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Soxhlet and High-Pressure Processing (HPP) Method: Antimicrobial Study on the *Chromolaena odorata* and *Azadirachta indica* Leaves Extracts Against Six (6) Opportunistic Bacteria

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Abstract

Chromolaena odorata and *Azadirachta indica* are well-known for their antimicrobial properties and have long been utilized in traditional medicine for treating various skin ailments. This study aimed to compare the efficacy of two extraction methods, Soxhlet extraction and High-Pressure Processing (HPP), in extracting bioactive components from *C. odorata* and *A. indica*, and their resulting antimicrobial activity. The antimicrobial properties of the extracted samples were assessed using the Kirby-Bauer disc diffusion method and minimum inhibitory concentrations (MICs). The maximum inhibition zones against *E. coli* were observed for Soxhlet-extracted *C. odorata* (17.25 mm) and *A. indica* (16.67 mm). The MICs were recorded as 0.78% for *C. odorata* and 3.13% for *A. indica*, which were notably higher than those of the HPP extracts. For the HPP extracts, treatment at 600 MPa for 10 minutes showed lower MICs against *E. coli*, with values of 100.0% and 12.5% for *C. odorata* and *A. indica*, respectively. Overall, the antimicrobial activity of the Soxhlet-extracted samples was significantly greater than that of all HPP samples. Although Soxhlet extraction demonstrated higher antimicrobial activity, HPP offers advantages as an efficient herb extraction method due to its ability to extract essential compounds from *C. odorata* and *A. indica* in a short period and its non-thermal nature, which reduces the degradation of bioactive chemicals. Therefore, these findings hold significant promise, particularly in the cosmeceutical and health product sectors, where the potential of *C. odorata* and *A. indica* as natural antimicrobial agents and wound healers could contribute to minimizing harm to human health.

1. Introduction

Chromolaena odorata is a weed belonging to the Asteraceae family. It is also known as 'pokok kapal terbang' by traditional practitioners in Malaysia and is frequently used for burns treatment, skin infection and soft tissue wounds. Many studies have reported that several parts of *C. odorata* has a broad range of properties like anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant [1],[2],[3]. Furthermore, several active phytochemical components for instance alkaloids, flavonoids, flavanone, essential oils, phenolics, saponins, tannins, and terpenoids also have been discovered in *C. odorata* [3].

Neem, the former name of *Azadirachta indica* is an omnipotent tree that is categorized under mahogany family, Meliaceae. In Malaysia, *A. indica* is known as 'pokok semambu'. This plant has been extensively used as traditional medicine since prehistoric times for humankind to treat skin diseases such as leprosy, ulcers, gastrointestinal issues, oral care, urinary tract issues, hair problems, diabetes, high blood pressure, and cholesterol [4]. This neem tree is claimed to be antibacterial, antiviral, anticarcinogenic, and antioxidant [4] following its Sanskrit name, 'Arishtha' meaning 'reliever of sickness' [5]. Moreover, the bioactive compounds, include alkaloids, flavonoids, triterpenoids, phenolic compounds, steroids, carotenoids and ketones [6] in *A. indica* have been discovered for benefit to society.

Soxhlet extraction is one of the most popular techniques for extraction functional compounds from plants. It is a very helpful technique that has been used for decades to extract and recover important analytes from various solid matrices. Today, it is still the standard extraction technique for solid samples against which other leaching techniques are measured. Solid samples, however, require suitable sample treatment prior to extraction in order to achieve an adequate recovery of analytes. Zygler and his colleagues (2012) reported that the extraction efficiency is primarily dependent on three interrelated factors: solubility, mass transfer, and matrix effects [7]. Consequently, it is evident to examine the practical concerns associated with extraction processes, such as matrix properties, solvent selection, liquid-solid ratio, temperature, pressure, and extraction and evaporation times.

High-pressure-assisted extraction (HPE) is a novel extraction technology to extract active ingredients in medicinal herbs plant. HPE, which also known as high pressure processing (HPP) has the potential to improve the extraction efficiency by increasing the mass transfer rates and the possibility of breaking cell walls, membranes, and organelles of plant tissues, with lower solvent consumption at the short processing time. Currently, the HPE operation in industry typically use hydrostatic pressure of 100 to 600 MPa, depending on the product being processed [8]. Compared to conventional extraction, such thermal extraction, HPP is promising as an alternative extraction method used in various applications, such as extracting lycopene from ketchup, anthocyanins from grape skin, flavonoids from litchi peel, ginsenosides from ginseng root and catechins, and caffeine and polyphenols from tea [9].

A wound describes as a break in the typical arrangement of skin cells and a disruption in the skin's role in linking and protect underlying tissues and organs [10]. Nowadays, chronic wounds have become the main attention worldwide due to the high cost of treating the wound. Wounds, can lead to skin infections involving bacterial diseases, which may require a long time to heal. Generally, humans need antimicrobials to protect them against microorganisms, including bacteria, fungi and viruses. Several medicinal herbs may serve as a natural source of antimicrobials due to some bioactive compounds that have been presented in them. Significant compounds in medicinal herbs can inhibit or decrease the bacteria's growth rate, thus providing the herbs for use in the treatment field. It is because the availability of antimicrobial agents is necessary as they aid in the treatment of fighting the infectious disease caused by pathogenic bacteria.

Numerous studies have reported that *C. odorata* and *A. indica* play a crucial role in treating various types of diseases, which have the potential to be antimicrobial agents. Pathogens, the microorganisms responsible for causing illness, exhibit diverse types and varying degrees of severity in the diseases they induce. As infectious agents, pathogens transmit diseases to their hosts, prioritizing their own survival and reproduction. The human body's immune system serves as a defence mechanism against infections, effectively combating some viruses while others pose potentially lethal threats. Five main types of pathogens exist: bacteria, viruses, fungi, protists, and parasitic worms. Symptoms such as fever, malaise, and rash often indicate the immune system's response to infection. Antibiotics are powerful medications used to combat bacterial infections by either destroying or inhibiting bacterial growth, allowing the body's natural defences to eliminate the infection. However, the rise of antibiotic resistance among certain microbes poses a significant challenge.

To address this concern, several pathogenic bacteria, including Escherichia coli, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus cereus, and Staphylococcus aureus, have been studied to assess their resistance to antibiotics. This study aims to investigate the antimicrobial potential of extracts from C. odorata and A. indica using two different extraction methods: Soxhlet extraction and High-Pressure Processing (HPP). Additionally, the study seeks to assess the capacity of C. odorata and A. indica extracts to substitute synthetic antimicrobial agents in healthcare products, potentially contributing to advancements in the healthcare industry, particularly in the cosmeceutical segment.

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2. Materials and Methods

2.1 Material and Sample Preparation

In this research, *Chromolaena odorata* and *Azadirachta indica* were collected from the Nasuha Herbs & Spice Farm in Muar, Johor, Malaysia. *C. odorata* and *A. indica* were washed under running tap water, cut only healthy plants, and brushed away insects. The leaves were rinsed and dried in ovenat temperature of 45°C for 4–7 days or until completely dry [11], then pulverized using a grinder. The respective powders were weighed and stored in an air-tight container until used [12].

2.2 Extraction (Soxhlet and High-Pressure Processing)

2.2.1 Soxhlet Extraction

Ten (10) g of powdered sample were extracted with 250 ml of 95% ethanol (solvent) by using assembled Soxhlet apparatus. The heating mantle was adjusted to stage 4 for 16 hours. The solvent evaporates from isomantle to the condenser, while the thimble reservoir collected condensate. The solvent is poured back into the flask at the syphon, restarting the cycle. The resulted dark-green extract was then evaporated using a rotary evaporator under pressure of 58 mbar at 60°C. Crude extracts werecollected until 2 to 3 ml of viscous paste left in the glass bottom flask [13].

2.2.2 High Pressure Processing (HPP)

About 7.5 g powdered *C. odorata* and *A. indica* were added with 150 mL distilled water in the bottle. The samples subjected to HPP with pressure of 200 MPa at 5, 10, and 15 minutes and 600 MPa at 10 minutes at ambient temperature. The temperature within the pressure chamber was monitored using thermocouples that were immersed in the pressure medium (distilled water) where it positioned at the middle and top of the vessel. On the cycle report, the compression time, decompression time, and average temperature were collected directly from a control system handled by a computer running software [14]. The resulted extracts then were cooled in ice water after HPP treatment until used. The experiment used triplicate *C. odorata* and *A. indica* samples from the same batch.

2.3 Antimicrobial Activity Testing

The antimicrobial activity the extracted samples, *C. odorata* and *A. indica* were investigated by using Kirby-Bauer Disk Diffusion Method [15]

2.3.1 Subculturing Bacteria

The microbes (Escherichia coli, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiellapneumoniae, Bacillus cereus and Staphylococcus aureus) were subcultured on a sterile nutrient agar plate by using streak plate method, then incubated at 37°C for 24 hours [15].

2.3.2 Media Preparation

The media used for determining the antimicrobial activity of extracted samples, C. odorata and A. indica against Escherichia coli, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus cereus and Staphylococcus aureus was Mueller-Hinton Agar (MHA). The Mueller-Hinton Agar media was prepared by dissolving 38g of agar powder in 1000 mL distilled water. It was autoclaved at 121°C for 15 minutes, and let to be cooled in room temperature before pouring into petri dishes. The agar is ready to be used once it is in solid form [15].

2.3.3 Broth Preparation

The Mueller-Hinton Broth was used to determine the Minimum Inhibitory Concentration. The broth was prepared by dissolving 21g of broth powder in 1000 mL distilled water. It was then poured into the test tubes and sterilized using autoclave at 121°C for 15 minutes. The medium should be stored at below 25°C once it has prepared [15].

2.3.4 Antibacterial Susceptibility Test Using Herbs Plant Extract

A sterile cotton swab dipped into the standardized inoculum, and excess medium is removed by pressing the swab against the tube wall. Then, the entire surface area of the MH agar plate was swabbed by rotating the plate approximately 60 degrees each time to ensure a uniform distribution of the inoculum, and allowed to dry at room

temperature for at least 3 to 5 minutes inside the biosafety cabinet [15]. The Whatman antibiotic disc (6.0 mm diameter) were soaked with the extracts of *C. odorata* and *A. indica*, then were placed on Mueller-Hinton Agar, thus left to dry. For positive control, an effectiveantibiotic named Streptomycin was used while sterile distilled water as negative control. The plates were incubated at 37°C for 24 hours to observe the inhibition zone generated by the extract.

2.3.5 Determination of Minimum Inhibitory Concentration (MIC)

There were eleven (11) test tubes used to determine the Minimum Inhibitory Concentration (MIC).The test tubes were pipetted with 1 mL of Mueller-Hinton Broth, exclude the negative control (2 mL).The first test tube was pipetted with 1 mL of *C. odorata* and *A. indica* extracts, then performing serial dilution for the next tubes. 0.1 mL of tested bacteria was injected into each test tube exclude positive control. The MIC values conducted were 1 MIC, ½ MIC, ¼ MIC, 1/8 MIC, 1/16 MIC, 1/32 MIC, 1/64MIC, 1/128 MIC and 1/256 MIC [16].

3. Results and Discussion

3.1 Antimicrobial Analysis of *Chromolaena Odorata* and *Azadirachta indica*

For this experiment, *C. odorata* and *A. indica* extracts were obtained through two different extraction method, Soxhlet and HPP extraction. The antibacterial properties of extracted *C. odorata* and *A. indica* were determined by using the agar-disc diffusion method against different types of bacteria which were *E. coli, S. epidermidis, P. aeruginosa, K. pneumoniae, B. cereus* and *S. aureus*. For the agar-disc diffusion method, the antibacterial activity of *C. odorata* and *A. indica* extracted were evaluated based on the diameters of clear inhibition zone around the disc located on the agar. No inhibition zone showing that there is no antibacterial activity.

3.1.1 Comparison of Inhibition Zone of *Chromolaena Odorata* Between Soxhlet and HPP extraction

Table 1 indicate the comparison of inhibition zone (diameter) for both extracted samples by using Soxhlet and HPP method, against *E. coli, S. epidermidis, P. aeruginosa, K. pneumoniae, B. cereus* and *S. aureus*. The result revealed that the ethanolic extract of *C. odorata* are very effective suppressing thebacterial growth. As shown in Table 1, the ethanolic extract of *C. odorata* had the maximum inhibition zone against *E. coli* (17.25 ± 2.14 mm), whereas HPP extract of *C. odorata* (600 MPa for 10 minutes)showed a maximum zone of inhibition against *K. pneumoniae* (7.75 ± 1.39 mm). However, *C. odorata*extract through Soxhlet showed minimum inhibition zone against *K. pneumoniae* (8.60 ± 0.53 mm). Meanwhile, the HPP extract for *C. odorata* had minimum inhibition zone against *P. aeruginosa* (2.67± 4.62 mm) when pressure applied was 200 MPa for 15 minutes.

Based on the diameter of inhibition zone, the *C. odorata* extract obtained from Soxhlet showed significant difference in antimicrobial activity ($p < 0.05$) against *E. coli* as compared to HPP and untreated sample. The result is in line with the result obtained by Farahida et al., (2020) who studied antimicrobial potential of *C. odorata* extract against *E. coli* and *S. epidermidis* considering their inhibition diameter [17]. They also reported that extraction time plays a significant role on the antimicrobial activity of herbs plant [17].

In the case of HPP extraction, high pressure (600 MPa) executed more antimicrobial activity in *C. odorata* as presented in Table 1. According to Alexandre and his co-workers (2017), high pressure extraction at 600 MPa resulted in improved extraction, up to a maximum of 35% total flavonoids, which one of bioactive compounds that assist in antimicrobial properties [17]. Therefore, the maximum inhibition zone was generated by HPP extraction at 600 MPa maybe due to high contain of total flavonoids in *C. odorata*, which gave high antimicrobial activity compare to pressure of 200 MPa. Overall, the *C. odorata* extract obtained from Soxhlet showed better capability in inhibiting the tested bacteria as compared to HPP and untreated sample.

Table 1 *The inhibition zone (mm) of untreated, Soxhlet-treated and HPP-treated of Chromolaena odorata against six different bacteria*

Extraction Method	Duration	Inhibition Zone Measurement Against Different Bacteria (mm)					
		E. coli	*S. epidermidis*	*P. aeruginosa*	*K. pneumoniae*	*B. cereus*	*S. aureus*
Untreated		4.33±3.75^{b}	5.67 ± 4.93^{ab}	4.50 ± 3.91^{ab}	2.00 ± 3.46 ^{b}	0.00 ± 0.00 ^{b}	6.00 ± 0.00 ^{b}

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Soxhlet	16 hrs	*17.25 ± 2.14 [a]	13.33 ± 2.08[a]	14.97 ± 0.95[a]	*8.60 ± 0.53[a]	13.17 ± 1.76[a]	11.50 ± 0.50[a]
200 MPa	5 min	0.00 ± 0.00 [b]	5.50 ± 4.77[ab]	5.50 ± 4.77 [ab]	6.17 ± 0.29 [ab]	0.00 ± 0.00[b]	6.58 ± 0.38 [b]
	10 min	4.83 ± 4.19 [b]	7.17 ± 0.29 [ab]	5.33 ± 4.73 [ab]	6.67 ± 0.29[b]	4.17 ± 3.62[b]	6.00 ± 0.00 [b]
	15 min	0.00 ± 0.00 [b]	0.00 ± 0.00 [b]	*2.67 ± 4.62[b]	4.5 ± 3.97 ab	4.33 ± 3.79[b]	6.00 ± 0.00 [b]
600MPa	10 min	4.67 ± 4.04 [b]	7.5 ± 0.866 [ab]	5.50 ± 4.77 [ab]	*7.75 ± 1.39 [ab]	4.67 ± 4.04[b]	7.67 ± 1.52 [b]

[ab]Mean values (means ± standard deviation), where n = 3 and the different letters in each column indicate a significant difference ($p<0.05$) based on Tukey's HSD test (Minitab v21.1.0, Statistical Software).

3.1.2 Comparison of Inhibition Zone of Azadirachta indica Between Soxhlet and HPP Extraction

Table 2 showed the comparison diameter of inhibition zone between the different extraction of *Azadirachta indica* against *E. coli, S. epidermidis, P. aeruginosa, K. pneumoniae, B. cereus* and *S. aureus*. The maximum inhibition zone was identified on *A. indica* through Soxhlet extraction against *E. coli* (16.67 ± 1.16 mm), and HPP extracted sample treated at 600 MPa for 10 minutes showed the largest inhibition zone against *P. aeruginosa*, with a diameter of 8.75 mm. Meanwhile, the sample against *S. aureus* obtained from Soxhlet extraction method showed minimum inhibition zone with a recorded value of 9.00 mm. The smallest inhibition zone for HPP extracted sample was found as 4.00 mm against *E. coli* and *S. aureus* when the pressure applied is 200 MPa, with 5 and 10 minutes, respectively.

Similar to *C. odorata*, the *A. indica* extract obtained from Soxhlet also showed the highest antimicrobial activity against *E. coli* (16.67 mm diameter) compared to HPP and untreated sample. The result obtained showed a significance difference between these treatments ($p < 0.05$). This may occur due to less impurity of extract obtained, in this case ethanol has assisted *A. indica* in inhibiting bacterial growth. In the case of HPP extraction, a higher pressure (more than 400 MPa) promotes the antimicrobial activity than at lower pressure. According to Shouqin et al., (2004), the protein will be denatured, and the cell membranes will be damaged under high pressure, then more solvent will enter the interior of cells, allowing more essential compounds (EC) to be extracted more easily [18]. Therefore, high pressure of 600 MPa could execute high antimicrobial activity than pressure of 200 MPa maybe because there is more EC in *C. odorata* when it subjected to 600 MPa. However, the ANOVA data showed the pressure difference considered as insignificant as they are in same grouping. As a result, the *A. indica* extract is very effective in inhibiting bacterial growth under Soxhlet extraction.

Table 2 *The inhibition zone (mm) of untreated, Soxhlet-treated and HPP-treated of Azadirachta indica against six different bacteria*

Extraction Method	Period	Inhibition Zone Measurement Against Different Bacteria (mm)					
		E. coli	*S. epidermidis*	*P. aeruginosa*	*K. pneumoniae*	*B. cereus*	*S. aureus*
Untreated		7.50± 0.50[b]	5.50± 4.82[bc]	5.00± 4.33[b]	7.63 ± 0.55[b]	7.67 ± 1.53[a]	7.50± 0.50[ab]
Soxhlet	16 hrs	*16.67± 1.16[a]	12.33 ± 1.16[a]	12.50 ± 1.80[a]	11.50 ± 0.87[a]	10.00 ± 1.00[a]	*9.00 ± 0.00[a]
200 MPa	5 min	*4.00 ± 3.46[b]	8.00± 0.00[ab]	8.50 ± 0.50[ab]	6.67± 0.58[b]	7.17 ± 0.76[a]	6.47± 0.45[ab]
	10 min	7.50 ± 1.32[b]	7.00± 1.00[ab]	8.17 ± 1.04[ab]	7.00 ± 1.73[b]	7.00 ± 1.00[a]	*4.00 ± 3.46[ab]
	15 min	6.83 ± 1.04[b]	0.00 ± 0.00[c]	8.50 ± 0.50[ab]	6.83 ± 0.76[b]	7.00 ± 1.00[a]	6.17 ± 0.29[ab]
600MPa	10 min	6.83 ± 0.76[b]	7.33± 0.58[ab]	*8.75 ± 1.25[ab]	7.00 ± 1.00[b]	7.50 ± 1.32[a]	0.00± 0.00[c]

[abc]Mean values (means ± standard deviation), where n = 3 and the different letters in each column indicate a significant difference ($p<0.05$) based on Tukey's HSD test (Minitab v21.1.0, Statistical Software)

3.2 Minimum Inhibitory Concentration (MIC)

3.2.1 Comparison of MIC Between the Different Extraction of *Chromolaena odorata* Extract

The Minimal Inhibitory Concentration (MIC) was determined through macrodilution method towards *C. odorata* sample by using two (2) different extraction methods, which are Soxhlet and HPP. The sample used for MIC determination of *C. odorata* extract was chosen among the best samples from Antibacterial Susceptibility Testing with a higher inhibition zones measurement, in this case the extract obtained from Soxhlet. For HPP extraction, the pressure of 200 MPa and 600 MPa for 10 minutes respectively were chosen for MIC testing. Table 3 shows the MIC identified for *C. odorata* sample under Soxhlet and HPP extraction.

From the result obtained, the concentration of MIC recorded for Soxhlet- extracted *C. odorata* against tested bacteria, was 0.78%, while the concentration of MIC for HPP extraction was 50.0%. However, no significant differences were observed for HPP extracts using 200 MPa and 600 MPa against tested pathogens. In the comparison, the Soxhlet-extracted sample had lower value of MIC (0.78%) than HPP-extracted samples against *E. coli* and *S. epidermidis*. Further, Huang et al., (2019), who studied high-pressure extraction effect on phenolic compounds of Djulis hull that are known to have antimicrobial activity, the HPE samples had higher phenolic and flavonoids content than conventional extraction [8]. Contrarily to our results, the *C. odorata* extract from Soxhlet showed a higher antimicrobial activity than the samples extracted from HPP method. This may occur due to less concentrated of *C. odorata* extract after HPP treatment, which gave minimal antimicrobial activity of *C. odorata* against tested bacteria. Besides, the HPP samples have been left for a week before proceeding to microbiological analysis. The samples should be freeze-dried, and frozen at -80 ºC until use for further analysis [19].

Table 3 *Minimum inhibitory concentration (%) of Soxhlet-treated and high pressure processing-treated of Chromolaena odorata against different bacteria*

Extraction Method	Condition	Minimum Inhibitory Concentration (%)					
		E. coli	*S. epidermidis*	*P. aeruginosa*	*K. pneumoniae*	*B. cereus*	*S. aureus*
Soxhlet	16 hrs	0.78	0.78	0.78	0.78	0.78	0.78
200 MPa	10 min	50.00	100.00	100.00	100.00	100.00	100.00
600 MPa	10 min	100.00	50.00	100.00	100.00	100.00	100.00

3.2.2 Comparison of MICs Between the Different Extraction of *Azadirachta Indica* Extract

From Table 4, the lowest value of MIC reported for *A. indica* under Soxhlet extraction was 3.13%, while *A. indica* extract through HPP extraction was found as 12.5%. As compared to previous Antibacterial Susceptibility Testing, *A. indica* from Soxhlet are more potent as antibacterial agent than HPP-treated extract. However, Khan et al., (2019) reported High Pressure Extraction (HPE) was observed to be quicker and more productive method of extraction than that of conventional extraction techniques [20],[21]. The result obtained from this study is in contradiction, which might be due to extraction solvent. It is because the solvent used for HPP is water. There is study where extraction of polyphenols from green tea, by using 0.5% ethanol as an aid has increase by 50% more as compared to the water [21]. Besides, the results is contradicted with theory due to the HPP samples having less concentration, which may give less effect on antimicrobial activity of *A. indica.* After HPE, the extract should undergo vacuum concentrated, freeze-dried, and the analysis were performed at 4ºC. That is why extracted *A. indica* from HPP disable to compete the *A. indica* from Soxhlet.

In comparison of pressure used, high-pressure HPP could give greater antibacterial activity than low-pressure HPP in theory. Similar result was observed by Cardoso and his colleagues (2013) when the amount of total phenolic compounds content was increased when the pressure is doubled up from initial levels of 10–20 MPa [21],[22]. However, the result obtained shows insignificant differences between 200 MPa and 600 MPa. Shouqin et al., (2004) reported that the majority of HPP extraction is for tiny compounds, meaning that essential compounds (EC) will not alter during HPP at room temperature [18]. However, the extract of interest, EC, may

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undergo reaction changes such as stereoisomerization, pericyclic reaction, anionic reaction, synthesis, hydrogenation, inorganic and bioinorganic reaction when subjected to high pressure [18]. Therefore, it can be said that there might be have reaction changes on EC, which give effect on antimicrobial activity of *A. indica* extract for pressure at 600 MPa.

Table 4 *Minimum inhibitory concentration (%) of Soxhlet-treated and high pressure processing-treated of Azadirachta indica against different pathogenic bacteria*

Extraction Method	Period	Minimum Inhibitory Concentration (%)					
		E. coli	*S. epidermidis*	*P. aeruginosa*	*K. pneumoniae*	*B. cereus*	*S. aureus*
Soxhlet	16 hrs	3.13	3.13	6.25	6.25	6.25	6.25
200 MPa	10 min	25.00	25.00	25.00	12.5.00	12.50	25.00
600 MPa	10 min	12.50	25.00	50.00	25.00	50.00	50.00

4. Conclusion

This study showed that, both *C. odorata* and *A. indica* leaves extracts, have strong antimicrobial activity, especially towards Gram-negative bacteria (*E.coli, P. aeruginosa and K. pneumoniae)* and Gram-positive bacteria *(S. epidermidis, B. cereus and S. aureus*). Soxhlet extraction yields bioactive components with higher antimicrobial activity compared to HPP for both *C. odorata* and *A. indica*, as evidenced by larger inhibition zones and lower minimum inhibitory concentrations (MICs). While HPP extracts showed lower antimicrobial activity compared to Soxhlet extraction, HPP is highlighted as an efficient herb extraction method due to its shorter processing time and non-thermal nature, which helps in preserving bioactive chemical compounds. The study implies the need for further research to explore and optimize extraction methods to enhance the antimicrobial activity of HPP extracts and to better understand the specific bioactive compounds responsible for the observed effects. The findings show the potential applications of *C. odorata* and *A. indica* extracts as natural antimicrobial agents which could offer benefits in minimizing harm to human health, compared to synthetic antimicrobial agents.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Noor Akhmazillah Mohd Fauzi, Faiz Razali;* ***data collection:*** *Nur Helwa Kamaruzzan, Faiz Razali;* ***analysis and interpretation of results:*** *Noor Akhmazillah Mohd Fauzi, Nur Helwa Kamaruzzaman, Angzzas Sari Mohd Kassim;* ***draft manuscript preparation:*** *Sity Aisyah Mansur, Aliff Hisyam A Razak, Nur Helwa Kamaruzzaman. All authors reviewed the results and approved the final version of the manuscript.*

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