I. INTRODUCTION

1.1 Background

Microbial pathogens in food cause foodborne illnesses and poisoning. These incidences have only worsened as bacteria have developed resistance against chemical preservatives like benzoic and sorbic acids (Liu et al., 2017). Chemical preservatives also present drawbacks due to their reported detrimental effects on human health, primarily the carcinogenic and teratogenic impacts of chemical synthetic preservatives (Tshabalala et al., 2021). As an alternative, natural substitutes like spices for their uses as preservatives are explored. Natural preservatives, particularly spices such as clove, are favored because they are rich sources of phytochemicals and bioactive compounds derived from their plant origins. These bioactive compounds not only excel at inhibiting microbial growth, but also have anti-inflammatory, anti-hypertensive, and antioxidant properties for the human body (Tshabalala et al., 2021).

Clove (*Syzgium aromaticum)* has been researched for its antimicrobial activities against foodborne pathogens owed to its main active component, eugenol. Eugenol, rich in antioxidants such as flavonoids and other phenolic compounds, has antioxidant activities against hydroxyl radicals and acts as an iron chelator (Batiha et al., 2020; Wani et al., 2022). Other compounds in clove responsible for the antioxidant activity are eugenol acetate and β-caryophyllene. Clove has been classified as the best of 100 dietary sources in terms of polyphenolic and antioxidant compound content, making it a potential natural antioxidant (Pérez-Jiménez et al., 2010).

Clove extracts have been proven to have antimicrobial activity against food pathogens like *Salmonella enterica, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis,*

Escherichia coli, and *Listeria monocytogenes* (Liu et al., 2017; Wani et al., 202). Its mechanism of action is by destroying cell walls and membranes of microorganisms and permeating the cytoplasmic membranes or by entry to the cells. Following this, it can then inhibit the normal synthesis of DNA and proteins. Furthermore, clove solvent extracts were found to be most effective in comparison to 12 other spices against microbes like *Pseudomonas aerogomonas* and *Escherichia coli* (Keskin & Toroglu, 2011). Following other studies (Alcantara et al., 2020; Gonelimali et al., 2018; Rosario et al., 2021; Tekin et al., 2015), the effectiveness of clove extracts against microbes can be determined using techniques such as agar well diffusion, and determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

The antimicrobial and antioxidant activity of clove extracts is further enhanced by ultrasound-assisted extraction (UAE) using ultrasonic treatment. The extracts obtained using this method have demonstrated more potent and high efficiency against both Gram-positive and Gram-negative microbes (Mehta et al., 2022). UAE is based on the principle of acoustic cavitation and stands out as a sustainable method requiring less energy and solvent. It is also safe, economical, and easily reproducible. Efficient UAE depends on several factors, such as amplitude, temperature, time, solvent: sample ratio, and solvent concentration. According to Medina-Torres et al. (2017), the solute/solvent ratio is one of the most critical factors during mass transfer.

Subsequently, the antioxidant activity and content of clove extracts can be measured and quantified using analyses such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), total phenolic content (TPC) and total flavonoid content (TFC). While several studies have reported better antimicrobial and antioxidant activity for plant material extracted using UAE, fewer studies have reported results in terms of optimizing varying plant concentrations (Farahani et al.,

2021; Frohlich et al., 2022; Irfan et al., 2022; Rosarior et al., 2021). Hence, cloves have been found to be rich in flavonoids and other antioxidants when extracted using solvents, treatments, and plant concentrations (Adaramola & Onigbinde, 2016; Rosarior et al., 2021).

Several different plant concentrations have been studied using UAE to establish the optimum condition. In cloves, a ratio of 1:20 g/mL (plant concentration 5%) has been found to be most effective by Tekin et al. (2015). A review conducted by Medina-Torris et al. (2017) has found that a ratio of 1:40 g/mL is the most optimum when it comes to the UAE. At or beyond 1:20 g/mL, the yield is found to decrease (Keshavarz & Rezaei, 2020). Hence, this research aims to determine the plant concentration and consequently, the effect of these different plant concentrations on the phenolic content, flavonoid content, antioxidant activity, and antimicrobial activity of ethanolic clove extracts when extracted using UAE.

1.2 Research aim

1) To determine the effect of plant concentration on extraction yield.
2) To evaluate the antimicrobial properties of different concentrations of ethanolic clove extracts using well diffusion, minimum inhibitory concentration and minimum bactericidal concentration (MIC/MBC), on *E. coli* and *S. aureus.*
3) To evaluate the antioxidant properties of different concentrations of ethanolic clove extracts using DPPH assay.
4) To evaluate the TPC and TFC of different concentrations of ethanolic clove extract.

1.3 Research Scope

In this study, the antioxidant and antimicrobial properties of ultrasound-treated clove extracts were evaluated. Cloves were extracted at three different concentrations, namely 1:40, 1:20, 1:10. The extraction yield was calculated to determine the optimum extraction method and concentration. Following this, the total phenolic (TPC) and total flavonoid (TFC) content was calculated using colorimetric methods to quantify the phenolic and flavonoid content of the clove extracts. In addition, the antioxidant activity of the clove extracts was evaluated using the DPPH assay. Lastly, antimicrobial assays were performed using the well diffusion method, as well as the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined.

1.4 Hypotheses

H_0 : The different plant concentrations in UAE do not show any significant difference on the extraction yield, antioxidant and antimicrobial activity, and phenolic and flavonoid content of ethanolic clove extracts.

1) H_1: There are significant differences in extraction yield among different plant concentrations.
2) H_1 : There is a significant difference in the antimicrobial activity among different plant concentrations.
3) H_1 : There are significant differences in antioxidant activity among different plant concentrations.
4) H_1 : There are significant differences in the phenolic and flavonoid content among plant concentration.