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A Systematic Review: Optimization of Extracts *Psidium Guajava* L. By Using Ultrasound-Assisted with Soxhlet Extraction in Application of Food Preservation

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Abstract: Natural antimicrobial compounds extracted from medicinal plants has brought a lot of attention to researchers worldwide since synthetic antimicrobial agents can lead to health issues. Natural antimicrobials from extracted plant has characteristic to inhibit the growth of pathogenic microorganism that can cause diseases. Even so, the yield of natural antimicrobial agent depend on the employment of suitable extraction technique. In this systematic review, ultrasound assisted with Soxhlet extraction conducted on *Psidium guajava* L. was studied. Highest extraction efficiency obtained by using combination of ultrasound assisted with Soxhlet extraction in which utilised by 95.00 % of ethanol and highest concentration of quercetin produce in fresh leaves were found in this research with (40.96±1.29) % and (63.90±8.50) %, respectively. Therefore, the revised outcome of this systematic analysis give possible value of *Psidium guajava* L. as an antimicrobial agent which can be applied in food processing. Based on four databases implemented in this research (PubMED, Tandfonline, Google Scholar and Science Direct), the search strategy was built with the search terms ("*Psidium guajava* L.", "antimicrobial activity", "ultrasound-assisted extraction", and "Soxhlet extraction"). The unified data was analysed using PRISMA rules, and 504 papers were separated after the identical articles were eliminated. 401 papers were eventually excluded when they did not match the inclusion requirements and 103 articles were re-evaluated for eligibility again. Optimum condition for ultrasound-assisted with Soxhlet extraction were evaluated by varying ultrasound-assisted extraction temperature and time and also altering the temperature of Soxhlet extraction. The findings of this systematic review has shown that the optimum condition of extracts *Psidium guajava* L. is at 60 °C of sonication temperature and 35 minutes of extraction time. Whereas the extraction that undergoes in Soxhlet will be at 45 °C. Best solvents between methanol and ethanol which applied in Soxhlet extraction were studied. Thus, according to the systematic study, ethanol was chosen as the suitable solvent in extraction of *Psidium guajava* L. due to high production of total phenolic compound was produce

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(40.50±0.88) mg GAE/g. In short, ultrasound assisted with Soxhlet extraction can enhance the production of antimicrobial activity from extracts of *Psidium guajava* L.

Keywords: *Psidium guajava L.*, Antimicrobial activity, Ultrasound-assisted extraction, Soxhlet extraction, Minimum Inhibitory Concentration

1. Introduction

Currently, most researchers are aware about the benefit of modern therapeutic agent which derived from medicinal plant extracts in Malaysia. Over the past decades, herb plants have drawn great attention medicinal practitioners in seeking novel pharmaceutical agents [1]. Malaysia is one of the country in Asia that is popular with application of traditional medicine practices to cure various diseases. According to the World Health Organization, most of the world population depends on medicinal herbs as their primary medicines. Phytochemical compounds from plant-derived are currently being applied as a single structure medical agents to cure and enhance health of the people. Malaysia have more than 1300 medicinal plant species and their therapeutic properties have become a great attention to the development of the country for various field such as pharmaceutical, food industry, and textiles. Example of medicinal plants that growth rapidly in Malaysia are *Psidium guajava* L. (Guava leaves), *Carica papaya* (Papaya), *Zingiber officinale Rosc*. (Ginger) and etc. [2]

Malaysia consist of various biodiversity where medicinal plant materials were commonly applied in traditional uses for medication and food preservation due to the antimicrobials characteristic that derived from the medicinal plants. Antimicrobial compounds obtained from plant extracts are able to inhibit the growth of microorganism and thus Malaysia have been enlisted as one of the country that can further develop the studies in antimicrobial activity from medicinal plant extracts as many medicinal herbs are grow in Malaysia [3]. Previous study showed that wide application of herbal plants have been used due to its antimicrobial property that can cease the percentage of drug-resistance pathogens [3]

According to [4], the significance of *Psidium guajava* L. has been scientifically discovered and is also play important role in medicinal field. The properties of antispasmodic and antimicrobial activities from *Psidium guajava* L. are being investigated to be used in the treatment of diarrhea and dysentery [5]. High concentration of flavonoids have been found in *Psidium guajava* L. as this compound does consist of antimicrobial and antioxidant properties. *Psidium guajava* L. is used in this study because this plant is easy to obtain and widely available in large quantities. This plant herb is suitable to grow naturally in Malaysia forest. Therefore, the potential of antimicrobial activity and total flavonoid contents from *Psidium guajava* L. extracts are studied.

Extraction method using ultrasound- assisted Soxhlet extraction is studied to obtain desired plant materials. Ultrasound was applied to reduce the number of cycle that required by conventional Soxhlet extraction to complete the process. Ultrasound-assisted Soxhlet extraction is an advanced method for extraction technique to replace conventional Soxhlet extraction due to the application of high frequency pulse to produce local hotspots associated with high shear stress and temperature [6]. The use of ultrasound in ultrasound-assisted Soxhlet application is to travel the waves through the solvent which generates cavitational bubbles in which this principle is capable to damage the cell walls of the plant and thereby facilitate releasing of bioactive compound [7]. In addition, the application of this plant extracts is tested on raw chicken meat in order to scientifically proved the presence of antimicrobial activity and flavonoid contents in *Psidium guajava* L.

The purpose of this research studies is to show existing scientific data on optimization of extracts *Psidium guajava* L. by using combination of ultrasound-assisted extraction for food preservation. At the end of this study, all findings were summarized and tabulated to relate the research discovery with main aims of the studies.

Synthetic preservatives have been used to overcome this problem as it can enhance shelf-life of food. However, due to the ingredients used in synthetic preservatives might give negative effect to human health, thus consumer demand another approach that safer to consume. Due to this issue, the researchers have been investigated the use of natural antimicrobial agent that derived from plant herbs [8]. SLR (systematic literature review) was implemented in this research study in order to identify and evaluate natural antimicrobial agent in *Psidium guajava* L. Based on the studies, natural agent from plant herbs provide the ability to increase shelf-life of food and enhance food quality [9].

From previous study, conventional Soxhlet extraction is commonly used in extraction of bio-active compound, but this method required some limited considerations. Soxhlet extraction is time consuming and the sample also exposed to a high temperature for a period of time that can cause thermal destruction of bioactive compound. Extraction method of ultrasound-assisted with Soxhlet extraction was reviewed in this SLR for the purpose to find optimum conditions for extraction of *Psidium guajava* L., thus enhance its extraction rate.

2. Materials and Methods

Illustration of the procedure for the search study and selection process is shown in Figure 1. A systematic review is implemented for the review study on optimization extracts of *Psidium guajava* L.by using ultrasound-assisted with Soxhlet extraction in application of food preservation according to PRISMA. According to [10], five stages in methodology were suggested to guide the review design, which are search strategy, inclusion criteria, exclusion criteria, study selection and data organization and reporting.

2.1 Search strategy

The searching process was conducted based on four objectives to recover relevant articles from databases namely Pubmed, Tandfonline and Google Scholar. Search methods have been tailored to accommodate the topic heading and keywords of each database. The search terms include "*Psidium guajava* L." and "antimicrobial activity", "*Psidium guajava* L." and "ultrasound-assisted extraction", "Soxhlet extraction" and "*Psidium guajava* L.", and "raw meat" and "*Psidium guajava* L.". At first stage which is in identification, the potential articles obtained from PubMED, Tandfonline and Google Scholar were 778. Then, during the screening process 504 of articles were removed.

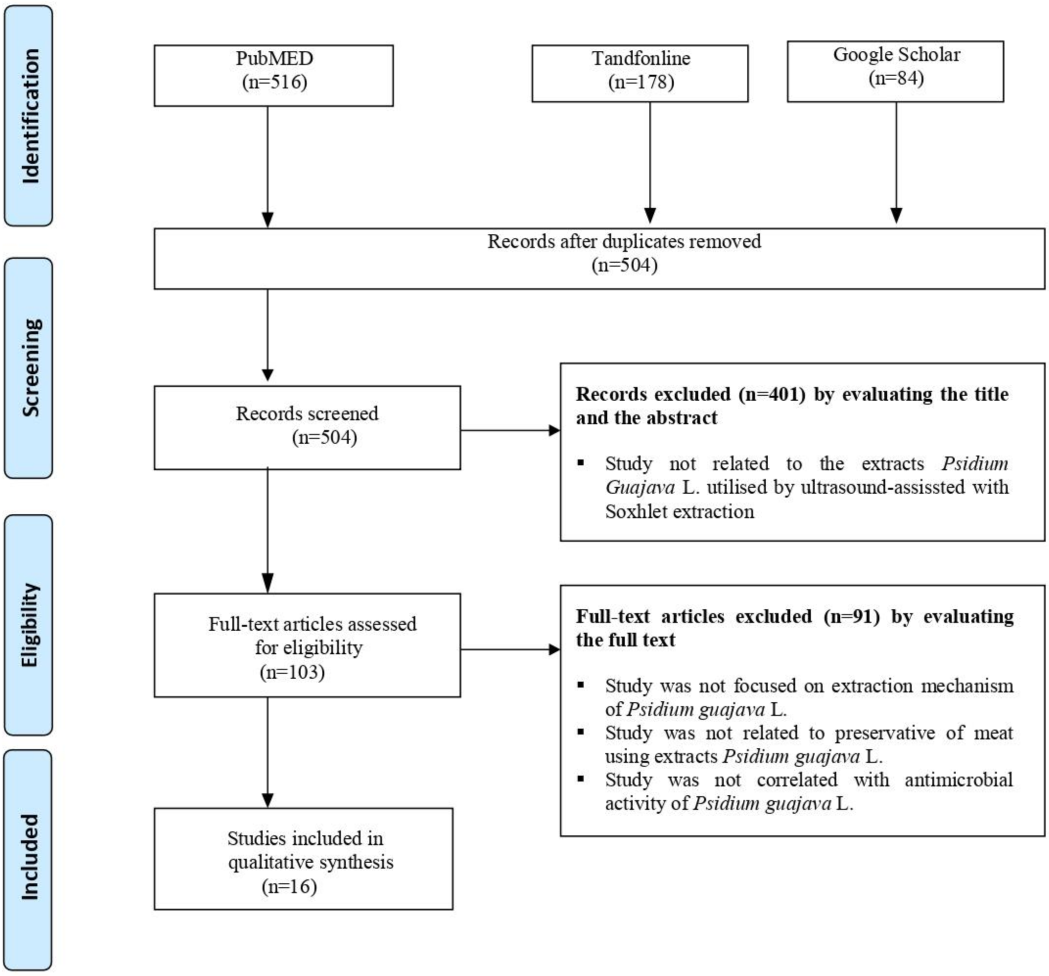

Figure 1: Flowchart of search strategy and selection process [10]

2.2 Inclusion criteria

The inclusion criteria was defined as follows: content of the selected paper, publication of the timeline and language. A timeline from 2000 to 2020 was chosen since it is compatible with the definition of the maturity of analysis without diminishing the merit of publishing before 2000 [11]. In addition, during this timeline release, major paper such as [12], [13], [14], [15] and [16] consist of a significant methodological principles of combination ultrasound-assisted with Soxhlet extraction that should be considered inthis studies. During this timeline, the number of related publications is higher as more important issues are studied and more evidence-driven subjects were investigated. This study covered the extraction method ultrasound-assisted with Soxhlet extraction that explored the antimicrobial activity of *Psidium guajava* L. From the databases, this analysis included that *Psidium guajava* L. has the properties of antimicrobial activity that can be used as food preservation in food processing. The articles published in English have been taken into account. The importance of choosing publications in language that easily to understand can reduce complexity, increase burdens of the study and also time consuming [17].

2.3 Exclusion criteria

Next, by reviewing titles and abstracts as well as findings and methodology pages can independently screened relevant articles. The research omitted literature reviews on antimicrobial activity and phenolic compound from other plant species. Whereas the analysis from research on the antioxidant of *Psidium guajava* L. or bioactive chemical components of guava were eliminated in this review. Moreover, study that were not related to the extracts *Psidium guajava* L. in which utilized by ultrasound-assisted with Soxhlet extraction were also excluded.

2.4 Study selection

The prime literature scan was executed by authors. Initially, all duplicate articles were filtered out by a computer and followed by hand scan to ensure that no duplicate articles were present. By screening the title, abstract, and recovery of the full article from the database scan, possible related papers were selected. Subsequently, due to inclusion and exclusion requirements, the following irrelevant papers were dismissed. Then, full-text papers were downloaded and checked for eligibility. At this stage, the study that were not correlated with antimicrobial activity on raw meat were excluded. Thus, total of papers that being eliminated in eligibility step were 91 article.

2.5 Data organization and reporting

The author separately evaluated the characteristics of the remaining posts which mainly focus on the abstract, process and key outcomes. At this point, author evaluated the papers qualitatively by categorizing them into three categories which were poor, moderate, or high quality. If the articles achieved high quality categories, it will be included in qualitative synthesis. This report only collected data from selected studies that meet the objectives. In particular, the information which collected from each analysis was organized according to details on the name of the author, year of publication, and parameters involved. The inclusion studies were documented according to PRISMA guidance. On top of that, the author also implemented standard deviation technique that was conducted in Excel for the calculation of variation within data set where the command to obtain standard deviation for the population is = STDEVP [18].

3. Results and Discussion

This chapter addressed the results of the systematic review study which focused on the research subject, based on methodology outlined in this section. All 16 findings were reported and further research was outlined in Tables 1-8.

3.1 Comparison of extraction efficiency for extracted *Psidium guajava* L. based on ultrasound-assisted with Soxhlet extraction

The studies of extraction efficiency for extracted *Psidium guajava* L. have produced a wide range of extraction yields efficiency for combination of ultrasound-assisted with Soxhlet extraction that utilized by different types of solvent [19] [14] [20] [21] All results for extraction efficiency shows a huge increment after undergoes Soxhlet treatment for this combination method as the ultrasound Soxhlet extraction give higher yield under milder extraction conditions [22]. According to the studies conducted by [23], the highest percentage of extraction efficiency was obtained when 95.00 % ethanol was used as solvent (40.96±1.29) %, while for 95.00 % of methanol gave (17.30±1.20) %. Meanwhile the lowest extraction efficiency among all 95.00 % ethanol for ultrasound-assisted extraction was achieved at 6.92±0.57 %. But, once the sample continue to be extracted by Soxhlet, the efficiency percentage rise to 21.00±1.00 % which almost fourth times higher than first extraction stage and it has been proved through the studies by [21]. Extraction of *Psidium guajava* L. that utilised by ultrasound-assisted extraction was chosen to be combined with conventional Soxhlet due to its characteristic that can easily integrated with other extraction technique in order to enhance the extraction efficiency and this finding was also demonstrated in the studies done by [24]. This finding shows that extraction technique and extraction efficiency of *Psidium guajava* L. extracts were interconnected. From the study, this combination method was suitable to be applied in extraction of *Psidium guajava* L. due to the capability of ultrasound-assisted with Soxhlet extraction that exhibit an enhancement in extraction efficiency.

3.2 Optimization of combination ultrasound-assisted with Soxhlet extraction

The optimum condition of combination ultrasound-assisted with Soxhlet extraction for extracts *Psidium guajava* L. was analysed in this extensive SLR analysis. The optimization of sonication temperature and frequency were further investigated in this section. The best solvent used in Soxhlet extraction was explored by evaluating the effects of methanol and ethanol to extracts *Psidium guajava* L.

3.2.1. Effect of extraction temperature of ultrasound-assisted extraction on polyphenol yield from *Psidium guajava* L.

From the studies done by [16] [25] the performance effect of extraction temperature show highest flavonoids contents that were extracted from *Psidium guaja* L. was obtained at 60 °C. While according to the research conducted by [15], the maximum yield of phenolic compound was observed at 80 °C. This could be caused by increasing in electrical acoustic intensity that was produce during the extraction where it is able to slightly increase the extraction yield. Although, low extraction temperature only give small effect extraction yield, but too high of temperature also could lead to decrease in yield of phenolic compounds due to the degradation of some thermolabile. Because of the sono-chemical impact on the molecular and microstructure of the cell wall, the extraction process by ultrasound-assisted extraction has been enhanced. High sonication temperature provides the mechanical effect that result in cell wall destruction and gives greater solvent penetration into the cellular materials [26]. Based on the comprehensive SLR analysis on the optimum temperature of ultrasound-assisted extraction, temperature of 60 °C is chosen as the ideal condition to extract guava leaves due the highest yield of bioactive compound that were accessed in studies done by [16] [25] The extraction temperature employed in this method was able to produce high yield because of the pulse of high frequency (20kHz) used in ultrasound-assisted extraction that produce local hotspots with high shear stress and temperature on a macroscopic scale by generating cavitation bubbles [27].

3.2.2. Effect of extraction time of ultrasound-assisted extraction on polyphenols yield from *Psidium guajava* L

Several researchers [16], [15], [23] reported that increasing extraction time could increase total phenolic yield. However, based on the studies demonstrated by [15], a notable drop of the total phenolic yield was observed as the extraction time was further increased to 45 min. It was assumed that extended of extraction time could lead to more oxygen exposure and rise the probability of oxidation of phenolic compounds [28]. Apart from that, at initial stage, the extraction yield can be improved as ethanol concentration was maintained and increased the extraction. Even so, further increase in ultrasonic time did not indicate any improvement in the overall phenolic content after more than 40 minutes [12]. In both studies of [15] and [16] proved that, the highest reading of total phenolic yield percentage was evaluated at 35 min of extraction time.

Extraction time of 35 minutes was selected as the optimum condition for extraction time in ultrasound-assisted extraction to produce high yield of phenolic compounds in *Psidium guajava* L. as it is compatible with finding of research done by [16] and [15]. The phenolic content and antimicrobial ability of plants extracts rely mostly on extraction compositions and circumstances. Generally, the findings show that the solvent extraction has had the most proclaim effect on the quality extracts, accompanied by the extraction duration and then by their interaction [29].

3.2.3. Effect of extraction temperature of Soxhlet extraction on polyphenols yield from *Psidium guajava* L.

The effect of extraction temperature of Soxhlet on polyphenols extraction yield from *Psidium guajava* L. with ethanol as solvent was analyzed in this research studies. Total phenolic compound was evaluated through this integrative studies in order to identify the optimum condition for Soxhlet extraction temperature. Based on the comprehensive SLR analysis, the highest yield was produced at temperature of 45±5 °C [16] followed by 40±5 °C [30] and 35±5 °C [15] with total phenolic compound of 176±10 mg GA/100 g, 172±10 mg GA/100 g and 136±2.54 mg GA/100 g respectively. As the temperature and time increased, the overall phenolic content also increased. Even so, according to [30] yield of total phenolic compound will be decreased as it was subjected to higher temperatures. This may be attributed to an increase in the rate of mass transfer at high temperatures, resulting in greater solubility in solvent [20].

Hence, the finding that can be concluded based on analysis of SLR research was the extraction temperature of 45±5 °C was assumed as optimum temperature for Soxhlet extraction in order to extracts *Psidium guajava* L. This temperature condition was chosen due to high yield of phenolic contents produced and it was proved by [16] Theoretically, plant tissues are mitigated under elevated tempertures and the weak connection damage the membrane of the cells [31]. Futhermore, the heat under the temperature of the degradation phenolic compounds may facilitate solute extraction by enhancing the cell walls penetrablility to the solvent as it lead to the increase of diffusion coefficients and decreasing the viscosity of the solvent during extraction [32].

3.2.4. Effect of different solvents in Soxhlet extraction on polyphenols extraction yield from *Psidium guajava* L.

The correlation between effects of methanol and ethanol as solvent used on extraction yield of polyphenols from *Psidium guajava* L. were evaluated in this research studies. Evaluation effect of these solvents to the yield of total phenolic compound was to determine the suitable solvent for *Psidium guajava* L. extracts which could give the highest extraction yield. The research study reported by [15], [33] and [13], state ethanol solution shows higher yield of total phenolic compounds when the extraction temperature was remained constant at 40±5 °C. According to [13], a significant difference for yield of total phenolic compound that used ethanol and methanol as extraction solvent with value of 120±2.39 and 90±2.07 mg GAE/g. Then, the yield of overall phenolic compound have 9 mg GAE/g difference between ethanol and methanol in which the value of the phenolic compound produced by these solvent were 40.50±0.88 mg GAE/g and 31.50±1.60 mg GAE/g respectively [33]

Ethanol was chosen as the best solvent to extract *Psidium guajava* L. due to high yield of total phenolic compound was produced. This finding is in line with the previous research by [13]. This was due to the characteristic of ethanol as a good solvent for polyphenols extraction and it was also safe to be consume by human. On top of that, its characteristic as a polar solvent and ability to break down water-soluble molecules make ethanol to be a good solvent compared to methanol [34].

3.3 Analysis of antimicrobial activity

Antimicrobial activity and total flavonoid contents obtained from extraction of *Psidium guajava* L. utilized by ultrasound-assisted extraction combined with Soxhlet were further discussed in this SLR research studies. Reactivity of Gram-positive bacteria and Gram-negative bacteria against extracts *Psidium gujava* L. were analyzed to identify antimicrobial activity guava leaves.

3.3.1. Minimum inhibitory concentrations (MICs)

Staphylococcus aureus (*S. aureus*) and *Escherichia coli* (*E. coli*) were some of the bacteria that could cause foodborne illness. In studies conducted by [35], [36] and [37], *E. coli* seems to have bigger measure of inhibition zone as Gram-negative bacteria has a resistant characteristic against antibiotic due to their impermeable cell wall structure. These type of bacteria composed of three layers that was structured in an envelope. The outer membrane (OM) of at first layer which act as a defensive and have special characteristic can differentiates Gram-negative bacteria from Gram-positive bacteria [38]. [39] also stated that Gram-positive bacteria is more susceptible to the antimicrobial agent in extract *Psidium guajava* L. The major source of resistance to a wide class of antibiotics is the outer membrane of Gram-negative bacteria. Any modification of Gram-negative bacteria in the outer membrane, such as modifying the hydrophobic properties or mutations in porins and other components, may produce resistance [39]. Minimum inhibitory concentrations (MICs) were classified as the lowest antimicrobial concentration that will slow down the growth of visible *S. aureus* and *E. coli*. A wide range of MIC between *S. aureus* and *E. coli* with concentration of 0.418 mg/ml and 250 mg/ml respectively were compared in the studies done by [37], [36], [35], E. coli exhibit higher MIC compared to *S. aureus* which means that more antimicrobial needed to inhibit growth of *E. coli* as this bacteria able to resist antimicrobial property in extracts *Psidium guajava* L. [37].

Table 1: Analysis of ultrasound-assisted extraction to extract *Psidium guajava* L

Authors and Year	Method	Solvents	Outcomes
Ida Madiha et al., (2017)	Combination of ultrasound-assisted with Soxhlet extraction	Methanol (95%)	Extraction of *Psidium guajava* L give 13.71% of extraction efficiency
Porwal et al., (2012)	Combination of ultrasound-assisted with Soxhlet extraction	Ethanol (95%)	Extraction of *Psidium guajava* L give 14.08% of extraction efficiency
Safdar et al., (2017)	Combination of ultrasound-assisted with Soxhlet extraction	Methanol (95%)	Extraction of *Psidium guajava* L give 13.3% of extraction efficiency
		Ethanol (95%)	Extraction of *Psidium guajava* L give 15.8% of extraction efficiency
Shekar et al., (2016)	Combination of ultrasound-assisted with Soxhlet extraction	Methanol (95%)	Extraction of *Psidium guajava* L give 11.3% of extraction efficiency
		Ethanol (95%)	Extraction of *Psidium guajava* L give 12.26% of extraction efficiency

Table 2: Effect of extraction temperature of ultrasound-assisted extraction

Authors and Year	Method	Extraction Temperature (°C)	Outcomes
Zeng et al., (2020)	Combination of ultrasound-assisted with Soxhlet extraction	20-40	▪ Increment of temperature resulting in increase of extraction yield
		30-60	▪ Highest flavonoid content was observed at 60 °C
		40-80	▪ Significant increase in temperature lower the extraction yield
		80-100	▪ Increase temperature to a maximum value at 100 °C, declined the production of phenolic compound
Li et al. (2019)	Combination of ultrasound-assisted with Soxhlet extraction	20-40	▪ Performed minimized effect in Anthocyanins yield
		30-60	▪ Increasing in temperature will enhance extraction yield
		40-80	▪ A maximum increase of phenolic compound was evaluated at 80 °C
		80-100	▪ A vast increment of temperature diminished the yield
Luo et al., (2018)	Combination of ultrasound-assisted with	20-60	▪ Highest extraction yield was obtained at 60 °C
		60-80	

	Soxhlet extraction	80-100	▪ Decreasing of the extraction yield due to increase of temperature ▪ A minor decrease in extraction yield was obtained at this temperature range

Table 3: Effect of extraction time of ultrasound-assisted extraction

Authors and Year	Method	Extraction Time (min)	Outcomes
Zeng et al. (2020)	Combination of ultrasound-assisted with Soxhlet extraction	15-25 35-45	▪ A significant increment of total phenolic yield was obtained during 15 – 25 min ▪ There was no significant high yield obtained, but the highest yield was produced at 35 min.
Li et al. (2019)	Combination of ultrasound-assisted with Soxhlet extraction	5-35 35-45	▪ The extraction yield was increased over the time ▪ A maximal extraction yield was observed at 35 min and depleted when the extraction time was further continue to 45 min.
Liu et al. (2014)	Combination of ultrasound-assisted with Soxhlet extraction	0-8 5-10	▪ The yield of total phenolic compound increase over the time and slightly decline at 8 min and above. ▪ Highest yield was obtained at 5 min.

Table 4: Effect of extraction temperature of Soxhlet extraction

Authors and Year	Method	Extraction Temperature (°C)	Outcomes
Zeng et al. (2020)	Combination of ultrasound-assisted with Soxhlet extraction	40±5	Extraction of *Psidium guajava* L. using ethanol produce 176±10 (mg GA/100g) of total phenolic compound
Li et al. (2019)	Combination of ultrasound-assisted with Soxhlet extraction	45±5	Extraction of *Psidium guajava* L. using ethanol produce 172±10 (mg GA/100g) of total phenolic compound
Castro-Vargas et al. (2010)	Combination of ultrasound-assisted with Soxhlet extraction	35±5	Extraction of *Psidium guajava* L. using ethanol produce 156±2.54 (mg GA/100g) of total phenolic compound

Table 5: Effect of different solvent of Soxhlet extraction

Authors and Year	Method	Types of Solvents	Outcomes
Li et al. (2019)	Combination of ultrasound-assisted with Soxhlet extraction	Methanol Ethanol	Extraction of *Psidium guajava* L. using methanol produce 90±2.07 (mg GA/100g) of total phenolic compound Extraction of *Psidium guajava* L. using ethanol produce 120±2.39 (mg GA/100g) of total phenolic compound

Do et al., (2014)	Combination of ultrasound-assisted with Soxhlet extraction	Methanol	Extraction of *Psidium guajava* L. using methanol produce 31.5±1.60 (mg GA/100g) of total phenolic compound
		Ethanol	Extraction of *Psidium guajava* L. using ethanol produce 40.5±0.88 (mg GA/100g) of total phenolic compound
Seo et al., (2014)	Combination of ultrasound-assisted with Soxhlet extraction	Methanol	Extraction of *Psidium guajava* L. using methanol produce 90±2.07 (mg GA/100g) of total phenolic compound
		Ethanol	Extraction of *Psidium guajava* L. using ethanol produce 120±2.39 (mg GA/100g) of total phenolic compound

Table 6: Antimicrobial studies on *Psidium guajava* L

Authors and Year	Method	Study Population	Outcomes
Munda et al. (2019)	Agar well diffusion and minimum inhibitory concentrations (MIC)	*S. aureus* and *E. coli*	The MIC value of *Psidium guajava* L. against *S. aureus* and *E. coli* ranged from 0.418-100 mg/ml and 1.974- 250 mg/ml respectively
Ara Farhana (2017)	Agar well diffusion and minimum inhibitory concentrations (MIC)	*S. aureus* and *E. coli*	Gram-positive bacteria is more susceptible to the tested of extract *Psidium guajava* L. compared to Gram-negative bacteria
Dhiman et al. (2011)	Agar well diffusion and minimum inhibitory concentrations (MIC)	*S. aureus* and *E. coli*	The inhibition zone at concentration of 3 (μg/ml) cannot be measured due to insufficient concentration of extract *Psidium guajava* L., while All tested samples are able to inhibit *S. aureus* and *E. coli*

3.4 pH values

The normal pH of meat can range from 5.2 to 5.7 [40]. From the study by [16], the pH values of the control sample and PGLE samples that were obtained started from days 0 to days 16 seem to rise from 5.84 ± 0.02 to 6.59 ± 0.02 and from 5.82 ± 0.03 to 5.68 ± 0.02 respectively. *Psidium guajava* L. extract (PGLE) on raw meat has a strong antibacterial activity because PGLE can function against pathogenic bacteria and indirectly extend shelf life of the meat The studies of PGLE on raw meat [16] showed increment of pH value for sample which treated by PGLE as it keep rising up until at days 12 and decreased back to normal pH at days This might be due to the susceptibility of microorganism in meat against antimicrobial agent in *Psidium guajava* L. that contributed in delaying the bacterial growth and prolong shelf life of meat [41]. The finding of the comparison pH value for sample that was treated with PGLE with control sample were compatible with the studies by [42], as antimicrobial activity from *Psidium guajava* L. are able to preserve the shelf life of meat.

3.5 Color values

In meat industry, the color plays the uttermost role in both consistency and customer choice. Redness, yellowness and lightness of meat products are known to be one of the most illuminating standards where customers judge their acceptability. The changes in color parameters of meat that being treated with PGLE during storage at 4 °C were analysed using the research studies conducted by [16]. Redness is the most vital parameter among the color values in determining meat oxidation, as a drop redness lowers the market acceptability of meat products. [16], reported that lightness of control sample displayed a huge decrease over the days from 54.97±0.06 to 35.41±0.02. Meanwhile redness of treated sample exhibit insignificant reduction of color started from days 0 to days 16. [43] found that the interdependence between lipid oxidation and color oxidation in meat may lead to a decrease in red colour intensity during storage. Changes of L* values of meat products are primarily due to the moisture and fat content where the water and free fat on the surface influences light reflection and thus, these variables make the meat lighter in color. In particular with redness and lightness, the yellowness had a decreasing tendency with further increased the storage time and this decline was more notable for control when compared to treated samples [44]. [16] concluded that antimicrobial agents that contain in extracts of *Psidium guajava* L. are able to preserve raw meat.

Table 7: Analysis of pH values for *Psidium guajava* L extracts on raw meat

Authors and Year	Method	Study Population	Outcomes
Zhang et al. (2016)	The pH of meat sample is measure with pH meter	100g sample of meat from Luoyang, China	The best pH result of treatment sample with *Psidium guajava* L. extracts reached a level of just 5.68±0.02 relative to that assessed for control sample, 6.59±0.02

Table 8: Analysis of color values for *Psidium guajava* L extracts on raw meat

Authors and Year	Method	Study Population	Outcomes
Zhang et al. (2016)	The color of meat sample is measured with Color Difference Meter (WSC-S)	100g sample of meat from Luoyang, China	The meat sample that treated with *Psidium guajava* L. extracts displayed an extreme red color compared to control sample.

4. Conclusion

Based on the studies, extraction temperature, frequency and type of solvent used are significantly influenced on the phenolic compounds. On top of that, extracts of *Psidium guajava* L. has the ability to inhibit the growth of pathogenic bacteria which could lead to various foodborne illnesses as guava leaves contain natural antimicrobial agent that exhibit antimicrobial properties against *Staphylococcus aureus* and *Escherichia coli*. The discovery of this study revealed that *Escherichia coli* was more resistance to ethanolic extracts of *Psidium guajava* L. compared to *Staphylococcus aureus.* A slight decrease in pH value and color intensity of meat indicate the presence of antimicrobial agents in treated sample. Hence, *Psidium guajava* L. can be applied in food processing industry that act as natural antimicrobial agents to preserve food.

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References

[1] Mitra, R., Orbell, J., & Muralitharan, M. S. (2007). Agriculture — Medicinal Plants of Malaysia. *Asia-Pacific Biotech News*, *11*(02), 105–110. https://doi.org/10.1142/s0219030307000110

[2] Sultana, N., Alsarhan, A., Al-Khatib, A., & Kadir, M. (2014). Review on Some Malaysian Traditional Medicinal Plants with Therapeutic Properties. *Journal of Basic & Applied Sciences*, *10*(November 2017), 149–159. https://doi.org/10.6000/1927-5129.2014.10.20

[3] Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, *25*(2), 361–366. https://doi.org/10.1016/j.sjbs.2017.02.004

[4] Gutiérrez, R. M. P., Mitchell, S., & Solis, R. V. (2008). Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, *117*(1), 1–27. https://doi.org/10.1016/j.jep.2008.01.025

[5] Daniels, D., Biswas, B., Rogers, K., Mclaughlin, F., Daniels, D., & Yadav, A. (2015). Antimicrobial Activities of Leaf Extracts of Guava (Psidium guajava L .) on Two Gram-Negative and Gram-Positive Bacteria *International Journal of Microbiology*, *2013*(October 2013), 1– 8. https://doi.org/10.1155/2013/746165

[6] Katherine B.Louie, S. M. (2020). Mass Spectrometry for Natural Product Discovery. *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*. doi: https://doi.org/10.1016/B978-0-12-409547-2.14834-6

[7] Samar Al Jitan Saeed, A. A. (2018). Chapter 13 - Phenolic Acids From Plants: Extraction and Application to Human Health. *Studies in Natural Products Chemistry*, *58*, 389-417. doi: https://doi.org/10.1016/B978-0-444-64056-7.00013-1

[8] Access, O., Fajardo, S., García-Galvan, R., F., Barranco, V., Galvan, J. C., & Batlle,F. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, *I* (tourism), 13. https://doi.org/http://dx.doi.org/10.5772/57353

[9] Review, M. (2019). *Natural products in food preservation* *. *26*(February), 41–46. Samar Al Jitan Saeed, A. A. (2018). Chapter 13 -Phenolic Acids From Plants: Extraction and Application to Human Health. *Studies in Natural Products Chemistry*, *58*, 389-417. doi: https://doi.org/10.1016/B978-0-444-64056-7.00013-1

[10] Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A., Clarke, M., Devereaux, P. J., Kleijnen, J., & Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *PLoS Medicine*, *6*(7). https://doi.org/10.1371/journal.pmed.1000100

[11] Kraus, S., Breier, M. y Dasí-Rodríguez, S. (2020). (2020). El arte de elaborar una revisión bibliográfica sistemática en la investigación sobre el espíritu empresarial. *International Entrepreneurship and Management Journal*, *16*, 1023–1042.

[12] Wang, J., Zhao, Y. M., Tian, Y. T., Yan, C. L., & Guo, C. Y. (2013). Ultrasound-assisted extraction of total phenolic compounds from inula helenium. *The Scientific World Journal*, *2013*, 1–5. https://doi.org/10.1155/2013/157527

[13] Seo, J., Lee, S., Elam, M. L., Johnson, S. A., Kang, J., & Arjmandi, B. H. (2014). Study to find the best extraction solvent for use with guava leaves (Psidium guajava L.) for high antioxidant efficacy . *Food Science & Nutrition*, *2*(2), 174–180. https://doi.org/10.1002/fsn3.91

[14] Safdar, M. N., Kausar, T., Jabbar, S., Mumtaz, A., Ahad, K., & Saddozai, A. A. (2017). Extraction and quantification of polyphenols from kinnow (Citrus reticulate L.) peel using ultrasound and maceration techniques. *Journal of Food and Drug Analysis*, *25*(3), 488–500. https://doi.org/10.1016/j.jfda.2016.07.010

[15] Li, J., Wu, C., Li, F., Yu, R., Wu, X., Shen, L., Liu, Y., & Zeng, W. (2019). Optimization of ultrasound-assisted water extraction of flavonoids from Psidium guajava leaves by response surface analysis. *Preparative Biochemistry and Biotechnology*, *49*(1), 21–29. https://doi.org/10.1080/10826068.2018.1466158

[16] Zeng, W., Li, F., Wu, C., Ge, Y., Yu, R., Wu, X., Shen, L., Liu, Y., & Li, J. (2020). Optimization of ultrasound-assisted aqueous extraction of polyphenols from Psidium guajava leaves using response surface methodology. *Separation Science and Technology (Philadelphia)*, *55*(4), 728–738. https://doi.org/10.1080/01496395.2019.1574830

[17] Melitz, J. (2018). English as a lingua franca: Facts, benefits and costs. *World Economy*, *41*(7), 1750–1774. https://doi.org/10.1111/twec.12643

[18] Pincher, A. C. (2020, October 27). *How to calculate standard deviation in Excel*. Retrieved from:https://www.jotform.com/blog/how-to-calculate-standard deviation/#:~:text=Say%20there's%20a%20dataset%20for,as%20well%20as%20the%20average.

[19] Shekar, B. R. C., Nagarajappa, R., Singh, R., Suma, S., & Thakur, R. (2016). Antimicrobial efficacy of the combinations of Acacia nilotica, Murraya koenigii (Linn.) Sprengel, Eucalyptus, and Psidium guajava on primary plaque colonizers: An in vitro study. *Indian Journal of Dental Research*, *27*(4), 415–420. https://doi.org/10.4103/0970-9290.191892

[20] Ida Madiha, Y., Rukayadi, Y., & Norhayati, H. (2017). Effects of extraction conditions on yield, total phenolic contents and antibacterial activity of methanolic Cinnamomum zeylanicum Blume leaves extract. *International Food Research Journal*, *24*(2), 779–786.

[21] Porwal et al. (2012). A comprehensive study on different methods of extraction from Guajava leaves for curing various health problem. *Int J Eng Res*, *2*(6), 490–496.

[22] Djenni, Z., Pingret, D., Mason, T. J., & Chemat, F. (2013). Sono-Soxhlet: In Situ Ultrasound-Assisted Extraction of Food Products. *Food Analytical Methods*, *6*(4), 1229–1233. https://doi.org/10.1007/s12161-012-9531-2

[23] Liu, C. W., Wang, Y. C., Lu, H. C., & Chiang, W. D. (2014). Optimization of ultrasound-assisted extraction conditions for total phenols with anti-hyperglycemic activity from Psidium guajava leaves. *Process Biochemistry*, *49*(10), 1601–1605. https://doi.org/10.1016/j.procbio.2014.06.009

[24] Louie, K. B., Kosina, S. M., Hu, Y., Otani, H., de Raad, M., Kuftin, A. N., Mouncey, N. J., Bowen, B. P., & Northen, T. R. (2020). Mass Spectrometry for Natural Product Discovery. In *Comprehensive Natural Products III* (3rd ed.). Elsevier Ltd. https://doi.org/10.1016/b978-0-12-409547-2.14834-6

[25] Luo, Y., Peng, B., Liu, Y., Wu, Y., & Wu, Z. (2018). Ultrasound extraction of polysaccharides from guava leaves and their antioxidant and antiglycation activity. *Process Biochemistry*, *73*(August), 228–234. https://doi.org/10.1016/j.procbio.2018.08.003

[26] Md Salehan, N. A., Sulaiman, A. Z., & Ajit, A. (2016). Effect of temperature and sonication on the extraction of gallic acid from Labisia Pumila (Kacip Fatimah). *ARPN Journal of Engineering and Applied Sciences*, *11*(4), 2193–2198.

[27] Al Jitan, S., Alkhoori, S. A., & Yousef, L. F. (2018). Phenolic Acids From Plants: Extraction and Application to Human Health. In *Studies in Natural Products Chemistry* (1st ed., Vol. 58). Elsevier B.V. https://doi.org/10.1016/B978-0-444-64056-7.00013-1

[28] Chew, K. K., Ng, S. Y., Thoo, Y. Y., Khoo, M. Z., Wan Aida, W. M., & Ho, C. W. (2011). Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Centella asiatica extracts. *International Food Research Journal*, *18*(2), 571–578.

[29] Falleh, H., Ksouri, R., Lucchessi, M. E., Abdelly, C., & Magné, C. (2012). Ultrasound-assisted extraction: Effect of extraction time and solvent power on the levels of polyphenols and antioxidant activity of Mesembryanthemum edule L. Aizoaceae shoots. *Tropical Journal of Pharmaceutical Research*, *11*(2), 243–249. https://doi.org/10.4314/tjpr.v11i2.10

[30] Castro-Vargas, H. I., Rodríguez-Varela, L. I., Ferreira, S. R. S., & Parada-Alfonso, F. (2010). Extraction of phenolic fraction from guava seeds (Psidium guajava L.) using supercritical carbon dioxide and co-solvents. *Journal of Supercritical Fluids*, *51*(3), 319–324. https://doi.org/10.1016/j.supflu.2009.10.012

[31] Che Sulaiman, I. S., Basri, M., Fard Masoumi, H. R., Chee, W. J., Ashari, S. E., & Ismail, M. (2017). Effects of temperature, time, and solvent ratio on the extraction of phenolic compounds and the anti-radical activity of Clinacanthus nutans Lindau leaves by response surface methodology. *Chemistry Central Journal*, *11*(1), 1–11. https://doi.org/10.1186/s13065-017-0285-1

[32] Norshazila, S., Koy, C. N., Rashidi, O., Ho, L. H., Azrina, I., Nurul Zaizuliana, R. A., & Zarinah, Z. (2017). The effect of time, temperature and solid to solvent ratio on pumpkin carotenoids extracted using food grade solvents. *Sains Malaysiana*, *46*(2), 231–237. https://doi.org/10.17576/jsm-2017-4602-07

[33] Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis*, *22*(3), 296–302. https://doi.org/10.1016/j.jfda.2013.11.001

[34] Dzah, C. S., Duan, Y., Zhang, H., Wen, C., Zhang, J., Chen, G., & Ma, H. (2020). The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. *Food Bioscience*, *35*(June 2019), 100547. https://doi.org/10.1016/j.fbio.2020.100547

[35] Munda, S., Dutta, S., Pandey, S. K., Sarma, N., & Lal, M. (2019). Antimicrobial Activity of Essential Oils of Medicinal and Aromatic Plants of the North East India: A Biodiversity Hot Spot. *Journal of Essential Oil-Bearing Plants*, *22*(1), 105–119. https://doi.org/10.1080/0972060X.2019.1601032

[36] Ara Farhana, J. (2017). Antibacterial Effects of Guava (<i>Psidium guajava</i> L.) Extracts Against Food Borne Pathogens. *International Journal of Nutrition and Food Sciences*, *6*(1), 1. https://doi.org/10.11648/j.ijnfs.20170601.11

[37] Dhiman, A., Nanda, A., Ahmad, S., & Narasimhan, B. (2011). In vitro antimicrobial activity of methanolic leaf extract of Psidium guajava L. *Journal of Pharmacy and Bioallied Sciences*, *3*(2), 226–229. https://doi.org/10.4103/0975-7406.80776

[38] Silhavy, T. J., Kahne, D., & Walker, S. (2010). The Bacterial Cell Envelope1 T. J. Silhavy, D. Kahne and S. Walker, . *Cold Spring Harb Perspect Biol*, *2*, 1–16. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2857177/pdf/cshperspect-PRK-a000414.pdf

[39] Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules*, *25*(6). https://doi.org/10.3390/molecules25061340

[40] Processing, M., For, T., Producers, S.-T. M., & Repository, D. (2002). *pH Measurement To Determine Freshness Of Meat Products*.

[41] Quinto, E. J., Caro, I., Villalobos-Delgado, L. H., Mateo, J., De-Mateo-silleras, B., & Redondo-Del-río, M. P. (2019). Food safety through natural antimicrobials. *Antibiotics*, *8*(4), 1–30. https://doi.org/10.3390/antibiotics8040208

[42] Rubab, M., Chelliah, R., Saravanakumar, K., Kim, J. R., Yoo, D., Wang, M. H., & Oh, D. H. (2020). Phytochemical characterization, and antioxidant and antimicrobial activities of white cabbage extract on the quality and shelf life of raw beef during refrigerated storage. *RSC Advances*, *10*(68), 41430–41442. https://doi.org/10.1039/d0ra06727j

[43] Lynch, M. P., & Faustman, C. (2000). Effect of aldehyde lipid oxidation products on myoglobin. *Journal of Agricultural and Food Chemistry*, *48*(3), 600–604. https://doi.org/10.1021/jf990732e

[44] Cierach, M., & Niedźwiedź, J. (2014). Effects of three lighting intensities during display on discolouration of beef semitendinosus muscle. *European Food Research and Technology*, *239*(3), 377–383. https://doi.org/10.1007/s00217-014-2231-y