CHAPTER 1

INTRODUCTION

1.1. Research Background

Colon cancer has become one of the global health burdens, being placed as the third-most commonly diagnosed cancer worldwide. Dietary and environmental factors have been correlated with the risk of colon cancer development (Kuipers et al., 2015); thus, proper diet consumption and particular dietary agents may potentially be cancer treatment. Fermented food and beverages have shown a crucial role in human diet, and its health benefits have been well-documented, including anticancer properties (Melini et al., 2019; Tamang et al., 2016; Şanlier et al., 2017), increasing its popularity and consumption rates worldwide (Kapp & Sumner, 2019). Lately, the growing interest in consuming fermented food and beverages has been slightly shifted toward dairy-free and plant-based products (Pimentel et al., 2021; Dey, 2018; Kumar et al., 2015); one of the products is kombucha tea.

Kombucha is a traditional yet popular fermented non-alcoholic beverage that is widely consumed worldwide, especially for health or medicinal purposes. This slightly acidic and carbonated beverage is prepared through fermentation of tea with the addition of sugar and symbiotic culture of acetic acid bacteria (AAB), lactic acid bacteria (LAB), and osmophilic yeasts, or also known as SCOBY (Jayabalan et al., 2014; Leal et al., 2018). There are a variety of substrates besides tea that can be utilized as a base for kombucha preparation, including medicinal herbs (Velićanski et al., 2013), lemon balm (Velićanski et al., 2014), coconut water (Watawana et al., 2015a), grape juice (Ayed et al., 2016), and snake fruit juice (Zubaidah et al., 2020). However, black tea along with white sugar are the most preferred ingredients and considered the finest substrates (Chakravorty et al., 2019). In appearance, kombucha tea consists of two discrete portions: the acidic tea broth or liquid phase and floating cellulose or biofilm on the surface. The former plays a critical role in limiting any contamination by pathogenic bacteria due to its acidic pH (Watawana et al., 2015b; Kaewkod et al., 2019), while the

biofilm produced encapsulates the microbial community and enhancces the interaction between bacteria and yeast (Marsh et al., 2014).

As fermentation is such a dynamic process, there is no exact microbial composition inside the kombucha due to its great varieties as well as dependence on inoculum source and fermentation condition (Leal et al., 2018). The most dominant bacteria genera identified in kombucha culture belong to *Komagataeibacter* (or known as *Gluconacetobacter* or *Acetobacter*) and *Gluconobacter*. Numerous species are included in these group of AAB, such as *Komagataeibacter xylinus*, *Komagataeibacter intermedius*, *Komagataeibacter rhaeticus*, and *Gluconobacter oxydans*; all of which have been found abundantly in kombucha biofilm and tea broth (Yamada et al., 2012; Chakravorty et al., 2016; May et al., 2019; Chakravorty et al., 2019). Alongside bacteria species, a more variable and broader spectrum of yeasts has been described. This includes those in the genera *Zygosaccharomyces*, *Dekkera* or *Brettanomyces*, *Pichia*, *Candida*, and *Saccharomyces* (Jayabalan et al., 2014; Coton et al., 2017; May et al., 2019).

Fermentation of the kombucha process begins with the breakdown of sucrose into its monomers, glucose and fructose, by the invertase enzyme of yeasts. These sugar monomers are then transformed into carbon dioxide and ethanol before bacteria utilize ethanol and generate acetic acids, lowering the pH of kombucha. AAB also makes use of glucose in the production of gluconic acid, and glucuronic acid (Jakubczyk et al., 2020; May et al., 2019). Besides those aforementioned organic acids and sugars, other components like vitamins (C, B1, B2, B6, and B12), amino acids, active enzymes, unidentified metabolic products of bacteria and yeast, and polyphenols could also be observed (Jayabalan et al., 2014). The latter is known to act as potent antioxidants, preventing diseases correlated with oxidative stress, such as cancer (Dayem et al., 2016), cardiometabolic-related disease (Fraga et al., 2019), or neurodegenerative disorders (Ataie et al., 2016).

Although there is not enough evidence on how kombucha positively affects human health, some in vitro and in vivo studies have described kombucha's therapeutic value. It includes antimicrobial effects on numerous Gram-positive and Gram-negative bacteria due to its acetic acid

content (Battikh et al., 2012; Sreeramulu et al., 2001; Kumar & Joshi, 2016), higher antioxidant activities than tea associated with the presence of metabolic enzymes and D-saccharic acid-1,4-lactone (DSL) (Jayabalan et al., 2014; Leal et al., 2018), and hepatoprotective properties (Abshenas et al., 2011; Wang et al., 2013). Not to mention, kombucha has also been claimed to have antiproliferative activities against several cancer cell lines, including colon cancer cells, by inducing cancer cell apoptosis or downregulating the angiogenic factors (Cetojevic-Simin et al., 2008; Srihari et al., 2013; Deghrigue et al., 2013; Dehnavi et al., 2020).

As there is no standardized methodology and microbial composition regulation on kombucha production, bioactive compounds may differ between different kombucha preparations. More comprehensive research on diverse microbial species of kombucha is required to specify bioactive compounds in kombucha culture. One of the most identified species in kombucha, bacteria *Komagataeibacter intermedius* and yeast from genus *Dekkera* have been known to contribute in cellulose expression and biofilm production (Santos et al., 2015; Joseph et al., 2007), but the metabolic products and how it benefits human health have not been characterized yet. Therefore, this research aims to evaluate the chemical characteristics as well as antiproliferative activity on HT-29 colon cancer cells from kombucha prepared by using two different culture conditions, single bacteria *K. intermedius*, and mix culture of bacteria *K. intermedius* and yeast *Dekkera bruxellensis*.

1.2. Research Objectives

The objectives of this research are set as follows:

- To prepare kombucha tea using two different culture conditions, single bacteria *K. intermedius*, and mix culture of bacteria *K. intermedius* and yeast *D. bruxellensis*.
- To evaluate chemical characteristics (pH, total acid, total polyphenol and flavonoids content, total reducing sugar, and scavenging rate) of fermented kombucha solutions prepared using two different culture conditions.

- To investigate the antiproliferative activity of kombucha tea (single culture of *K. intermedius* and mix culture of *K. intermedius* and *D. bruxellensis*) against human colon adenocarcinoma cells (HT-29).

1.3. Scope of Work

The scope of works of this research are:

- The culture used to prepare kombucha in this study are single bacteria *K. intermedius* and a combination of bacteria *K. intermedius* and yeast *D. bruxellensis* in black tea. The fermentation is performed under room temperature for 14 days.
- The kombucha tea prepared using two different culture conditions is harvested on day 0, 1, 2, 4, 6, 8, 12, and 14 of fermentation to analyze their biological and chemical characteristics.
- Several biological and chemical characteristics are analyzed, including:
    - pH analysis using a pH meter
    - Growth density determination using a spectrophotometer
    - Total acid determination by measuring the amount of NaOH titrated
    - Total polyphenols determination using Folin-Ciocalteau method
    - Total flavonoids determination using aluminum chloride ($AlCl_3$) method
    - Antioxidant activity (scavenging rate) using 2,2-diphenyl-1-picryl- hydrazyl-hydrate (DPPH) assay
    - Reducing sugar analysis using 3,5-dinitrosalicylic acid (DNS) assay
- The antiproliferative activity of kombucha cultures against HT-29 cells is assessed by using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Human embryonic kidney cells (HEK293T) is used as control.
- DNA fragmentation assay is performed to distinguish between apoptosis- and necrosis-related cell death.