CHAPTER I: BACKGROUND

1.1. Introduction

The utilization of naturally occurring bioactive compounds has been increasing in recent years. These compounds possess various pharmacological activities to improve the health state or act as prophylactic agents. Kombucha tea is a fermented beverage that employs the symbiotic relationship of bacteria and yeast consortium (SCOBY) to produce secondary metabolites that consist of bioactive compounds which offer health benefits. The end product of the fermentation results in a refreshing, fizzy, slightly acidic, and sweet beverage. Kombucha tea has been claimed to act as an antimicrobial, antioxidant, anti-inflammatory, and anticancer agent, which resulted from the polyphenol obtained from the tea and the metabolites produced from the fermentation process. However, further studies are needed to validate the true chemical properties and the potency of kombucha tea for health.

Kombucha is a beverage derived from fermented tea added with sugar and a symbiotic culture of bacteria and yeast, or what is often called SCOBY. This fermented tea originated from Northeast China around 220 B.C.E and became popular due to its healing properties (Jayabalan, Malbasa, Loncar, Vitas, & Sathiskumar, 2014). The symbiotic cultures used in kombucha fermentation are a consortium of acetic acid bacteria and osmophilic yeast species. *Acetobacter* and *Gluconobacter* are the two most predominant bacteria genera found in kombucha culture, whereas *Zygosaccharomyces* spp. are the main yeast genus. The combination of bacteria and yeast inside the tea will result in a symbiotic process that produces cellulose layers on the top of the culture medium (Villarreal Soto et al., 2018).

The bacteria and yeast are involved in a symbiotic relationship in a way that they are able to utilize different substrates but in a complementary way. The yeasts break down sucrose into simpler forms, which are glucose and fructose, by their enzyme invertase. Then, through glycolysis, they will mainly utilize fructose as an energy source and produce ethanol. The acetic acid bacteria, on the other side, make use of glucose to produce gluconic acid and ethanol to produce acetic acid. The production of these secondary metabolites during the fermentation process will result in an acidic pH of the kombucha, which contributes to the antimicrobial activity against pathogenic bacteria and hence, prevents contamination of the tea fungus. The product from the sugar tea fermentation will result in a refreshing, fizzy, acidic, and slightly sweet taste, which comes from the presence of alcohols, aldehydes, ketones, esters, and amino acids (Kumar & Joshi, 2016; Kaczmarczyk & Lochynski, 2014).

Production of biofilm layer on the surface of the culture involves the cellulose precursor synthesis, which is the uridine diphospho-glucose (UDPGlc). Acetic acid bacteria inside the culture are responsible for polymerizing glucose residues into β-1,4-glucan chains. As a result, the semi-solid biofilm layers are constructed, providing the bacteria an appropriate and reserved environment for them to grow rapidly and allow them to utilize carbon supply from various sources, including glucose, sucrose, ethanol, and glycerol (Villarreal Soto et al., 2018).

Kombucha has been claimed to alleviate various health conditions such as gastric problems, diabetes, cardiovascular diseases, hypertension, pulmonary diseases, and cancer. The beneficial effects that kombucha offers have been linked to the presence of polyphenols and secondary metabolites that are produced during the fermentation process. The polyphenols, which come from the tea, are suggested to act as an antioxidant as they are able to neutralize harmful free radicals and reduce inflammation. The most substantial constituents of kombucha are the organic acids, which are mainly composed of acetic acid, lactic acid, glucuronic acid, and gluconic acid. The lactic acid contained in kombucha has been linked to antimicrobial activity, whereas gluconic acid plays a major role as a detoxifying agent (Kaczmarczyk & Lochynski, 2014). In cancer studies, there's growing evidence that kombucha may also have a role as an antiproliferative in certain cancer cell lines. It was assumed that kombuchas are able to modulate immunity and prevent cancer proliferation (Vina, Semjonovs, Linde, & Denina, 2014; Watawana, Jayawardena, Gunawardhana, & Waisundara; 2015).

Cervical cancer is listed as the fourth most common cancer in women worldwide. As of 2018, the World Health Organization (WHO) estimated there are over 500,000 women diagnosed with cervical cancer, and the disease itself is responsible for more than 300,000 deaths (Arbyn et al., 2019). Like any other cancer, the management of cervical cancer commonly involves treatment such as surgery, chemotherapy, and radiation therapy. Though many noninvasive treatments are currently available, the side effects from the therapy may result in a poor overall health state (Devlin, Denson, & Whitford, 2017). Hence, the study of natural-derived anticancer compounds with fewer adverse effects are growing in recent years.

The antiproliferative effects of kombucha against cervical cancer cell lines have been previously investigated in several types of research. In 2008, Cetojevic-Simin and the team compared the activity of kombucha prepared from winter savory and black tea. The study revealed a significant increase of antiproliferative activity in savory winter kombucha against the cervical cancer (HeLa) cell line. Another study revealed antitumor properties of lemon balm kombucha towards HeLa cells at low concentration (Četojević-Simin et al., 2010). Antiproliferative activity of kombucha was compared among green and black tea in a study conducted by Cardoso et al. The results revealed that green kombucha tea exhibited a higher antitumor activity signified by a lower IC50 (Rezende Cardoso et al., 2019).

Due to the increasing appearance of kombucha's health benefit, an experimental setup is constructed to prove and elucidate the truly existing properties of kombucha tea. It is important to bear in mind that the microbial compositions among different SCOBY are varying; hence, the result from one study can't be entirely compared to another. This research aimed to characterize the chemical properties of kombucha beverages prepared using known bacteria and yeast cultures. The purpose of using these known strains is to be able to control the fermentation process better and adjust to the most favorable condition in order to enhance the potent anticancer activity. The parameters in this study include their pH, total titratable acidity, total phenolic content, flavonoids content, and antioxidant activity. Moreover, the antiproliferative activity of kombucha against the HeLa cell line is investigated as well. The kombucha is fermented with *Komagataeibacter intermedius* and *Dekkera bruxellensis*, which are the predominant microorganisms in SCOBY isolated in the previous research by Biotechnology Department i3L.

1.2. Objective

The objective of the experiment is to characterize the chemical properties of kombucha beverages prepared through two distinct methods with the inoculation of known cultures, while also identifying the antiproliferative activities against HeLa cell line.

1.3. Scope of Work

The scope of work that is going to be covered in this experiment includes the preparation of Kombucha tea fermentation with two different cultures. The first method is the inoculation of pure bacterial culture *Komagataeibacter intermedius* and the second method is through the inoculation of pure bacterial culture *Komagateibacter intermedius* and yeast culture *Dekkera bruxellensis*. These cultures were previously isolated from SCOBY by Biotechnology Department i3L.

The chemical analysis will then start with the measurement of total titratable acidity from the fermentation process. The Total Polyphenol Content (TPC) and Total Flavonoid Content (TFC) will be measured during the fermentation process (day 0, 1, 2, 4, 6, 8, 12, 14). Next, the determination of antioxidant activity will be conducted through the DPPH method. The analysis will proceed to the determination of reducing sugar content during the fermentation process. At last, the analysis of antiproliferative activity on HeLa cell line through MTS assay will be conducted, followed by DNA fragmentation assay to determine the cell death pathway.