplasma_	28	0.26	2	0.01	4	0.03
Aerococcus_	11	0.1	0	0	10	0.09

** Underscore (_) alone means the species could not be classified.

DISCUSSION

Effect of the intervention yogurt on fecal gut biomarkers and its implications to health: There are several gut microbial biomarkers that can be used to predict host health, metabolic disorders and disease status. They include and are not limited to the following: (i) Number and diversity of microbial community (ii) Profile of

beneficial /commensal bacteria (iii) Profile of pathogenic and dysbiotic microbial communities (iv) The Firmicutes to Bacteroidetes ratio (v) Bacteria metabolites [8,11].

Polyphenols, which are abundant in purple leaf tea have been reported to exert prebiotic-like effects, by modulating pre-existing dysbiosis, stimulating the growth of beneficial bacteria and inhibiting pathogenic bacteria [38-39]. In the current study, the total number of microbial communities increased from the baseline level cohort rats after the 28 days of the experimental diet intervention with PYPT, but with no significant change in microbial diversity. Note that, lowering of microbial diversity is considered a biomarker of an unhealthy gut microbiome or dysbiosis of the gut [10- 11]. Some *Lactobacillus* and *Bifidobacterium* strains are the major bacteria associated with health-promoting gut biomarkers. They are also used as probiotics in the production of some beverages and functional foods [29, 40-41]. In the present study there were 10 commensal gut health-promoting biomarker bacteria classified at species level (Figure 4). The major ones included *Lactobacillus reuteri, L. helveticus, Bifidobacterium animalis Faecalibacterium prausnitzii, Prevotella copri and Roseburia*. *Lactobacillus helveticus*, a lactic acid bacterium that is naturally found in the human gut, is Generally Recognized as Safe (GRAS) status for use in probiotic and nutraceutical food products. It also has proven health promoting properties of preventing gastrointestinal infections and enhancing protection against pathogens as well as modulating host immune responses [42-43]. *Bifidobacterium animalis- lactis* (*BB-12®*: Chr. Hansen's Limited) collection of dairy cultures which was used in the fermentation of the yogurt has proven high stability in foods and as frozen dried powders]. It is known to protect humans against antibiotic-associated disruption of microbiota composition and functions [44- 46]. The presence of *B. animalis* in the rat fecal matter at the end of the experimental days confirms the Bifidobactreium probiotic activity by successfully going through the gut digestive system, surviving the stomach's low pH and resisting the bile salts. This result concurs with a study which found an increase in the levels of Bifidobacterium in the human gut of 20 athletes after a three-week intervention study on consumption of the probiotic yogurt [46]. *Roseburia*, a beneficial gut butyrate metabolite producing bacteria increased from baseline level after intervention with the PYPT. An *in vitro* study with Crohn's disease patients supplemented with *Roseburia* was reported to enhance intestinal epithelial barrier integrity [47]. However, *Faecalibacterium prausnitzii,* also known for beneficial butyrate-producing probiotic activity [48] decreased in levels in the current study from the baseline level rats to the intervention rats. Although *Prevotella* genera have a high genetic diversity, with some possessing potential role of intestinal pathobionts that is linked with chronic inflammatory conditions, some strains have been shown to be positive gut biomarker and are used in dietary supplements due to the ability to breakdown complex polysaccharides such as dietary fiber [49]. In the current study, *Prevotella copri* numbers increased remarkable to almost double in the intervention group of rats from the baseline level but reduced in the control cohort rats. Other species of interest are the two starter culture bacteria (*Lactobacillus acidophilus and Streptococcus thermophile)* used in the yogurt production. The increase in unclassified *Lactobacillus* and the decrease in *Streptococcus* clearly indicate the former bacteria's ability to exhibit probiotic activity that withstands gut enzymatic activity, while the latter does not.

Another key gut bacteria biomarker is the phyla ratio of Firmicutes to Bacteroidetes (F/B). An increased and decreased F/B ratio is associated with the

development of obesity or IBD, respectively both in mice and humans [11]. In the current study, the F/B ratio started at baseline level at (1:2), but at the end of the experimental yogurt trial, both the control and intervention rats' feces had an equal ratio of (1:1). The change in the intervention rats may be attributed to the starter culture bacteria used in the formulated probiotic yogurt. It had both the genera *Lactobacillus*- (phyla Firmicutes) and *Bifidobacterium* (phyla- Actinobacteria*)*, as well as the prebiotic activity of tea polyphenols.

The profile of a host's gut microbiota provides to a better understanding of the pathogenesis and dysbiosis that causes dysregulation of bodily functions and diseases. These gut microbiota irregularities are noticeable due to (i) reduction of beneficial bacteria, (ii) increase in pathogenic bacteria, and (iii) decrease in overall bacterial diversity [6, 10]. Foods of animal origin (milk and cheese), though very nutritious are notorious in promoting proliferation of opportunistic pathogens. Staphylococcal food poisoning via the production of heat-stable enterotoxins, serve as a reservoir of multi-drug resistant strains and is responsible for some human endocarditis, peritonitis, septic shock, urinary tract infection, pelvic inflammatory disease and wound infections [50- 51]. These results are consistent at species level with an unclassified *Staphylococcus*- and *Staphylococcus sciuri* which recorded counts of (26 and 2) respectively at baseline level, zero in the intervention group, while the control group of rats had *(*12 and 11) respectively.

Some *Helicobacter* genera such as species *Helicobacter pylori* (H. pylori) are known pathogens causing peptic ulcers [49-]. *In vitro* and *in vivo* experiments as well as clinical trials prove that some foods have anti *Helicobacter pylori* effects [52-54]. The current study classified *H. rodentium (*pathogenicity is less known in human) with a baseline count of 7 and 2 at the end of the intervention experiment. The genus *Mycoplasma,* though not classified at species level in the current study are not quite common pathogens except for the *Mycoplasma pneumonia* and *Mycobacterium tuberculosis.* These are highly contagious in humans, causing pneumonia respiratory infection and leaky gut. [55]. Their number at genus level dropped significantly from baseline count of 28 (0.26 %) to 2 (0.01%) at intervention cohort rats while in the control cohort to count to 4 (0.03%). *Clostridium* genus count was low and could not be classified at species level. The unclassified species of *Clostridium* in the current study had a baseline count and abundance of (2 ,0.02 %) respectively which increased to (4, 0.04% at the control group and none at the PYPT intervention group of rats. *Clostridium difficile* and *Clostridium perfringens* have some of the most potent toxins responsible for severe pathologies in human and animal diseases. *Clostridium difficile* is related to antibiotic-associated diarrhea [56]. *Aerococcus urinae* and *Aerococcus sanguinicola* have been increasingly recognized as causative agents of urinary tract infection [57-58]. In the current study, the specific species of *Aerococcus* could not be classified, but the baseline cohort rats count and abundance (11, 0.1 %) respectively, had no significant difference with the control cohort of rats (10, 0.09%) while none was detected in the intervention group of rats.

CONCLUSION

The current study findings demonstrate that probiotic yogurt fortified with purple leaf tea yogurt increases the abundance of healthy *Lactobacillus* and *Bifidobacterium* genera, while reducing the pathogens, *Staphylococcus, Helicobacter, Mycoplasma and Aerococcus*. However, the combination of yogurt probiotics and tea bioactive compounds do not seem to influence the diversity of microorganisms. Nevertheless, since the bioactive

compounds in purple tea such as the anthocyanins and other polyphenols have other reported health benefits beyond the gut, there is great future of the usage of the yogurt as a nutritious and functional food for health. However, more studies using different probiotic microbes and formulations of the yogurt can be conducted for optimization of the health benefits. The health efficacy of the yogurt can be confirmed by scientifically proven placebo-controlled human clinical studies.

List of Abbreviations: ABT: Acidophilus-Bifidus-Thermophilus, MSG: Monosodium Glutamate, PCR: Polymerase Chain Reaction, PYPT: Probiotic Yogurt with Purple Tea, SAFARI: Small Animal Facility for Research and Innovation, F/B: Firmicutes to Bacteriodetes

Author's Contributions: Muchiri Mary (MM), Chege Peter. (CP) and Mucheru Patrick (MP) conceptualized of the study: M. conducted the experiments and wrote the original draft paper: MM. wrote original study, and received funding, applied for NACOSTI study license, revised original script and supervision: CP contributed to the study design, and obtaining animal study ethical clearance, supervision and review. All authors agreed on the goal of the study, contributed to the article and approved the submitted version.

Acknowledgements: The authors wish to convey their appreciation to the staff of the Small Animal Facility for Research and Innovation (SAFARI), Jomo Kenyatta University, Inqaba Biotech Limited and the National Research Fund (NRF), Kenya and Karatina University for the technical/labor support during the study. All authors contributed to the design, data analysis and the final manuscript.

Conflicts of Interest: The author declares no conflict of interest.

REFERENCES

1. Dingbo Lin, Denis M. Medeiros, The microbiome as a major function of the gastrointestinal tract and its implication in micronutrient metabolism and chronic diseases. *Nutrition Research,* 2023, 112: 30-45, DOI: https://doi.org/10.1016/j.nutres.2023.02.007.
2. Vemuri R, Shankar EM, Chieppa M, Eri R, Kavanagh K.: Beyond Just Bacteria: Functional Biomes in the Gut Ecosystem Including Virome, Mycobiome, Archaeome and Helminths. *Microorganisms*. 2020 Mar 28;8(4):483. DOI: https://doi:10.3390/microorganisms8040483
3. Szydłowska, A.; Sionek, B.: Probiotics and Postbiotics as the Functional Food Components Affecting the Immune Response. *Microorganisms*. 2023 11,104. DOI: https://doi.org /10.3390/microorganisms11010104
4. Embleton, N. D., Berrington, J. E., Dorling, J., Ewer, A. K., Juszczak, E., Kirby, J. A., Lamb, C. A., Lanyon, C. V., McGuire, W., & Probert, C. S. Mechanisms affecting the gut of preterm infants in enteral feeding trials. *Frontiers in Nutrition* 2017, *4*, 14. DOI: https://doi.org/10.3389/fnut.2017.00014
5. Hati S., Prajapati JB. Use of Probiotics for Nutritional Enrichment of Dairy Products. *Functional Foods in Health and Disease* 2022; 12(11): 713-733. DOI: https://www.doi.org/10.31989/ffhd.v12i11.1013
6. Moran-Ramos, S., Lopez-Contreras, B. E., Villarruel-Vazquez, R., Ocampo-Medina, E., Macias-Kauffer, L., Martinez-Medina, J. N., Villamil-Ramirez, H., León-Mimila, P., Del Rio-Navarro, B. E., & Ibarra-Gonzalez, I. Environmental and intrinsic factors shaping gut microbiota composition and diversity and its relation to metabolic health in children and early adolescents: A population-based study. *Gut Microbes*, 2020, 11(4), 900-917 DOI: https://doi.org/10.1080/19490976.2020.1712985.
7. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol.* 2021 Jan;19(1):55-71. DOI: https://doi:org/10.1038/s41579-020-0433-9
8. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, Mele MC. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms* 2019, 10;7(1):14.

DOI: https://doi:org/10.3390/microorganisms7010014

9. Castaneda L, Singharaj B and Martirosyan D. Functional foods, conventional treatment and bioactive compounds, and assistance in the management of inflammatory bowel disease. *Bioactive Compounds in Health and Disease* 2018, 1(4); 40-59 DOI: https://doi.org/110.31989/bchd.v1i4.409
10. Underwood, M. A.: Intestinal dysbiosis: novel mechanisms by which gut microbes trigger and prevent disease. *Preventive medicine* 2014, 65, 133-137
DOI: https://doi: 10.1016/j.ypmed.2014.05.010.
11. Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa S, Navarrete P, Balamurugan R.: The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients? *Nutrients* 2020, 12(5):1474.
DOI: https://doi:.3390/nu12051474
12. Winiarska-Mieczan A, Tomaszewska E, Donaldson J, Jachimowicz K. The Role of Nutritional Factors in the Modulation of the Composition of the Gut Microbiota in People with Autoimmune Diabetes. *Nutrients* 2022 14(12):2498. DOI: https:// doi:10.3390/nu14122498.
13. Soumya N.P., Mini S., Sivan S., Mondal S.: Bioactive compounds in functional foods and their role as therapeutics. *Bioactive Compounds in Health and Disease* 2021. 4(3); 24-39.
DOI: https://doi.org/10.31989/bchd.v4i3.786
14. Damián MR, Cortes-Perez NG, Quintana ET, Ortiz-Moreno A, Garfias Noguez C, CruceñoCasarrubias CE, Sánchez Pardo ME, Bermúdez-Humarán LG. Functional Foods, Nutraceuticals and Probiotics: A Focus on Human Health. *Microorganisms*, 2022, 21;10(5):1065.
DOI: https://doi:10.3390/microorganisms10051065.
15. Martirosyan D.M., Lampert T., Ekblad M. Classification and regulation of functional food proposed by the Functional Food center. *Functional Food Science*, 2022, 2(2): 25-46.
DOI: https://doi.org/10.31989/ffs.v2i2.890.
16. Martirosyan, D. M., Stratton S. Advancing functional food regulation. *Bioactive Compounds in Health and Disease* 2023; 6(7): 166-171.
DOI: https://www.doi.org/10.31989/bchd.v6i7.1178
17. Martirosyan D., Kanya H., Nadalet C. Can functional foods reduce the risk of disease? Advancement of functional food definition and steps to create functional food products. Functional Foods in Health and *Disease* 2021; 11(5): 213-221. DOI: https://www.doi.org/10.31989/ffhd.v11i5.788
18. Food and Agriculture Organization FAO. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. 2001, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy
19. Xiaoqin Li, Shan Hu, Jiawei Yin, Xiaobo Peng, Lei King, Linyan Li, Zihui Xu, Li Zhou, Zhao Peng, Xiaolei Ze, Xuguang Zhang, Qiangchuan Hou, Zhilei Shan & Liegang Liu (2023) Effect of synbiotic supplementation on immune parameters and gut microbiota in healthy adults: a double-blind randomized controlled trial, *Gut Microbes*, 15:2, 2247025
DOI: https://doi.org/10.1080/19490976.2023.2247025
20. Samat K., Tamara T., Maxat S., Nurislam M., Zhanel P., Dinar T., Indira U., Zhanagul K., Akmaral Z., Zharkyn J., Saniya K., Almagul K. Comparative Analysis of the Effect of Fermented Derivatives from Bactrian Milk on the Gut Microbiome. Functional Foods in Health and Disease 2023; 13(12): 676-689. DOI: https://www.doi.org/10.31989/ffhd.v13i12.1247
21. Sanders ME, Benson A, Lebeer S, Merenstein DJ, Klaenhammer TR.: Shared mechanisms among probiotic taxa: implications for general probiotic claims. Curr Opin Biotechnol. 2018 Feb; 49:207-216.
DOI: https://doi:10.1016/j.copbio.2017.09.007.
22. Hati S., Prajapati JB. Use of Probiotics for Nutritional Enrichment of Dairy Products. Functional Foods in Health and Disease2022; 12(11):713-733
DOI: https://www.doi.org/10.31989/ffhd.v12i11.1013
23. Ha HK, Hong JY, Ayu IL, Lee MR, Lee WJ. Development and evaluation of probiotic delivery systems using the rennet-induced gelation of milk proteins. J Anim Sci Technol. 2021 Sep;63(5):1182-1193.
DOI: https://doi:10.5187/jast.2021.e92.
24. Szydłowska A, Sionek B: Probiotics and Postbiotics as the Functional Food Components Affecting the Immune Response. *Microorganisms*: 2023, 11(1):104.
DOI: https://doi.org/10.3390/microorganisms11010104
25. Kerio LC, Wachira FN, Wanyoko JK, Rotich MK. Total polyphenols, catechin profiles and antioxidant activity of tea products from purple leaf coloured tea cultivars. *Food Chem.* 2013, 15;136(3-4):1405-13.
DOI: https://doi.org/10.1016/j.foodchem.2011.08.005
26. Wang, C.; Han, J.; Pu, Y.; Wang, X. Tea (*Camellia sinensis):* A Review of Nutritional Composition, Potential Applications, and Omics Research. *Appl. Sci*. 2022, 12, 5874.
DOI: https://doi.org/10.3390/app12125874

27. Chen X.M., Ma Z., and Kitts D.D. Demonstrating the relationship between the phytochemical profile of different teas with relative antioxidant and anti-inflammatory capacities. *Functional Foods in Health and Disease* 2017, 7(6); 375-395. DOI: https://doi.org/10.31989/ffhd.v7i6.342.
28. Yang C, Zhang J, Zhang L, Huang J, Wang Y. Mechanisms of body weight reduction and metabolic syndrome alleviation by tea. Mol Nutr Food Res. 2016, 60:160–74. DOI: https://doi.org/10.1002/MNFR.201500428
29. Pérez-Burillo S, Navajas-Porras B, López-Maldonado A, Hinojosa-Nogueira D, Pastoriza S, Rufián-Henares JÁ. Green Tea and Its Relation to Human Gut Microbiome. *Molecules.* 2021 Jun 26;26(13):3907. DOI: https://doi:10.3390/molecules26133907.
30. Liu JY, He D, Xing YF, Zeng W, Ren K, Zhang C, Lu Y, Yang S, Ou SJ, Wang Y, Xing XH. (2021). Effects of bioactive components of Pu-erh tea on gut microbiomes and health: A review. *Food Chem*. 15; 353:129439. DOI: https://doi: 10.1016/j.foodchem.2021.129439.
31. Thitirat Kaewsedam, Wirote Youravong, Zhenyu Li, Santad Wichienchot: Modulation of gut microbiota and their metabolites by functional mulberry juice non-thermally pasteurized using microfiltration. *Functional Foods in Health and Disease* 2022, 12(9): 547-563 DOI: https://doi:10.31989/ffhd. v12i9.980
32. Karori SM, FN Wachira, JK Wanyoko, RM Ngure. Antioxidant capacity of different types of tea products. *Afric Journ of Biotech* 2007, 6: 19 DOI: https://doi:10.5897/AJB2007.000-2358
33. Kerio, L.C., Wachira, F.N., Wanyoko, J.K. and Rotich, M.K. Characterization of Anthocyanins in Kenyan Teas: Extraction and Identification. *Food Chemistry,* 2012, 131, 31-38. DOI: https://doi.org/10.1016/j.foodchem.2011.08.005
34. Shimoda H, Hitoe S, Nakamura S, Matsuda H.: Purple Tea and Its Extract Suppress Diet-induced Fat Accumulation in Mice and Human Subjects by Inhibiting Fat Absorption and Enhancing Hepatic Carnitine Palmitoyltransferase Expression. *Int J Biomed Sci.* 2015, 11(2):67-75. www.ijbs.org Int J Biomed Sci vol. 11 no. 2 June 2015
35. Areba GO, Khalid R, Ngure RM, Maloba F, Nyaga N, Moseti KO, Ngotho M, Wanyoko JK, Karori SM, Wachira FN. Neuroprotective Effects of Tea against Cadmium Toxicity. *Bioactive Compounds in Health and Disease* 2019, 2(12): 230-246. DOI: https://doi.org/10.31989/bchd.v2i12.684
36. Chen SQ, Wang ZS, Ma YX, Zhang W, Lu JL, Liang YR, Zheng XQ. Neuroprotective Effects and Mechanisms of Tea Bioactive Components in Neurodegenerative Diseases. Molecules. 2018 Feb 25;23(3):512. DOI: https://doi:10.3390/molecules23030512
37. Faisal Khan, Asma Bashir and Fadwa Al Mughairbi:). Purple Tea Composition and Inhibitory Effect of Anthocyanin-Rich Extract on Cancer Cell Proliferation. *Med Aromat Plants (Los Angeles)* (20187:6 DOI: https://doi.0.4172/2167-0412.1000322
38. Muchiri N Mary, Asiko Lydia, Nduru Mbaka, Kilel Emily, Kamunya Samson, Kaburi Festus, Mwangi Simon and Chemutai Anne. Promotion of Kenyan Purple Tea Products Through Collaboration Between Academia and Tea Industry. *Tea Journal,* 018, 39: (1 & 2): 139-14.
39. Kilel, E.C., Faraj, A.K., Wanyoko, J.K., Wachira, F.N. and Mwingirwa, V.: Green Tea from Purple Leaf Coloured Tea Clones in Kenya—Their Quality Characteristics. 2013, *Food Chemistry*, 141, 769-775. DOI: https://doi.org/10.1016/j.foodchem.2013.03.05
40. Zhibin Liu, Jean-Paul Vincken, Wouter J.C. de Bruijn, Tea phenolics as prebiotics. *Trends in Food Science & Technology,* 2022, 127: 156-168 DOI: https://doi.org/10.1016/j.tifs.2022.06.007.
41. Benameur T, Porro C, Twfieg ME, Benameur N, Panaro MA, Filannino FM, Hasan A. Emerging Paradigms in Inflammatory Disease Management: Exploring Bioactive Compounds and the Gut Microbiota. Brain Sci. 2023 Aug 21;13(8):1226. DOI: https://doi:10.3390/brainsci13081226.
42. Yamashita, Maya, et al. "Anti-allergic effect of *Lactobacillus helveticus* SBT2171 on murine model of pollen allergy." *Functional Foods in Health and Disease* 2019, 9.3, 166-179. DOI: https:// doi:10.31989/ffhd.v9i3.586
43. Zago M, Massimiliano L, Bonvini B, Penna G, Giraffa G, Rescigno M. Functional characterization and immunomodulatory properties of *Lactobacillus helveticus* strains isolated from Italian hard cheeses. PLoS One. 2021 Jan 25;16(1):e0245903. DOI: https://doi: 10.1371/journal.pone.0245903
44. Jungersen M, Wind A, Johansen E, Christensen JE, Stuer-Lauridsen B, Eskesen D. The Science behind the Probiotic Strain *Bifidobacterium animalis* subsp. lactis BB-12(®). Microorganisms. 2014 Mar 28;2(2):92-110. DOI: https://doi:10.3390/microorganisms2020092.

45. Jena R, Choudhury PK. Bifidobacteria in Fermented Dairy Foods: A Health Beneficial Outlook. Probiotics *Antimicrob Proteins.*2023 Nov 18. DOI: https://doi:10.1007/s12602-023-10189-w.
46. Zhu J, Zhu Y, Song G. Effect of Probiotic Yogurt Supplementation (Bifidobacterium animalis ssp. lactis BB-12) on Gut Microbiota of Female Taekwondo Athletes and Its Relationship with Exercise-Related Psychological Fatigue. *Microorganisms*. 2023 May 26;11(6):1403. DOI: https://doi:10.3390/microorganisms11061403
47. Geirnaert A, Calatayud M, Grootaert C, Laukens D, Devriese S, Smagghe G, De Vos M, Boon N, Van de Wiele T.: Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. Sci Rep. 2017 Sep 13;7(1):11450. DOI: https://doi:10.1038/s41598-017-11734-8.
48. HE Leylabadlo, R Ghotaslou, MM Feizabadi, et., al.: The critical role of *Faecalibacterium prausnitzii* in human health: An overview. Microbial Pathogenesis 149, 2020, 104344 DOI: https://doi.org/10.1016/j.micpath.2020.104344
49. Precup G, Vodnar DC. Gut Prevolella as a possible biomarker of diet and its eubiotic versus dysbiotic roles: a comprehensive literature review. *British Journal of Nutrition* 2019, 122(2), 28 July 2019, pp. 131 – 140 DOI: https://doi.org/10.1017/S0007114519000680.
50. Szczuka E, Porada K, Wesołowska M, Łęska B. Occurrence and Characteristics of Staphylococcus aureus Isolated from Dairy Products. *Molecules*. 2022 Jul 21;27(14):4649. DOI: https://doi.org/10.3390/molecules27144649
51. Guo Y, Song G, Sun M, Wang J and Wang Y:) Prevalence and Therapies of Antibiotic Resistance in *Staphylococcus aureus*. Front. *Cell. Infect. Microbiol*. 2020, 10:107. DOI: https://doi.org/10.3389/fcimb.2020.00107.
52. Malfertheiner, P., Camargo, M.C., El-Omar, E. et al. *Helicobacter pylori* infection. *Nat Rev Dis Primers* 9, 19 (2023). DOI: https://doi.org/10.1038/s41572-023-00431-8
53. Lawal T. O., Patel S. R., Mahady G. B. Food and medicinal plants from Nigeria with anti-*Helicobacter pylori* activities induce apoptosis in colon and gastric cancer cell lines. Functional Foods in Health and Disease 2022; 13(7): 372-387. DOI: https://doi.org/10.31989/ffhd.v13i7.1105
54. Nair MR, Chouhan D, Sen Gupta S, Chattopadhyay S. Fermented Foods: Are They Tasty Medicines for *Helicobacter pylori* Associated Peptic Ulcer and Gastric Cancer? Front Microbiol. 2016 Jul 25; 7:1148. DOI: https://doi:10.3389/fmicb.2016.01148.
55. He J, Liu M, Ye Z, Tan T, Liu X, You X, Zeng Y, Wu Y. [Corrigendum] Insights into the pathogenesis of *Mycoplasma pneumoniae* (Review). *Mol Med Rep*. 2018 Mar;17(3):4155. DOI: https://doi:10.3892/mmr.2017.8324
56. Dilnessa T, Getaneh A, Hailu W, Moges F, Gelaw B. Prevalence and antimicrobial resistance pattern of *Clostridium difficile* among hospitalized diarrheal patients: A systematic review and meta-analysis. PLoS One. 2022 Jan 13;17(1):e0262597. DOI: https:// doi:10.1371/journal.pone.0262597
57. Sahu KK, Lal A, Mishra AK, Abraham GM.: Aerococcus-Related Infections and their Significance: A 9-Year Retrospective Study. *J Microsc Ultrastruct.* 2020 Nov 9;9(1):18-25. DOI: https://doi:10.4103/JMAU.JMAU_61_19.
58. Sahu, Kamal Kant; Lal, Amos; Mishra, Ajay Kumar; Abraham, George M.: Aerococcus-Related Infections and their Significance: A 9-Year Retrospective Study. *Journal of Microscopy and Ultrastructure* 9(1):p 18-25, Jan–Mar 2021. DOI: https://doi10.4103/JMAU.JMAU_61_19.