1. INTRODUCTION

1.1. Background

Nowadays, scientific practices demand more environmental friendly methods to achieve sustainability. To realize it, Green Chemistry and Green Analytical Chemistry (GAC) have become the key focus on conducting scientific experiments, even outside the field of environmental science. This applies to food analysis as well, where waste produced by the experiments could pose threats to the environment. The major principles of green chemistry were established in 1998 by Anastas and Warner. Even though until now there have been a few updates on the principles, these 12 original principles are still relevant until now:

1. Prevention: It is better to prevent waste than to clean or treat them once it has been created
2. Atom economy: Synthetic methods should be designed to maximize the utilization of all materials in the process to create a final product
3. Less hazardous chemical syntheses: Whenever possible, synthetic methods should be designed to use and generate substances that has little or no toxicity towards human health and environment
4. Designing safer chemicals: chemical products should be designed to affect the desired function while minimizing toxicity
5. Safer solvent and auxiliaries: The usage of auxiliary substances (e.g., solvents) should not be used whenever possible and harmless when used
6. Design for energy efficiency: Energy requirements of chemical processes should be minimized. Whenever possible, synthetic methods should be conducted at ambient temperature and pressure
7. Use of renewable feedstock: Raw materials of feedstock should come from renewable sources whenever practicable
8. Reduce derivatives: Unnecessary derivatization should be minimized or avoided since those steps require additional reagents and generate more waste
9. Catalysis: Catalytic reagents are superior to stoichiometric reagents

10. Design for degradation: Chemical products should be designed to be broken down into harmless substances at the end of their function and the degraded products do not linger in the environment
11. Real-time analysis for pollution prevention: Analytical methods need to be developed to allow real-time , in-process monitoring and control prior to the formation of hazardous substances
12. Inherently safer chemistry for accident prevention: Substances used in chemical processes should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

(Anastas & Warner, 1998)

Out of all usages of green chemistry, extraction of essential oils from plants using novel technology is considered important so as to minimize damages to the phytochemicals in the plants.In analytical science, while it is desirable to directly measure and analyze the samples directly without altering the samples in any way. However, this is almost impossible to achieve as there would be some pretreatments, such as centrifugation, filtration, extraction, concentration, etc. given to the samples prior to analysis. These aforementioned steps would be the most challenging steps to be made environmentally friendly as they involve some reagents which may be toxic to the environment (Armenta, *et al.*, 2015).

Essential oils can be defined as *volatile scented oil of direct biological origins*. Some authors even describe essential oils as *any oily and aromatic volatile liquid that can be harvested from any part of a plant*. Essential oils usually consist of mostly hydrocarbons with some functional components. These functional components vary in number depending on the type of essential oil itself. Some oils can have up to 300 different functional compounds while most oils are found to contain only 20-30 functional compounds. While some animals and microorganisms can produce essential oils, most essential oils are produced by plants and these oils are given names based on the plant from which the oil originated from, such as betel leaf oil, mint oil, rose oil, etc. Based on the quality of the aroma, essential oils can be divided into different categories such as earthy, minty, floral, spicy, herbaceous, citrus, etc. When grouped based on the rate of evaporation, essential oils can be categorized as top note (1-2 hours), middle note (2-4 hours), and base note (a few days) (Guha & Nandi, 2019).

Free radical species are side products of biological metabolism which damage body cells. Damages on body cells will cause body organs to malfunction and ultimately cause disease such as heart disease, inflammation, cancer, etc. (Fazal *et al.*, 2013). Plant phytochemicals gained attention potent antioxidants and they are recognized for their health boosting effects. Polyphenols have a lot of contributions towards cellular processes in the body. The phenolic compounds in the plant have antioxidant properties which can neutralize free radical species. For example, catechin is a phenolic compound that has several functions such as: increasing lipid oxidation and sensitivity of insulin (Kim *et al.*, 2007), neutralizing LDL (low-density lipoprotein) oxidation, thus preserving HDL (high-density lipoprotein) while at the same time removing LDL from the body. For more information, polyphenols can act as anticancer, anti-mutagenic and anti-inflammation.

Lately, the use of novel technology to extract the phytochemicals more efficiently have been developed. Some examples are microwave-assisted extraction (MAE), enzyme-assisted extraction (Li *et al.*, 2006), ultrasound-assisted extraction, and many other methods. These techniques can help preserving polyphenols and at the same time enable commercialization due to cost-lowering process which can be applied in industrial scale (Chavan & Singhal, 2013). Compared to MAE, Ultrasound-assisted extraction (UAE) is considered cheaper and it has fewer limitations such as the choice of solvent used. Solvent used in MAE has to be transparent to allow the microwave to penetrate through the solvent while in UAE, such limitation does not exist (Setyaningsih *et al*, 2019). In the past, the efficiency of extraction process was often low because of the high extraction temperature or long extraction time which may cause degradation to the phytochemicals. Nowadays, with ultrasound-assisted extraction, extraction efficiency can be improved, thus, more phytochemicals can be extracted out of a plant as compared to the previous extraction methods.

1.2. Literature Studies

1.2.1. Betel Leaves

Piper betel Linn (Betel vine) is an important remedial plant in Southeast Asia. This plant belongs to *Piperaceae* family and is cultivated widely in India, Nepal, Bangladesh, Burma, and Sri Lanka. Measuring 10-18 cm long and 5-10 cm wide, the leaves of *Piper betel* has numerous therapeutic effects. Due to that reason, betel leaves have been widely investigated for its contents, mainly the phytochemicals in the leaves. The spicy odour from the leaves are due to the phenol and terpens content in the leaves. Besides those two substances, table 1 shows the phytochemicals found in betel leaves and their pharmacological actions.

Table 1. Phytochemicals in betel leaves and their pharmacological actions

Phytochemical	**Molecular Formula**	**Pharmacological Actions**
1,8-cineol	$C_{10}H_{18}O$	Treatment of inflammatory diseases.
α-cadinene	$C_{15}H_{24}$	Anticancer activity.
α-humulene	$C_{15}H_{24}$	Anti-inflammatory, effective in reducing platelet activating factor.
Allylpyrocatechol diacetate	$C_{13}H_{14}O_4$	Antimicrobial activity against various obligate oral anaerobes.
Aromadendrene	$C_{15}H_{24}$	Antioxidant and anti-ageing.
β-elemene	$C_{15}H_{24}$	Anti-proliferative effect, used in chemotherapy for cancer treatment.
β-selinene	$C_{15}H_{24}$	Antibacterial properties.
Caryophyllene	$C_{15}H_{24}$	Antioxidant, anti-inflammatory, anticancer, and local anaesthetic.
Eugenol	$C_{10}H_{12}O_2$	Antiseptic and local anaesthetic, in dentistry.
Eugenyl acetate	$C_{12}H_{14}O_3$	Anti-virulence potential.
Germacrene D	$C_{15}H_{24}$	Analgesic and anti-inflammatory properties.
Globulol	$C_{15}H_{26}O$	Antimicrobial activity.
Hydroxychavicol	$C_9H_{10}O_2$	Antimutagenic activity.
Sabinene	$C_{10}H_{16}$	Antimicrobial activity.
α-Pinene	$C_{10}H_{16}$	Anti-inflammatory and antimicrobial.

(Kudva, *et al.*, 2018)

Polyphenolic compounds belong to one of the six groups of important bioactive groups in phytochemicals. Polyphenolic compounds are characterized by the benzene ring with one or more hydroxyl groups attached to it. Production of polyphenolic compounds starts with phenylalanine as a precursor progressing through 3 biosynthetic routes: 1) succinylbenzoate pathway, 2) acetate/malonate pathway, 3) acetate/melavonate pathway (Bhattacharya *et al.*, 2010). Polyphenolic compounds can be found in the form of flavonoids, tannins, phenolic acids, lignans, stilbenes, and coumarines (Holland *et al.*, 2017).

Phenolic compounds in betel leaves contribute to its antimicrobial activity. According to Ali *et al.*, 2018, in a fundamental study of *piper betel*'s antimicrobial activity, a toothpaste which is enriched with *piper betel* extract exhibit larger inhibition zone as compared to toothpaste with triclosan and fluoride alone. Extraction of polyphenol must be done at a lower temperature as polyphenol in plants degrade at high temperature. Depending on the type of the plants, thermal treatment on samples may degrade polyphenol or may increase the content of polyphenol (Sikora & Borczak, 2014).

1.2.2. Ultrasound-assisted Extraction

Application of ultrasound wave for food processing is vast. Ultrasound is described as a soundwave which operates at frequency of 16 kHz or higher. Normally, extraction from natural products requires hours of extraction process using conventional solid-liquid extraction methods, however with the application of ultrasound wave, food extraction can be done within minutes. This is due to ultrasound effects which disrupt cell wall and assist mass transfer. As far as green extraction theory goes, UAE also comes with other benefits such as reduced amount of solvent being used and safer, non-toxic solvents can be used.

Ultrasound wave can be further divided based on the energy used to induce the vibrational motion of medium molecules. Low-power ultrasound (LPU) will induce high frequency wave (>100 kHz) while high-power ultrasound (HPU) will induce low frequency wave (16/20-100 kHz). LPU is usually used in non-invasive analysis while HPU is more often used in industrial processes, including the ones being done in laboratory. HPU creates cavitation phenomenon. This is achieved through generation of microbubbles which disrupt biological cell walls and help solvent reach food components to be extracted. Ultrasound-assisted extraction can be done by employing an ultrasound bath or a probe (Lavilla & Bendicho, 2017)

a. Extraction Mechanism

Ultrasound's output source is none other than a vibrating body. This is when the surrounding medium vibrates to the ultrasound wave and transmits energy to the neighboring particles. The major parameters which play essential roles in ultrasound extraction process are ultrasound power, frequency, and amplitude. There are 3 ways of expressing the energy level at which an ultrasound propagates through a medium, namely ultrasound power (Watt), intensity (Watt/cm^2), and acoustic energy density (Watt/cm^3) (Esclapez *et al.*, 2011). Ultrasound wave can cause cavitation, mixing, crushing, vibration, etc. in the medium it is propagating on. For extraction process, the effective frequency used ranges from 20kHz to 50 kHz (Wen *et al.*, 2018).

It is generally believed that in UAE, cavitation, thermal and mechanical effects play important roles. These three effects lead to destruction of cell wall, reduction in particle size, and an increase in mass transfer rate without changing the structure and functions of the solutes (Ashokkumar, 2015). The cavitation effect arises from negative pressure when a liquid with certain critical value can form gas cavity in the local domain. This critical negative pressure where liquid forms gas bubbles is called cavitation threshold. These negative pressure may facilitate cavitation by creating many small bubbles called 'cavitation nuclei' (Ohl & Wolfrum, 2003). As ultrasound wave propagates through medium, a series of alternating pressure (negative and positive) compress and expand the liquid medium. This compression and rarefaction cycle creates a phenomenon called 'stable cavitation' where the cavitation bubbles vary with the frequency of the wave. As the extraction process proceeds, the bubbles grow in size until it reaches a critical value with a high temperature (5000 K) and high pressure (100 MPa) generated around cavitation zone. Due to the high temperature and pressure caused at the moment of the collapse, shear forces and turbulence are produced; this cavitation process is called 'transient cavitation'. The heat and volume generated, though large in values, are not sufficient to affect macroscopic system due to the small size of the parameters, yet they are still able to affect cell structure and facilitate in mass transfer (Brennen, 1995).

1.2.3. Application of Ultrasound-assisted Extraction on Food

Application of ultrasound wave in food industry is vast and one of them is extraction of bioactive compounds. A study conducted by Ferarsa *et al*., 2018 showed that there is a significant increase in the recovery of anthocyanin and phenolic compounds from eggplant. In this study, it was found out that ground eggplant peel subjected to 75°C, pH 2 for 60 minutes gave a total phenolic content 23.101 mg GAE/g DM. The same sample, when subjected to ultrasound wave for 30 minutes using acidified water as solvent, yielded 29.011 mg GAE/g DM or total phenolic content. Same effect of ultrasound wave on recovery yield of bioactive compound is observed on extraction of anthocyanin.

An optimization study with the use of response surface methodology was done on ultrasound-assisted extraction of arecanut (betel nut). In this research, three parameters were studied, namely, sonication power, duty cycle, and extraction time. In this study, polyphenols were to be separated from the alkaloid of arecanut, arecoline. The result of the study showed that maximum yield of total polyphenols (362.59 mg GAE/g DM), total tannins (89.24 mg GAE/g DM) and antioxidant activity by FRAP method (1039.11 mM TEAC) with minimum amount of arecoline (3.12 mg/g) was achieved using sonication power at 30W, duty cycle of 50%, and extraction time of 50 minutes (Chavan & Singhal, 2013). In a similar study Irakli *et al.*, 2018 conducted an experiment to find the optimal treatments for ultrasound-assisted extraction of phenolic compounds from olive leaves. It was found out that the highest yield of TPC and flavonoid was obtained when 50% acetone as solvent was used and the UAE was done for 10 minutes at 60°C.

1.3. Objectives

To find the optimal extraction parameters of betel leaves ultrasound-assisted extraction, encompassing extraction time, ultrasound bath power, and extraction temperature, on total phenolic compounds in the leaf extract.