Kombucha: An Exotic, Functional, Traditional Elixir of life

What is Kombucha?

Kombucha is a functional beverage prepared by the fermentation of sweetened tea (Camellia sinensis) by a Symbiotic Culture Of Bacteria and Yeasts (SCOBY). SCOBY is the starter culture whose function is to perform fermentation to obtain the kombucha. The SCOBY is made up of acetic acid bacteria, lactic acid bacteria, and yeasts. It serves an essential role in creating the delicious flavors and nutritional advantages of the favorite kombucha beverages. Detailed information about SCOBY is given below under the sub-title "Microbiology of Kombucha Fermentation".

Known for its strong taste and alleged health benefits, kombucha has a long history of popularity all over the world. Although this beverage is predominantly made with tea (black tea, green tea, oolong tea), it is possible to find other varieties infused with different flavors such as lemon balm, mint and jasmine, mango, cherry, pineapple, pomegranate, apple, orange, grape, passionfruit, peach (1).

Kombucha, although popularised in recent years, originated in the Tsin Dynasty in Northeast China around 220 BC and is known for its detoxifying and energizing properties, was brought to Japan in 440 AD by a Korean physician, Dr Kombu, to cure the digestive troubles of the Emperor Inkyo (2).

Subsequently, kombucha is believed to have travelled to Russia and Eastern Europe via trade routes extending beyond the Far East. Kombucha, gained popularity as a folk remedy in Germany in the 1920s, spread from Germany to western European countries such as France and their colonial countries in North Africa and the Middle East (2,3). Today this beverage is produced in large-scale for commercial use as well as in domestic conditions in many countries.

While the term "Kombucha" is the most commonly used name for the beverage, it is also known by other names such as Tea Kvass, Champignon de Longue Vie, Chainii Grib, Chainii Kvass, Ling Zhi, Kocha Kinoko, and Red Tea Fungus (4).

Processing Techniques of Kombucha

The main inputs for kombucha production are tea, sucrose and starter culture, known as SCOBY. Black, oolong and green teas, either individually or in combination, are the main ingredients used in making kombucha.

Kombucha production is done in two ways: home-made production and industrial production. In small-scale home-made production, fermentation and storage processes are carried out in glass containers, while stainless steel tanks are used in industrial-scale production(5).

In processing, generally 50-100 g of sucrose is dissolved in 1 L of boiling drinking water, so the sucrose concentration varies between 0.05g/mL and 0.10 g/mL. Depending on the quality of the tea used, 5 to 10 grams of tea leaves are added to the solution, stirred, infused for 5-10 minutes and filtered (3,4,6,7). As carbon source, the presence of sucrose in the solution initiates a cascade of metabolic processes that generate a carbonated and slightly acidic drink at the end of the primary fermentation cycle (8).

After filtration the mixture is poured into a sterile container and allowed to cool to room temperature (24±4°C). By adding about 50 g/L of freshly grown starter culture known as SCOBY in biofilm or cellulose-pellicle form and 200mL of previously fermented tea broth, the fermentation commences. The purpose of adding previously fermented tea broth is to lower the initial pH (≤ 4.6) to inhibit the growth of undesirable microorganisms. Each container is carefully covered with a hygienic cloth and fastened properly (7,8,9,10).

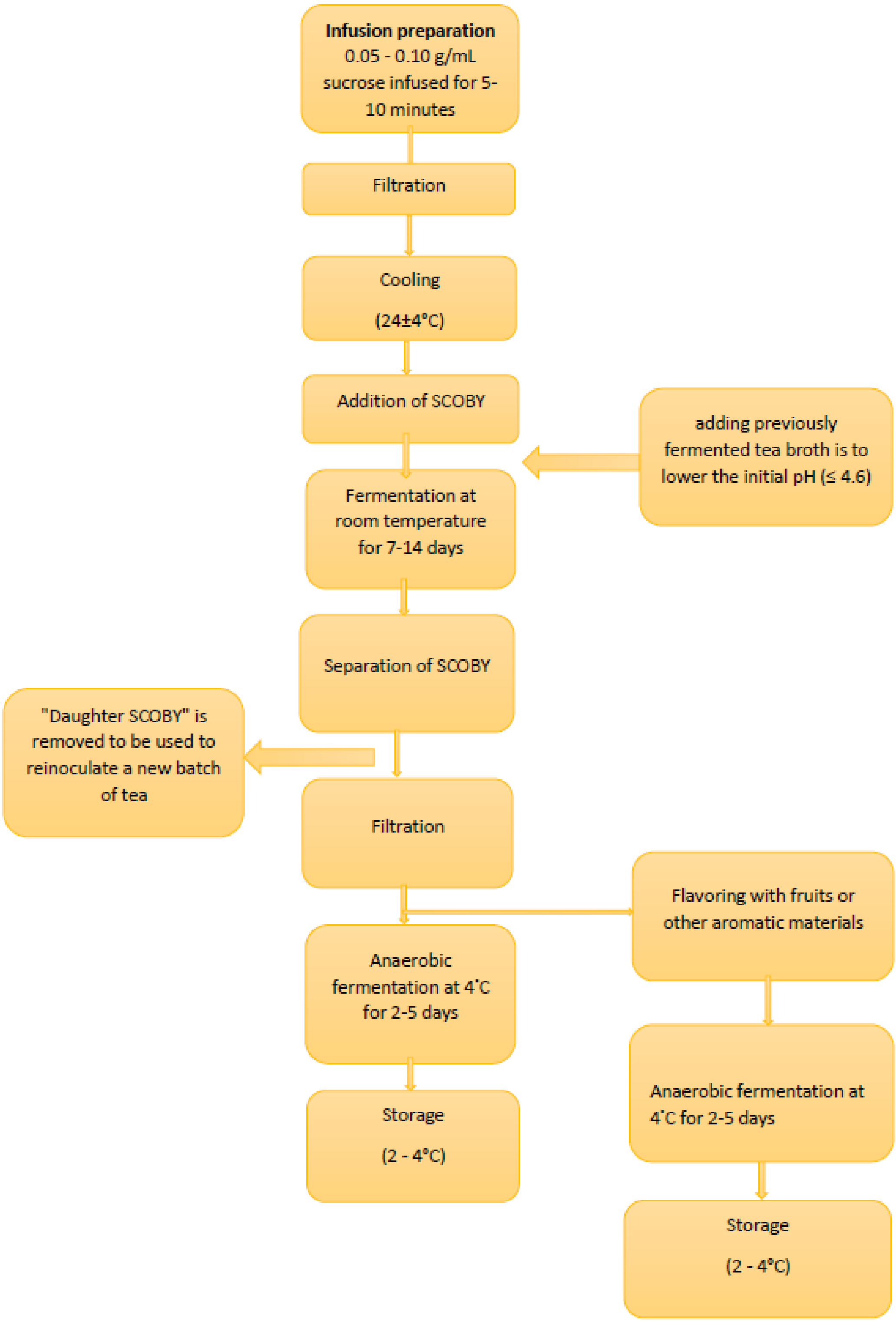

Figure 1. Flow chart of the kombucha production process

Initial fermentation takes about 7 to 14 days. As the fermentation proceeds, a new disc-shaped cellulose pellicle called the “Daughter SCOBY” forms on the surface of the fermented tea broth and the composition of the tea broth changes with both sucrose and pH decreases and the acidity increases due to the symbiotic activity of bacteria and yeast. The pH endpoint of >2.5 is recommended for the safe consumption of kombucha (1,2,6,7).

After inital fermentation the new disc-shaped cellulose pellicle called "Daughter SCOBY" is removed to be used to reinoculate a new batch of tea. “Mother Scoby”, initially used in inoculation, and its remnants are separated from kombucha broth by straining. Kombucha broth is filled into sealed container and properly stored for anaerobic fermentation at 4°C for 2-5 days before consumption (5,10,11).

Figure 2. General view of Mother SCOBY, Kombucha Broth and Daughter SCOBY

In order to improve sensory quality and carbonation, it is recommended to add flavored compounds, preferably in liquid form, to kombucha broth. Flavored compounds, such as fruits, herbs, and esential oils, are added to the kombucha broth which is then stored under anaerobic conditions at 4°C. Flavors take 2–5 days for a total incorporation into the broth, depending on how potent the compound is. At the end of 2-5 days kombucha is strained, filled into bottles, capped and labeled and then is maintained between 2 to 4°C to ensure freshness of the drinks (5,7,11).

In industrial scale production after the anaerobic fermentation of flavored kombucha, the broth is strained and pumped into kegs along with pressurized CO_2. The pressure carbonated kombucha is then bottled, capped, labeled, and boxed. A temperature of 2–4°C is maintained in the bottles and kegs to ensure freshness of the drinks (5,12,13).

Depending on the fermentation period the taste of the Kombucha changes from a pleasantly fruity sour-like sparkling flavor after a few days to a mild vinegar-like taste after a long incubation period (2).

Microbiology of Kombucha Fermentation

Kombucha is a product of the symbiotic association of specialized microorganisms that change rapidly in terms of quantitative and qualitative composition. SCOBY, used for the kombucha fermentation as starter culture, has a variable microbiological composition, depending on its origin, environmental conditions, geographical location and the medium used for the fermentation process. The most abundant bacteria in the symbiotic culture belong to the genera Acetobacter and Gluconobacter.

The most characteristic microorganisms in kombucha fermentations are Acetic Acid Bacteria (AAB) and yeasts. Lactic Acid Bacteria (LAB) can occur, but do not seem to be an essential part of the kombucha microbial ecosystem as they are not always found (8,9).

Yeasts play an important role in kombucha fermentation, and are one of the various types of microorganisms found in SCOBY. At the beginning of the fermentation process, yeasts hydrolyze sucrose into glucose and fructose by *invertase.* Since acetic acid bacteria cannot metabolize sucrose, yeasts perform this task and this hydrolysis reaction of sucrose forms the basis of the symbiosis that occurs in Kombucha fermentation. What is more, yeasts produce ethanol and CO_2 via glycolysis. However, high concentration of alcohol is harmful to microorganisms through modifications to the structures, functions, and integrities of cellular membranes. Thanks to the symbiotic activity of bacteria and yeasts, ethanol can be reduced by aldehyde and alcohol dehydrogenase produced by acetic acid bacteria that oxidize it and produce acetic acid via the Krebs cycle. (14).

Although dominant yeast genera appears to be substantially more variable from study to study, *Zygosaccharomyces, Candida, Torulaspora, Brettanomyces, Schizosaccharomyces, Hanseniaspora, Pichia*, and *Saccharomyces* are some of the genera described as important for Kombucha production (15).

The dominant bacteria of SCOBY are AAB, which are aerobic bacteria able to use alcohol as a substrate to form acetic acid. In contrast to yeast, these bacteria, require large amounts of oxygen for their growth and activity. The metabolic process is based on the conversion of acetaldehyde into ethanol and acetaldehyde hydrate into acetic acid by the enzyme *acetaldehyde dehydrogenase* (16).

During fermentation floating SCOBY keeps the acetic acid bacteria on the surface, allowing enough oxygen for their development and protecting the yeasts from the action of oxygen since they are housed in the lower part of the film, allowing the realization of anaerobic fermentation (17).

The acetic acid bacteria community is mostly represented by the genera *Acetobacter, Gluconobacter and Komagataeibacter (Gluconacetobacter).*

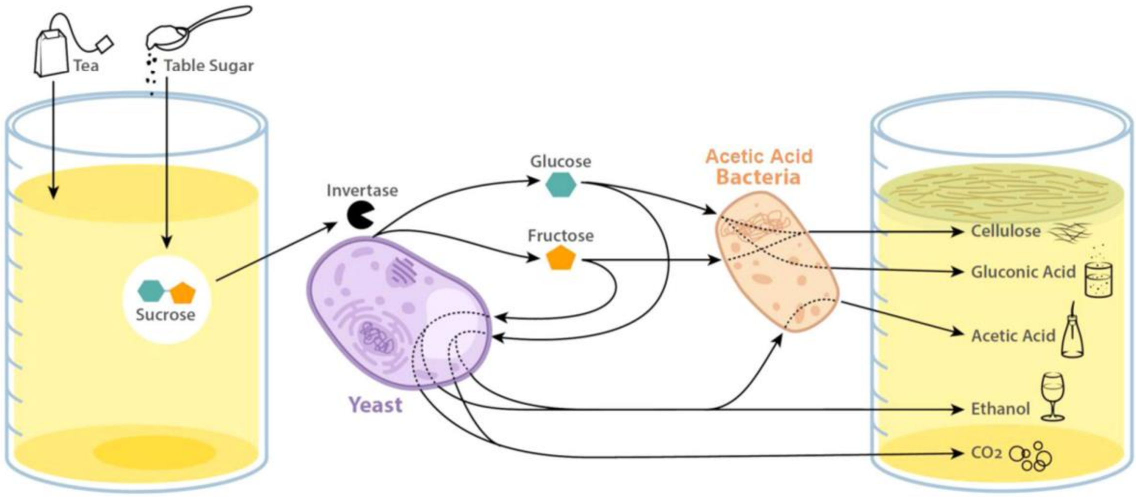

Figure 3. Kombucha metabolism and microbial interactions *(adapted from May et al.,2019)*

Komagataeibacter species are well-recognized bionanocellulose (BNC) producers. Formation of new cellulose-pellicle in kombucha broth is reliant upon the presence of at least one cellulose-producing acetic acid bacterium (AAB) of the *Komagataeibacter* (formerly *Gluconacetobacter*) genus. As well as generating the cellulose-pellicle, *Komagataeibacter* is responsible for production of organic acids integral to Kombucha's characteristic sweet and sour flavor profile. In a study that analyzed the relative abundance of major bacterial groups, a single species predominantly dominated both cellulose-pellicle

(SCOBY) and kombucha broth: the genus *Komagataebacter*. The relative proportion of *Komagataebacter* was determined to exceed 95% (8,9,16).

In addition to acetic acid and bacterial cellulose, AAB are also responsible for the production of glucuronic acid. This uronic acid is produced from glucose, which is metabolized into gluconic acid and then converted into glucuronic acid, which provides numerous health benefits to consumers. It is also worth noting that Gluconobacter strains can synthesize vitamin C (L-ascorbic acid) from D-sorbitol obtained from glucose (18).

It has been reported that lactic acid bacteria are generally absent or low in Kombucha broth. However, there are some studies indicating that higher amounts of lactic acid bacteria are present during fermentation when producing kombucha on an industrial scale (9).

Chemical composition of Kombucha

The results of the studies on the chemical components of kombucha drink showed that organic acids, especially acetic, gluconic and glucuronic acids, as well as citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, pyruvic and usnic acids are also present in kombucha broth. In addition to sugars (sucrose, glucose and fructose), whose amounts vary depending on the amount of sucrose used and the fermentation time, water-soluble vitamins (B1, B2, B6, B12, C), amino acids, biogenic amines, purines, pigments, lipids, proteins, hydrolytic enzymes, ethanol, acetic acid bacteria and lactic acid bacteria, carbon dioxide, polyphenols, minerals (manganese, iron, nickel, copper, zinc, lead, cobalt, chromium

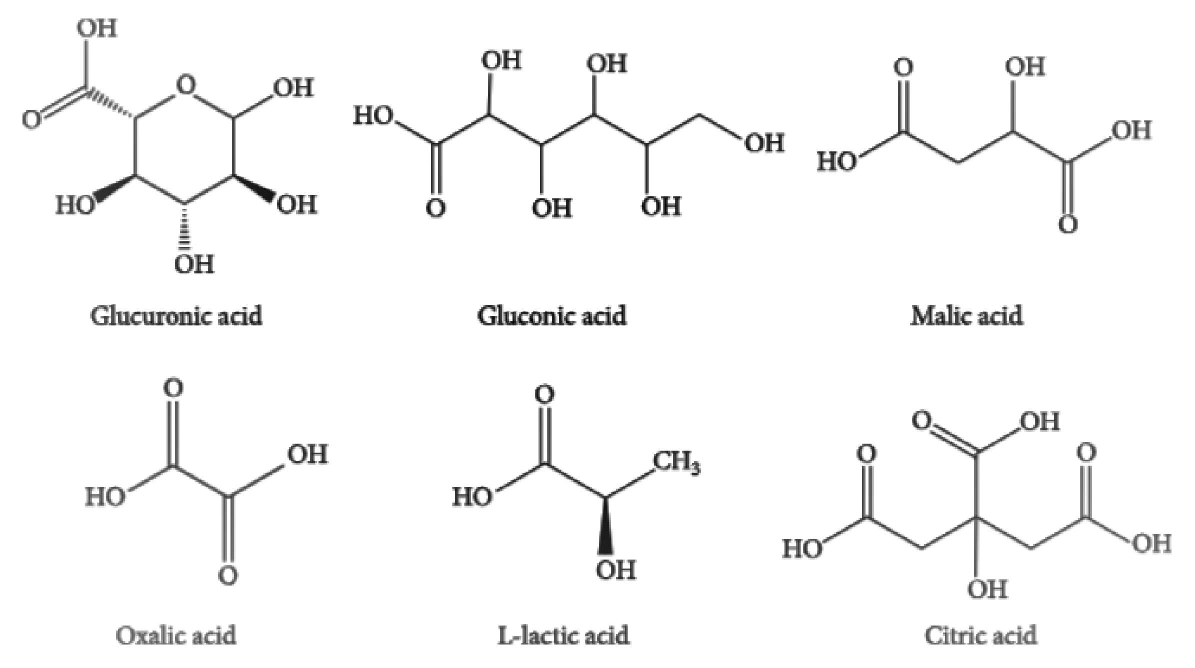

Figure 4. The most abundant organic acids found in kombucha as a result of fermentation

and cadmium), anions (fluoride, chloride, bromide, iodide, nitrate), phosphate and sulfate), D-saccharic acid-1,4-lactone (DSL), and metabolic products of yeast and bacteria are also important chemical components of kombucha broth (19,20,21,22,23).

Health Benefits of Kombucha

Kombucha, a promising functional beverage, is in the focus of an increasing number of researchers, entrepreneurs and especially consumers due to its antimicrobial, antioxidant, antidiabetic and anticancer effects. Kombucha also acts as a probiotic beverage, influencing the gastrointestinal microbial flora in human being and helping to balance the intestinal flora, facilitating the normalization of intestinal activities to some extent.

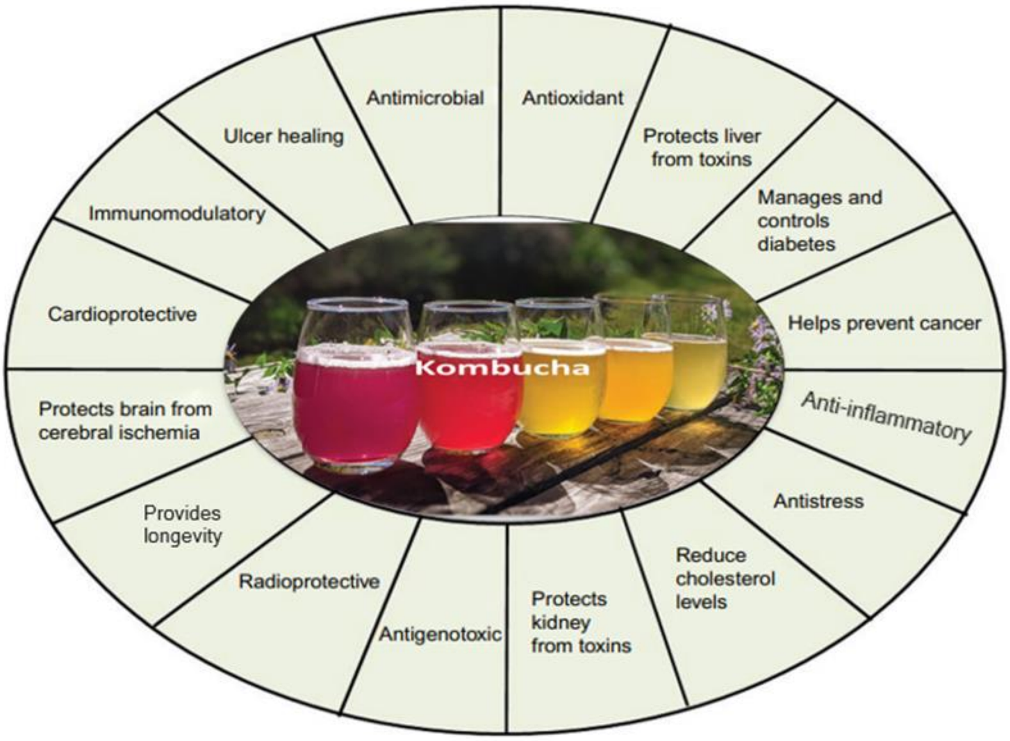

Figure 5. Health Benefits of Kombucha

- **Antioxidant Effect of Kombucha**

An antioxidant is a substance that, although present in low concentrations compared to an oxidizable substrate, significantly retards or inhibits the oxidation of the substrate. Free radicals, which cause cell damage and death through oxidation in the human body, are the principal cause of diseases such as Parkinson's disease, coronary heart disease, cancer, and diabetes. Antioxidants, on the other hand, have the ability to contact free radicals and oxidize them and inhibit other oxidation reactions that lead to harmful chain reactions. It is important to include antioxidant-containing foodstuffs in the daily diet to balance this oxidative stress caused by free radicals (24).

The antioxidant property of kombucha has been investigated by many researchers in the last two decades. Kombucha beverage has been shown to have a high radical scavenging activity when prepared using green tea, black tea and oolong tea. Tea leaves, one of the main inputs used in the production of kombucha, contain polyphenols, especially catechins. Polyphenols, which pass into the liquid phase during the infusion and fermentation stages, have the ability to scavenge free radicals and reactive oxygen species, that is, they have a high level of broad antioxidant properties. Polyphenols make up about 30% of the total dry weight of fresh tea leaves, and epigallocatechin, epigallocatechin-3-gallate, epicatechin-3-gallate, and epicatechin are the most prominent types of polyphenols found in tea leaves. When complex phenolic compounds are present in the acidic environment or when enzymes released by bacteria and yeasts are present in SCOBY, the breakdown of complex molecules into small molecules takes place, resulting in an increase in the total phenolic compounds found in kombucha broth. Therefore, when fermentation occurs, the total phenolic content increases. The production of compounds possessing radical scavenging properties depends on the fermentation period and origin of SCOBY that determines which metabolites are produced (25,26,27,28).

- **Anticancer Effect of Kombucha**

Phytochemicals are defined as non-nutrient bioactive plant components found in fruits, vegetables, grains and other plant-derived foods such as kombucha and are associated with reducing the risk of major chronic diseases. In recent years, the tendency to consume foods rich in bioactive compounds has been increasing rapidly. Kombucha is one of the most important of these.

Scientific studies have also claimed that Kombucha has anticancer effects. The Central Oncological Research Unit in Russia and the Russian Academy of Sciences conducted population studies on kombucha and found that daily consumption of kombucha broth was correlated with an extremely high resistance to cancer in 1951 (1).

In another study, kombucha prepared from green tea showed significant cytotoxic activity against human cancer cell lines A549 and Hep-2 (epidermoid carcinoma), while kombucha prepared from black tea was found to be effective only against Hep-2 (29). One of the most recent studies also showed that lyophilized kombucha extract prepared from fermenting black tea significantly reduced the survival of the prostate cancer cell line PC-3 by downregulating the expression of angiogenesis stimulators (30).

The possible mechanisms of the anticancer activity of kombucha are mainly considered to be inhibition of cancer cell proliferation, termination of metastasis, induction of cancer cell apoptosis and inhibition of gene mutation (30,31). It has also been reported that the polyphenols found in kombucha have antitumor properties and therefore act as a cancer-blocking agent (29).

- **Probiotic Effect of Kombucha**

Probiotics are a combination of live beneficial bacteria and/or yeasts that naturally live in your body. It is known that probiotic microbes play a vital role in the well-being of human health. Often, the bacterial component of a probiotic mix consists of Lactobacillus or Bifidobacterium, while this mix can also be supplemented with a few common yeast species such as Saccharomyces boulardii and Saccharomyces cerevisiae. Probiotic microorganisms provide balance in the intestinal microbiota, normalize the processes in the intestine and strengthen the immune system. In addition, they help improve digestion, fight harmful bacterial growth, provide mental clarity and mood stability, and relieve psychological conditions such as anxiety and depression. Many studies have claimed that this beverage is not only a probiotic, but also acts as a symbiotic, a combination of prebiotics and probiotics (1,32,33,34). This is because the bacteria and yeast found in kombucha drink act as probiotics, and the microcellulose present can aid the growth of beneficial microorganisms in the gut (33).

However, the production of kombucha is not standardized and the final composition of the beverage is highly dependent on the raw materials used and the physicochemical parameters adopted in the process (35).

- **Hepatoprotective Effect of Kombucha**

The liver plays a crucial role in many essential physiological processes and is vulnerable to a wide variety of toxic, microbial, metabolic, circulatory, and neoplastic insults. Liver damage or dysfunction is considered a serious health issue. Hepatoprotection, or antihepatotoxicity, is the ability of a chemical to prevent damage to the liver.

Many studies on cell lines and animal models have determined that Kombucha broth exhibits hepatoprotective activity against various environmental pollutants (2). Many scientific studies have been conducted to evaluate the ability of Kombucha broth to effectively attenuate physiological changes caused by hepatotoxic agents such as aflatoxin B1 (36), acetaminophen (37), cadmium chloride (38), and tert-butyl hydroxyperoxide (39). Pauline et al.(2001) determined in their in vivo study that Kombucha broth could prevent paracetamol-induced hepatotoxicity (40) Carbon tetrachloride (CCl_4) is a xenobiotic that induces lipid peroxidation and generates a free radical, which leads to liver damage. In another in vivo study, it was shown that kombucha consumption inhibited CCl_4 activity and prevented liver damage in rats (41).

Studies showing the protective effects of kombucha against thioacetamide-induced hepatotoxicity have shown that the antioxidant activity of polyphenolic substances in kombucha is responsible for this function. These studies also revealed that Kombucha prevents apoptotic cell death of hepatocytes triggered by liver exposure to environmental toxins (42). As a result, it can be concluded that kombucha can be used as a healing agent as well as protective against oxidative stress-mediated hepatotoxicity.

- **Antidiabetic Effect of Kombucha**

According to WHO statistics In 2019, diabetes was the direct cause of 1.5 million deaths, and 48% of all deaths due to diabetes occurred before the age of 70 years. Additionally 460,000 kidney disease deaths were caused by diabetes, and raised blood glucose causes around 20% of cardiovascular deaths (43).

Oxidative stress plays an important role in diabetes mellitus contributing to the progress of diabetic complications and different organ damage. Many studies have shown that some antioxidants play an important role in reducing oxidative stress in diabetes mellitus (6).

Srihari et al. (2013) found that there was a reduction in glycosylated hemoglobin and an increase in plasma insulin, hemoglobin, and tissue glycogen levels in diabetic rats induced by streptozotocin. The rats had consumed 6 mg/kg of body mass of lyophilized kombucha over 45 days of treatment. A significant reduction in enzymatic activities of glucose-6-phosphatase, fructose-1, 6-bisphosphatase, and hexokinase, the main enzymes responsible for the control of glycolysis and gluconeogenesis in liver and muscle, was also observed, indicating that kombucha has a potential to act at various points in the glucose regulatory pathways (44).

Another study observed a potential antidiabetic effect of kombucha in alloxan-induced diabetic rats. After 14 days of treatment with 150 mg/kg body weight lyophilized kombucha extract, the blood glucose level decreased by approximately 56.4% (45). Chakravorty et al. (2016) showed in their study that the microbial community of kombucha can play an important role in increasing the anti-diabetic property of kombucha (46).

- **Antimicrobial Effect of Kombucha**

Numerous studies have been conducted on kombucha showing an inhibitory effect on many pathogenic microorganisms of both Gram-positive and Gram-negative origin (1). Some of the pathogenic microorganisms in question are Helicobacter pylori (an organism that causes peptic ulcers), *Escherichia coli* (the causative organism of common diarrhea), *Entamoeba cloacae*, *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermis, Agrobacterium tumefaciens, Bacillus cereus, Aeromonas hydrophila, Salmonella typhimurium, Salmonella enteritidis, Shigella sonnei, Leuconostoc monocytogenes, Yersinia enterocolitica, Campylobacter jejuni, and Candida albicans* (1, 47, 48).

Antimicrobial activity of Kombucha broth is attributed to the low pH, mainly due to the presence of acetic acid and various organic acids, ethanol and catechins, as well as many large proteins produced during fermentation (47) Greenwalt et al. (1998) have tested the antimicrobial activity of Kombucha as well as normal tea extracts prepared at different concentrations and found that the inhibitory effects of Kombucha increased with the tea concentration (48).

It has been reported that inhibitory effect of kombucha, prepared from black tea, on *Candida albicans* but not against *Zygosaccharomyces bailii.* The 21-day fermented kombucha broth from both green and black teas inhibited the growth of *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida dubliniensis,* and *Candida sake* excepting *Candida krusei* (50).

The results of the study conducted by Yuniarto et al (2016) showed that kombucha can be used as a potential antifungal source against human pathogenic fungi including *Aspergillus flavus*, *Candida albicans* and *Microsporum gypseum* (51).

Antifungal activity of the ethyl acetate fraction of traditional kombucha was studied against a lipophilic fungus, i.e. the genus *Malassezia*, which plays a role in the development of seborrheic dermatitis. For this purpose, 23 clinical isolates of *Malassezia sp*. were identified in 19 patients with seborrheic dermatitis and used in the study. Antifungal activity of the ethyl acetate fraction of kombucha was studied at concentrations of 10, 40, and 80 mg/mL and found to display a concentration-dependent inhibition (52).

- **Detoxification Effect of Kombucha**

Detoxification is the physiological or medicinal removal of toxic substances from a living organism, including the human body, which is mainly carried out by the liver. The term ‘toxin’ means poisonous substances that are encompassing pollutants, synthetic chemicals, heavy metals, processed food and other potentially harmful products of modern life. Detoxification helps maintain a healthy liver and is also known to play a role in cancer prevention. Enzymes, bacterial acids and other secondary metabolites produced by microorganisms during the fermentation process in the preparation of kombucha have shown the ability to detoxify the body (1).

In fact, many of the enzymes and bacterial acids found in kombucha are very similar to the chemicals the body produces to detoxify itself. Therefore, including Kombucha in one's diet can result in a reduction of the detoxification burden that puts strain on the liver. Many scientific studies have reported that this ability is mainly due to the capacity of glucuronic acid to bind toxic molecules entering the body, and also to increase the excretion of these molecules from the physiology with the help of the kidneys and intestines (22). The strong binding process between the molecules of glucuronic acid (an organic acid that occurs in the oxidation reaction of glucose during the fermentation of Kambucha) and the toxin is known as glucuronidation (53). This acid is the most important detoxifier in the human body; thus, it has the ability to bind to toxins in the liver and encourage them to be eliminated from the body (54). Like glucuronic acid, malic acid is a byproduct of fermentation that helps detoxify the liver (27).

It is known that kombucha consumption supports detoxification of the liver, as well as helping to remove heavy metal substances and environmental pollutants from the human body through the kidneys (55). It is also useful in the biotransformation of endogenous metabolites such as bilirubin and excess steroid hormones (27). It has been reported that the detoxifying effect of kombucha helps to get rid of gout, rheumatism, arthritis and kidney stones, which are health problems associated with the accumulation of toxic substances in the body (1).

- **Other Therapeutic Effects**

The Antilipidemic and Cardioprotective effects of Kombucha were investigated and it was determined that it significantly lowered cholesterol, triglycerides, LDL and VLDL while increasing HDL levels. Likewise, a reduction in leakage of cardiac myocardial markers was observed. (56).

Marzban et al (2015) investigated the anti-inflammatory effect of kombucha and observed beneficial effects in autoimmune encephalomyelitis due to a lower incidence, reduced severity, and delay in disease onset. Mice treated with kombucha tea had significantly reduced serum NO and TNF-α levels (57).

The cytoprotective effect of kombuch was investigated and it was stated that supplementing tea with kombucha reduces phenol-induced cytotoxicity, but its protective role is dose-dependent (58).

In a study examining the anti-radioactive effect of kombucha, it was reported that significant potential was seen in terms of its ability to protect the cellular system from radiation-induced damage and scavenge free radicals (59). In a similar study, it was stated that kombucha supplementation could reduce the frequency of induced chromosomal abnormalities, however the radioprotective effect against ionizing radiation was dose-dependent (60).

Black tea compounds such as flavonoids, amino acids, and phenolic acids are transformed by the action of yeast and bacteria into metabolites that have been associated with the prevention of neurodegenerative diseases, reduced blood pressure, hypoglycemic effect, etc (16).

Caveat

Although there have been many scientific studies on the health benefits of Kombucha, known as the "Elixir of Long Life" by the Far Eastern people for about 2000 years, there are some precautions to be taken and some rules to be followed in order to benefit from this bio-tea without any problems.

Whether kombucha is produced on a small scale or on an industrial scale, all tools and equipment used must be of food grade, and hygiene rules must be followed 100% in the production area. It is appropriate to use sterilized glass containers in small-scale production, and stainless steel containers in industrial scale.

The quality and hardness of the water used should be suitable for the desired symbiotic activity of the bacteria and yeasts.

The fact that both SCOBY and the tea used as raw material are of the same quality and standard in each batch is the first condition necessary for the final product to be produced at the same quality and standard. The second condition that must be met for the production of standard products is the optimization of the production parameters. In other words, in order to produce kombucha of the same quality and standard in every batch, pH, titratable acidity value, fermentation time, fermentation temperature, final alcohol value (≤0.5%), type, quality and amount of sugar used should be kept under control. Aseptic bottling should be done and suitable storage conditions maintained until it reaches the consumer.

When all these conditions are met, food safety and quality will be ensured, so you can drink your delicious kombucha with pleasure, offer it to your friends, and market it. Enjoy your elixir of life !

Written by Dr Saziye ILGAZ

REFERENCES

1. Dufresne, C., & Farnworth, E. (2000). *Tea, Kombucha, and health: a review. Food Research International, 33(6), 409–421.* doi:10.1016/s0963-9969(00)00067-3

2. Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). *A Review on Kombucha Tea-Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus. Comprehensive Reviews in Food Science and Food Safety, 13(4), 538–550.* doi:10.1111/1541-4337.12073

3. Kim, J., & Adhikari, K. (2020). *Current Trends in Kombucha: Marketing Perspectives and the Need for Improved Sensory Research. Beverages, 6(1), 15.* doi:10.3390/beverages6010015

4. Jayabalan, R., & Waisundara, V. Y. (2019). *Kombucha as a Functional Beverage. Functional and Medicinal Beverages, 413–446.* doi:10.1016/b978-0-12-816397-9.00012-1

5. Soares, M. G., de Lima, M., & Reolon Schmidt, V. C. (2021). Technological aspects of kombucha, its applications and the symbiotic culture (SCOBY), and extraction of compounds of interest: A literature review. Trends in Food Science & Technology, 110, 539–550. doi:10.1016/j.tifs.2021.02.017

6. Chakravorty, S., Bhattacharya, S., Bhattacharya, D., Sarkar, S., & Gachhui, R. (2019). *Kombucha: A Promising Functional Beverage Prepared From Tea. Non-Alcoholic Beverages, 285–327.* doi:10.1016/b978-0-12-815270-6.00010-4 (Figure 1 health benefits)

7. Wang, B.; Rutherfurd-Markwick, K.; Zhang, X.-X.; Mutukumira, A.N. Kombucha: Production and Microbiological Research. Foods 2022, 11, 3456. https://doi.org/ 10.3390/foods11213456

8. May A, Narayanan S, Alcock J, Varsani A, Maley C, Aktipis A. 2019. Kombucha: a novel model system for cooperation and conflict in a complex multi-species microbial ecosystem. *PeerJ* 7:e7565 https://doi.org/10.7717/peerj.7565

9. Laureys, D., Britton, S. J., & De Clippeleer, J. (2020). Kombucha Tea Fermentation: A Review. Journal of the American Society of Brewing Chemists, 78(3), 165–174. doi:10.1080/03610470.2020.1734150

10. Nyhan, L.M.; Lynch, K.M.; Sahin, A.W.; Arendt, E.K. Advances in Kombucha Tea Fermentation: A Review. Appl. Microbiol. 2022, 2, 73–103. https://doi.org/10.3390/ applmicrobiol201000

11. Chandrakala, S. K., Lobo, R. O. & Dias, F. O. (2019). *Kombucha (Bio-Tea): An Elixir for Life? Nutrients in Beverages, 591–616.* doi:10.1016/b978-0-12-816842-4.00016-2

12. Watawana, M. I., Jayawardena, N., Gunawardhana, C. B., & Waisundara, V. Y. (2015). Health, Wellness, and Safety Aspects of the Consumption of Kombucha. Journal of Chemistry, 2015, 1–11. doi:10.1155/2015/591869

13. Dutta, H.& Paul, S. K. (2019). Kombucha Drink: Production, Quality, and Safety Aspects. Production and Management of Beverages, 259–288. doi:10.1016/b978-0-12-815260-7.00008-0

14. Tran, T., Grandvalet, C., Verdier, F., Martin, A., Alexandre, H. & Tourdot-Maréchal, R. (2020). *Microbiological and technological parameters impacting the chemical composition and sensory quality of kombucha. Comprehensive Reviews in Food Science and Food Safety.* doi:10.1111/1541-4337.12574

15. Harrison, K., & Curtin, C. (2021). *Microbial Composition of SCOBY Starter Cultures Used by Commercial Kombucha Brewers in North America. Microorganisms, 9(5), 1060.* doi:10.3390/microorganisms9051060

16. Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J.-P., & Taillandier, P. (2018). *Understanding Kombucha Tea Fermentation: A Review. Journal of Food Science, 83(3), 580–588.* doi:10.1111/1750-3841.14068

17. Coelho, R. M. D., Almeida, A. L. de, Amaral, R. Q. G. do, Mota, R. N. da, & Sousa, P. H. M. de. (2020). *Kombucha: Review. International Journal of Gastronomy and Food Science, 22, 100272.* doi:10.1016/j.ijgfs.2020.100272

18. Antolak, H.; Piechota, D.; Kucharska, A. (2021). Kombucha Tea—A Double Power of Bioactive Compounds from Tea and Symbiotic Culture of Bacteria and Yeasts (SCOBY). Antioxidants 2021, 10, 1541. https://doi.org/10.3390/ antiox10101541

19. Bauer-petrovska, B., & Petrushevska-tozi, L. (2000). Mineral and water soluble vitamin content in the Kombucha drink. International Journal of Food Science and Technology, 35, 201–205

20. Kumar, S. D., Narayan, G., & Hassarajani, S. (2008). Determination of anionic minerals in black and Kombucha tea using ion chromatography. Food Chemistry, 111, 784–788. https://doi.org/10.1016/j.foodchem.2008.05.012

21. Chen, C., & Liu, B. Y. (2000). Changes in major components of tea fungus metabolites during prolonged fermentation. Journal of Applied Microbiology, 89, 834–839. https://doi. org/10.1046/j.1365-2672.2000.01188.x

22. Jayabalan, R., Marimuthu, S., & Swaminathan, K. (2007). Changes in content of organic acids and tea polyphenols during Kombucha tea fermentation. Food Chemistry, 102(1), 392–398. https://doi.org/10.1016/j.foodchem.2006.05.032

23. Kumar, S. D., Narayan, G., & Hassarajani, S. (2008). Determination of anionic minerals in black and Kombucha tea using ion chromatography. Food Chemistry, 111, 784–788. https://doi.org/10.1016/j.foodchem.2008.05.012

24. Shebis,Y., Iluz, D., Kinel-Tahan, Y., Dubinsky,Z., and Yehoshua, Y. (2013). “Natural antioxidants: function and sources,” Food and Nutrition Sciences, vol. 4, no. 6, pp. 643–649, 2013.

25. Vanida Osiripun, & Tarit Apisittiwong. (2023). Polyphenol and antioxidant activities of Kombucha fermented from different teas and fruit juices. *Journal of Current Science and Technology*, *11*(2), 188–196. Retrieved from https://ph04.tci-thaijo.org/index.php/JCST/article/view/390

26. Kaewkod, T., Bovonsombut, S., & Tragoolpua, Y. (2019). *Efficacy of Kombucha Obtained from Green, Oolong, and Black Teas on Inhibition of Pathogenic Bacteria, Antioxidation, and Toxicity on Colorectal Cancer Cell Line. Microorganisms, 7(12), 700.* doi:10.3390/microorganisms7120700

27. Srihari, T. and Satyanarayana, U.(2012). “Changes in free radical scavenging activity of Kombucha during fermentation,” Journal of Pharmaceutical Sciences and Research, vol. 4, no. 11, pp. 1978–1981.

28. Jayabalan,R., Subathradevi, P., Marimuthu,S., Sathishkumar, M.,and Swaminathan, K.(2008). “Changes in free-radical scavenging ability of kombucha tea during fermentation,” Food Chemistry, vol. 109, no. 1, pp. 227–234.

29. Deghrigue, M., Chriaa, J.,Battikh,H., Abid, K., and Bakhrouf,A.(2013). “Antiproliferative and antimicrobial activities of Kombucha tea,” African Journal of Microbiology Research, vol. 7, pp. 3466–3470.

30. Jayabalan,R., Chen,P.N., Hsieh, Y.S.et al. (2011). “Effect of solvent fractions of kombucha tea on viability and invasiveness of cancer cells-characterization of dimethyl 2-(2-hydroxy-2-methoxypropylidine) malonate and vitexin,” Indian Journal of Biotechnology, vol. 10, no. 1, pp. 75–82.

31. Conney, A.H., Lu,Y.P., Lou, Y.R., and Huang, M.T. (2002). “Inhibitory effects of tea and caffeine on UV-induced carcinogenesis: relationship to enhanced apoptosis and decreased tissue fat,” European Journal of Cancer Prevention, vol. 2, pp. 28–36.

32. Bode, A.M. and Dong, Z. (2003). “Signal transduction pathways: targets for green and black tea polyphenols,” Journal of Biochemistry and Molecular Biology, vol. 36, no. 1, pp. 66–77.

33. Kozyrovska,N.O.,Reva,O.M., Goginyan,V.B., and De Vera,J.P. (2012). “Kombucha microbiome as a probiotic: a view from the perspective of post-genomics and synthetic ecology,” Biopolymers and Cell, vol. 28, no. 2, pp. 103–113.

34. Júnior, R. J. S.,Batista, R. A., Rodrigues, S. A., Filho, L. X., and Lima,Á.S.(2009). “Antimicrobial activity of broth fermented with Kombucha colonies,” Journal of Microbial & Biochemical Technology, vol. 1, no. 1, pp. 72–78.

35. Vargas, B. K., Fabricio, M. F., & Záchia Ayub, M. A. (2021). *Health effects and probiotic and prebiotic potential of Kombucha: A bibliometric and systematic review. Food Bioscience, 101332.* doi:10.1016/j.fbio.2021.101332

36. Jayabalan,R.,Baskaran, S., Marimuthu,S., Swaminathan, K.,and Yun,S.E. (2010). “Effect of kombucha tea on aflatoxin B1 induced acute hepatotoxicity in albino rats-prophylactic and curative studies,” Journal of Applied Biological Chemistry, vol. 53, no. 4, pp. 407–416.

37. Abshenas,J., Derakhshanfar,A., Ferdosi,M.H. and Hasanzadeh, S. (2012).“Protective effect of kombucha tea against acetaminophen-induced hepatotoxicity in mice: a biochemical and histopathological study,” Comparative Clinical Pathology, vol. 21, no. 6, pp. 1243–1248.

38. Ibrahim,N.K. (2011). “Possible protective effect of Kombucha Tea Ferment on cadmium chloride induced liver and kidney damage in irradiated rats,” World Academy of Science, Engineering and Technology, vol. 55, pp. 1097–1102.

39. Bhattacharya,S., Gachhui, R. and Sil,P.C.(2011). “Hepatoprotective properties of kombucha tea against TBHP-induced oxidative stress via suppression of mitochondria dependent apoptosis,” Pathophysiology, vol. 18, no. 3, pp. 221–234.

40. Pauline,T., Dipti,P., Anju,B. et al. (2001). “Studies on toxicity, anti-stress and hepato-protective properties of Kombucha tea,” Biomedical and Environmental Sciences, vol. 14, no. 3, pp. 207–213.

41. Murugesan,G.S., Sathishkumar,M., Jayabalan,R., Binupriya,A.R., Swaminathan,K. and Yun,S.E. (2009). “Hepatoprotective and curative properties of Kombucha tea against Carbon tetrachloride-induced toxicity,” Journal of Microbiology and Biotechnology, vol. 19, no. 4, pp. 397–402.

42. Kabiri,N., Setorki,M. and Darabi, M.A.(2013). “Protective effects of Kombucha tea and silimarin against thioacetamide induced hepatic injuries in wistar rats,” World Applied Sciences Journal, vol. 27, no. 4, pp. 524–532.

43. https://www.who.int/news-room/fact-sheets/detail/diabetes

44. Srihari, T., Karthikesan, K., Ashokkumar, N., & Satyanarayana, U. (2013). Antihyperglycaemic efficacy of kombucha in streptozotocin-induced rats. *Journal of Functional Foods*, **5**(4), 1794– 1802. https://doi.org/10.1016/j.jff.2013.08.008

45. Bhattacharya, S., Gachhui, R., & Sil, P. C. (2013). Effect of kombucha, a fermented black tea in attenuating oxidative stress mediated tissue damage in alloxan induced diabetic rats. Food and Chemical Toxicology, 60, 328– 340. https://doi.org/10.1016/j.fct.2013.07.051

46. Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D., & Gachhui, R. (2016). Kombucha tea fermentation: Microbial and biochemical dynamics. International Journal of Food Microbiology, 220, 63–72. doi:10.1016/j.ijfoodmicro.2015.12

47. Sreeramulu,G., Zhu,Y. and Knol,W. (2000). “Kombucha fermentation and its antimicrobial activity,” Journal of Agricultural and Food Chemistry, vol. 48, no. 6, pp. 2589–2594.

48. Sreeramulu,G., Zhu,Y. and Knol,W. (2001). “Characterization of antimicrobial activity in Kombucha fermentation,” Acta Biotechnologica, vol. 21, no. 1, pp. 49–56.

49. Greenwalt, C. J.; Ledford, R. A.; Steinkraus, K. H.(1998). Determination and characterisation of the antimicrobial activity of the fermented tea Kombucha. Lebensm. Wiss. -Technol. 31, 291-296.

50. BATTIKH, H., CHAIEB, K., BAKHROUF, A., & AMMAR, E. (2012). *ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF BLACK AND GREEN KOMBUCHA TEAS. Journal of Food Biochemistry, 37(2), 231–236.* doi:10.1111/j.1745-4514.2011.00629.x

51. Yuniarto, A.-, Anggadiredja, K., & Annisa Nur Aqidah, R. (2016). ANTIFUNGAL ACTIVITY OF KOMBUCHA TEA AGAINST HUMAN PATHOGENIC FUNGI. Asian Journal of Pharmaceutical and Clinical Research, 9(5), 253. doi:10.22159/ajpcr.2016.v9i5.1343

52. Mahmoudi, E., Saeidi, M., Marashi, M. A., Moafi, A., Mahmoudi, V., … Zeinolabedini Zamani, M. (2016). In vitro activity of kombucha tea ethyl acetate fraction against Malassezia species isolated from seborrhoeic dermatitis. Current Medical Mycology, 2(4), 30–36. doi:10.18869/acadpub.cmm.2.4.30

53. Vijayaraghavan,R., Singh,M., Rao,P.V.L. et al.(2000). "Sub-acute (90 days) oral toxicity studies of Kombucha tea," Biomedical and Environmental Sciences, vol. 13, no. 4, pp. 293–299.

54. Nguyen, N.K., Dong, N.T.N.,Le,P.H. and Nguyen,H.T.(2014). "Evaluation of the glucuronic acid production and other biological activities of fermented sweeten- black tea by Kombucha layer and the co-culture with different Lactobacillus Sp. strains," International Journal of Modern Engineering Research, vol. 4, no. 5, pp. 12–17.

55. Teoh,A.L., Heard, G.and Cox, J. (2004)."Yeast ecology of Kombucha fermentation," International Journal of Food Microbiology, vol. 95, no. 2, pp. 119–126.

56. Lobo, R.O.,& Shenoy, K. C. (2015). Evaluation of Kombucha Consumption Against Myocardial Infarction. IJSR - INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH. Volume : 4, Issue : 6, ISSN No 2277 – 8179.

57. Marzban, F., Azizi, G., Afraei, S., Sedaghat, R., Seyedzadeh, M. H., Razavi, A., & Mirshafiey, A. (2015). Kombucha tea ameliorates experimental autoimmune encephalomyelitis in mouse model of multiple sclerosis. Food and Agricultural Immunology, 26(6), 782–793. doi:10.1080/09540105.2015.1036353.

58. Yapar, K., Cavusoglu, K., Oruç, E., Yalcin, E. (2010).Protective effect of kombucha mushroom (KM) tea on phenol-induced cytotoxicity in albino mice. Journal of Environmental Biology, 31(5):615-21

59. Mondal, T., & Dey, S.K. (2016).Green tea kombucha, a good source of natural antioxidant and a probable radioprotector, Conference: 1st International Conference on Nano technology and Biotechnology (Nano Bio Con 2016)

60. Cavusoglu, K.,&Guler, P. (2010). Protective effect of kombucha mushroom (KM) tea on chromosomal aberrationsinduced by gamma radiation in human peripheral lymphocytes in-vitro. Journal of Environmental Biology, 31(5) 851-856.