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Soxhlet versus Ultrasound-Assisted Extraction of *Curcuma longa*: Phytochemical Profiling and Bioactivity Assessment

Zulkifli, M. H.; Abdul Latiff, M. F. N.; Hassan, S. N.*

Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuh Persiaran Tun Khalil, 26300 Kuantan, Pahang Malaysia.

ABSTRACT

Curcuma longa, a perennial plant from the Zingiberaceae family, is renowned for its medicinal properties. Traditionally, it has been a staple in Indian medicine for treating various health issues and is now a vital component in contemporary health supplements. The plant's active compounds, particularly curcumin, are known for their antibacterial, anti-inflammatory, and antioxidant properties. However, despite these recognized benefits, there remains a significant gap in scientific validation for many of these claims, especially concerning the optimal extraction methods to fully harness its bioactive compounds. This study primarily aimed to compare two extraction methods, Soxhlet and Ultrasonic-Assisted Extraction (UAE), to determine which is more effective in terms of efficiency and bioactivity. We then evaluated the extracts for their antioxidant, anti-inflammatory, and antibacterial activities to explore their potential pharmaceutical applications. C. longa extracts were obtained using both Soxhlet and UAE methods. The antioxidant potential was assessed using the hydrogen peroxide (H2O2) free radical scavenging assay, resulting in IC50 values of 269 µg/mL for Soxhlet and 295 µg/mL for UAE, indicating some antioxidant potential. The anti-inflammatory activity was measured using the BSA denaturation assay, with IC50 values of 301.99 µg/mL for Soxhlet and a lower value of 213.79 µg/mL for UAE, suggesting better anti-inflammatory properties for the latter. The antibacterial activity was tested using the disc diffusion method against S. aureus and E. coli. The UAE extract demonstrated superior antibacterial properties compared to the Soxhlet extract for S. aureus, with inhibition zones of 10 mm and 4.5 mm, respectively. However, the C. longa extract showed no inhibition zone for E. coli, likely due to the bacterium's bilayer peptidoglycan membrane structure.

Keywords: Anti-bacterial, Anti-inflammatory, Anti-oxidant, *Curcuma Longa*, LC-MS QTOF

*syaiful@umpsa.edu.my

1. INTRODUCTION

Curcuma longa is a tropical herb traditionally used for its healing properties in regions like India, China, and South-East Asia. This perennial herbaceous plant features aromatic, cylindrical rhizomes that are a vivid yellow-orange. It flourishes in warm, humid environments and is mainly grown for its rhizomes, which are extensively used in traditional medicine, culinary practices, and cosmetic products. Known as "Haldi" in India, "Jiang Huang" in China, and "Kunyit" in Malaysia [1], turmeric belongs to the Zingiberaceae family, characterized by its funnel-shaped flowers, lance-shaped leaves, and tuberous rhizomes. The rhizomes of C. longa contain numerous bioactive compounds, including curcuminoids, essential oils, and polysaccharides. Among these, the pharmacological effects of curcuminoids, particularly curcumin, have been most thoroughly

studied. Essential oils such as zingiberene, turmerone, and ar-turmerone enhance its aromatic and medicinal properties. Various parts of the plant, like rhizomes, leaves, or flowers, contain different bioactive chemical components [2].

Rhizomes are rich in curcumin, demethoxycurcumin, bisdemethoxycurcumin, and essential oils, while leaves contain flavonoids and phenolic compounds. Various techniques, including Soxhlet extraction (S.E.) and ultrasound-assisted extraction (U.A.E.), are employed to obtain extracts from *C. longa* [3]. Unlike traditional methods, U.A.E. is a modern extraction technique that significantly reduces both extraction time and operational costs, while yielding substantial quantities of bioactive compounds. *C. longa* has been extensively researched for its medicinal properties and is commonly utilized in creating therapeutic agents for treating inflammatory diseases, microbial infections, oxidative stress, and other ailments. Soxhlet extraction is a conventional method that involves using large volumes of solvent and requires extended periods to ensure thorough recovery of active compounds from solid materials [4]. Previous research indicates that *C. longa* possesses potent anti-bacterial, anti-inflammatory, and anti-oxidant properties. The constituents of *C. longa* include curcuminoids, essential oils, polysaccharides, alkaloids, and sterols, with curcuminoids and essential oils being the most frequently reported [5]. Curcuminoids, especially curcumin, exhibit strong anti-oxidant capabilities by neutralizing free radicals and enhancing the activity of anti-oxidant enzymes [6]. Essential oils have anti-microbial effects, effectively preventing the growth of bacteria such as *Staphylococcus aureus* and *Escherichia coli* [7]. Curcumin also shows promise in reducing inflammation by altering cell signalling pathways like NF-κB and inhibiting the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, thereby helping to alleviate chronic inflammation and related diseases [8].

The aim of this research is to perform a chemical analysis of C. longa extracts utilizing Liquid Chromatography – Mass Spectrometry Quadrupole Time-of-Flight (LC-MS QTOF). Following this, the study evaluates the anti-oxidant, anti-inflammatory, and anti-bacterial characteristics of *C. longa*. While earlier research suggests the medicinal advantages of *C. longa*, additional scientific evidence is necessary to substantiate these claims fully. Moreover, the anti-oxidant, anti-microbial, and anti-inflammatory properties of *C. longa* extracts are investigated through in-vitro experiments.

2. MATERIAL AND METHODS

2.1 Materials

Curcuma Longa rhizomes were purchased from nearby TMG Sdn. Bhd., a local supermarket located at Gambang, Pahang.

2.2 Ultrasonic-Assisted Extraction

An ultrasonic bath (Elmasonic S 10 H, Elma Schmibauer GmbH, Germany) was employed to extract *Curcuma longa*. The extraction process was conducted at a frequency of 37 kHz, with the bath operating at a power of 90 W. A total of 25 g of turmeric powder was used as the solute, which was dissolved in 500 ml of ethanol within an Erlenmeyer flask. This flask was then placed in the ultrasonic bath. The extraction was carried out for 1 hour and 15 minutes at a temperature of 40 °C. To prevent the loss of solvent, Parafilm was used to seal the Erlenmeyer flask during the experiment. Subsequently, the extract was filtered through a 0.45 μm filter, and the solvent was removed by vacuum evaporation using a rotary evaporator. Finally, the extract underwent LC-MS QTOF analysis for chemical profiling [9].

2.3 Soxhlet Extraction

The present study employed the Soxhlet extraction method. 25 grams of finely ground turmeric was inserted into a small container suspended within the extraction apparatus, using ethanol as the solvent. By maintaining the turmeric powder at a temperature of 80 °C for a duration of 8 hours, the applied method effectively isolated curcumin and oleoresin. Subsequently, the ethanol and extracted substances were separated from the remaining solid material, which was then utilized for LC-MS analysis to detect bioactive compounds in *Curcuma longa* [10].

2.4 Extraction Yield Calculations

The yields of extractions were calculated by applying Equation (1), where YT is the total extract yield, $X_{Extract}$ is the mass of crude extract (g) obtained and $X_{Turmeric}$ is the mass of *Curcuma Longa* powder used.

$$Total\ Yield\ (\%) = (X_{Extract}/X_{Turmeric}) \times 100 \quad (1)$$

2.5 The H_2O_2 Free Radical Scavenging Activity

The hydrogen peroxide (H_2O_2) scavenging capability of turmeric extract was assessed following the method outlined by Tuba et al. (2008), with some modifications. A stock solution of turmeric extract at 6 mg/mL was diluted to working concentrations of 125, 250, 500, and 1000 µg/mL. Ascorbic acid, used as a standard, was prepared as a 1 mg/mL stock in 0.1 M phosphate buffer saline (PBS) at pH 7.4 and diluted to the same concentrations. A 43.0 mM H_2O_2 solution in 0.1 M PBS was kept in an amber container to protect it from light degradation. For the assay, 2.4 mL of the 43.0 mM H_2O_2 solution was combined with 0.6 mL of either turmeric extract or ascorbic acid at the specified concentrations. Controls (H_2O_2 + PBS) and blanks (PBS only) were included to ensure accuracy. Absorbance was recorded at 230 nm using a UV-Vis spectrophotometer [10]. The percentage of H_2O_2 scavenging was determined using Equation (2) [10]:

$$H_2O_2\ scavenging\ effect\ (\%) = ((AC - AS)/AC) \times 100 \quad (2)$$

Which *AC* represents absorbance of control solution and *AS* represents absorbance of sample solution. The IC50 value of hydrogen peroxide (H_2O_2) was also measured.

2.6 Anti-inflammatory Bovine Albumin Serum (BSA) Denaturation Assay

The method described by [11] will be used to assess the inhibition of protein denaturation. For this study, the collected extracts will be dissolved in pure ethanol to reach a concentration of 1 mg/mL for each experiment. Both the test sample extracts and reference samples will be diluted to different concentrations (125–1000 µg/mL) in separate test tubes, using a 0.1 M PBS solution at pH 7.4. A 5 mL reaction solution will be prepared, consisting of a PBS mixture at pH 6.4, 0.02 mL of extract, and 0.2 mL of 1% bovine albumin. After mixing, the reaction solutions will be incubated for 15 minutes at 37 °C in a water bath, followed by heating for 5 minutes at 70°C. Once cooled, the turbidity of the reaction solutions will be measured with a UV/VIS spectrometer (Thermo Scientific Evolution 200, US) at 660 nm. Equation (3) will be used to calculate the percentage inhibition of protein denaturation:

$$Inhibition\ of\ denaturation\ (\%) = ((ARS - AMS)/ARS) \times 100 \quad (3)$$

Which *ARS* represents absorbance of reference sample solution and *AMS* represents absorbance of reaction mixture sample solution.

2.7 Determination of Anti-bacterial Activity

2.7.1 Bacterial Strain Preparation

Staphylococcus aureus, a Gram-positive bacterium, and *Escherichia coli*, a Gram-negative bacterium, were obtained from the Central Lab of University of Malaysia Pahang Al-Sultan Abdullah. Prior to conducting the experiment, the bacterial strains were sub-cultured for a period of 24 hours. Isolated colonies were selected using a sterile cotton swab and then suspended in a nutrient broth. After thorough mixing, the turbidity was measured using a UV-Vis spectrophotometer, ensuring it matched the McFarland standard of 0.08 – 0.10 [12].

2.7.2 Disc Diffusion Assay

The anti-bacterial evaluation was conducted using a disc diffusion method as described in [13 & 14]. Mueller-Hinton (MH) agar plates were prepared by dissolving 38 g/L of MH agar in deionized water, sterilizing it through autoclaving, and then pouring it into sterile petri dishes. The agar plates were inoculated by evenly spreading a standardized bacterial suspension with a sterile cotton swab, ensuring uniform coverage through multiple streaks. The inoculated plates were sectioned for testing turmeric extracts obtained through Soxhlet extraction (S.E.) and ultrasonic-assisted extraction (U.A.E.). Sterile filter paper discs were soaked with 10 µL of a 20 mg/mL turmeric extract solution. These treated discs were air-dried in a laminar flow cabinet under aseptic conditions. Erythromycin served as the positive control, while deionized water was used as the negative control. The prepared discs were carefully placed on the agar surface, and the plates were incubated at 37 °C. The anti-bacterial activity was determined by measuring the diameter of the inhibition zone in millimeters (mm).

3. RESULTS AND DISCUSSION

3.1 Extraction Method Impact on the Yield

The effectiveness of extracting plant compounds from raw materials depended on five primary factors: temperature regulation, applied pressure, choice of solvent, extraction technique, and the quantity of plant material used. In this research, *Curcuma longa* extracts yielded 6.84% and 8.84% through S.E. and U.A.E. extraction methods, respectively. U.A.E. proved more effective than other techniques because ultrasonic waves allowed the solvent to penetrate deeper into the plant material, facilitating the release of more active compounds. The findings of Botic et al. (2017) corroborate the enhanced performance of the ultrasonic-assisted extraction method. The lower efficiency of Soxhlet extraction might be attributed to the extended exposure of the sample to high temperatures, which could potentially degrade and damage delicate compounds [15 & 16].

The experiment demonstrates that U.A.E. required a shorter extraction time and was more effective with solvents compared to S.E., resulting in higher yields, as illustrated in Figure 1. U.A.E. is shown to be superior to traditional methods for extracting active compounds from *Curcuma longa* plants, as well as other rhizome plants like ginger (*Zingiber officinale roscoe*).

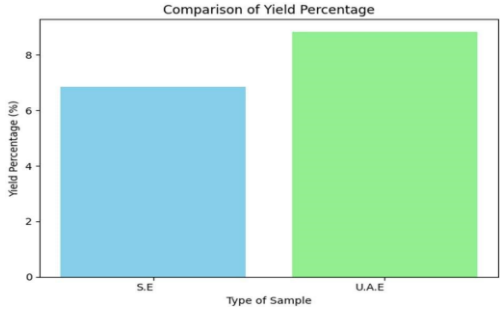

Figure 1. The comparison of yield percentages between Soxhlet extraction and Ultrasonic-assisted extraction methods

Table 1 The bioactive components identified in Soxhlet Extraction extract of Turmeric

Compound	Observed m/z	Observed RT (min)	Response
Mulberrofuran Q	637.1390	16.62	207
Yakuchinone A	311.1631	16.69	1065
Castalagin	933.0656	16.66	520
Kosamol A	525.2518	16.67	88
Punicalagin	1083.0573	16.68	474
Agrimonic Acid A	1149.0921	16.86	109
6-Gingerol	293.1742	17.79	4950
Tectochrysin	313.0722	17.98	90
4,5,6,7-Tetramethoxyflavone	341.1002	18.59	584

Table 2 The bioactive components identified in Ultrasonic-Assisted Extraction extract of Turmeric

Compound	Observed m/z	Observed RT (min)	Response
Mulberrofuran D	445.2384	16.54	167
Yakuchinone A	311.1659	16.68	89
Castalagin	933.0656	16.70	316
Kukoamine A	529.2980	16.56	221
Curcumin	367.1163	16.62	309
Demethoxycurcumin	337.1053	16.61	123
6-Gingerol	293.1752	16.61	178
Terflavin A	1131.0860	16.68	283
6-Hydroxy-2[2(4'methoxyphenyl)-ethyl] chromone	341.1048	18.61	1077

The S.E. extract featured notable metabolites, including Yakuchinone A (RT = 16.69 min; response = 1065), Kosamol A (RT = 16.67 min; response = 88), and 6-Gingerol (RT = 17.79 min; response = 4950). Yakuchinone A, a phenolic compound, was present in substantial quantities and is well-known for its anti-inflammatory and anti-oxidant properties, attributed to its capacity to inhibit

oxidative stress and prevent protein denaturation [17]. Kosamol A and 6-Gingerol also play a role in anti-inflammatory processes through similar pathways [18]. On the other hand, the U.A.E. extract exhibited a greater variety and preservation of bioactive compounds. Noteworthy metabolites included Curcumin (RT = 16.62 min; response = 309), Demethoxycurcumin (RT = 16.61 min; response = 123), and Mulberrofuran D (RT = 16.54 min; response = 167). Curcumin and its derivatives are highly regarded for their therapeutic benefits, especially in mitigating inflammation and oxidative stress by influencing cytokine activity. The shorter extraction duration and controlled temperature in UAE were more effective in preserving these temperature-sensitive metabolites compared to S.E..

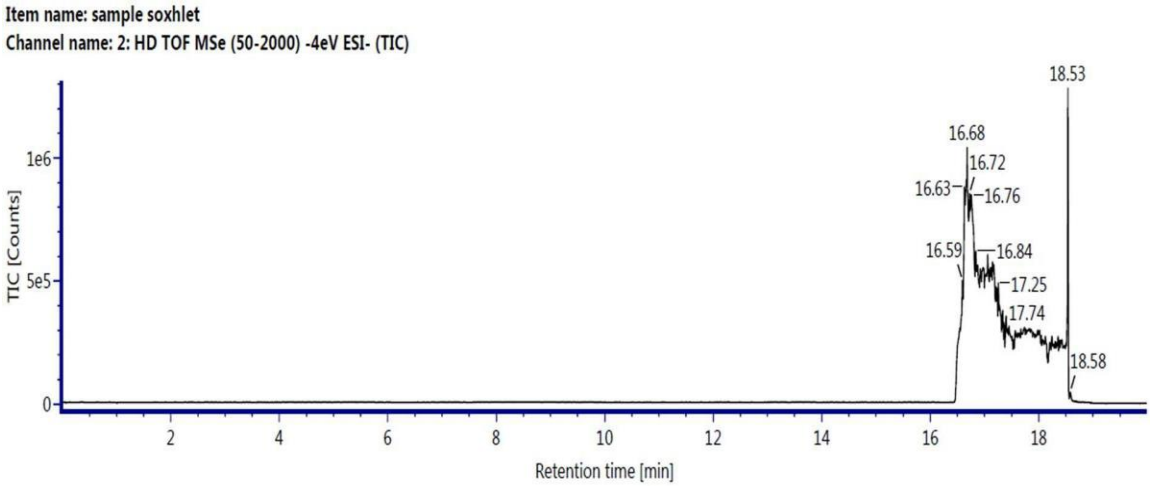

Figure 2 Total Ion Chromatogram of LC-MS QTOF of *Curcuma longa* in S.E.

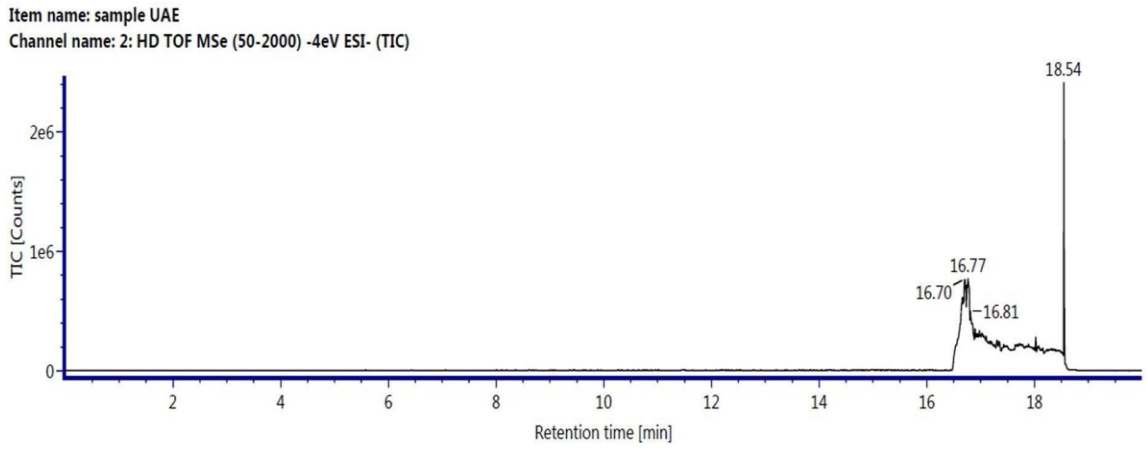

Figure 3 Total Ion Chromatogram of LC-MS QTOF of *Curcuma longa* in U.A.E.

The metabolites identified in samples are influenced by the specific extraction technique employed. S.E. was effective in isolating yakuchinone A through prolonged heating, but it adversely affected heat-sensitive curcumin when exposed to high temperatures. In contrast, ultrasonic extraction at lower temperatures in U.A.E. was more successful in preserving curcuminoids and phenolics in their natural form compared to traditional heat-based methods like Demethoxycurcumin and Curcumin.

The extraction parameters and choice of solvent also played a role in the chemicals extracted. U.A.E. improved solvent penetration into the plant matrix, aiding in the isolation of metabolites such as Curcumin and Mulberrofuran D. Meanwhile, ethanol, a moderately polar solvent, was effective in extracting hydrophobic molecules like 6-Gingerol and Yakuchinone A [19].
These results demonstrate that both S.E. and U.A.E. methods offer distinct benefits. S.E. is suitable for isolating compounds stable at elevated temperatures, whereas U.A.E. extraction maintains a wider range of bioactive compounds, especially those sensitive to heat [20]. The metabolite profiles suggest that *Curcuma longa* extracts, regardless of the extraction method, have considerable therapeutic potential due to their abundant phenolic and curcuminoid content.

3.2 Anti-oxidant properties of different extraction method of *Curcuma Longa*

The antioxidant activity was assessed by measuring the IC50 value of hydrogen peroxide (H_2O_2) for ascorbic acid, which served as the standard, and for *Curcuma longa* extracts obtained through both Soxhlet and Ultrasonic-assisted extraction methods. As depicted in Figure 4, the IC50 value for ascorbic acid is notably lower than those for both the S.E. and U.A.E. extracts, indicating that ascorbic acid exhibits the most potent scavenging activity among the samples. The IC50 value for the S.E. extract is 269 µg/mL, which is slightly less than that of the U.A.E. extract at 295 µg/mL. This suggests that the S.E. method produces an anti-oxidant extract with marginally greater strength than the U.A.E. method. Nonetheless, both extraction techniques display significant anti-oxidant properties. The slight variation in the anti-oxidant capacity of the S.E. may be attributed to its prolonged extraction time at elevated temperatures, which can enhance the release of bioactive compounds that contribute more to anti-oxidant properties [21]. Conversely, the U.A.E. method offers a quicker and more efficient process but results in slightly lower anti-oxidant activity due to its shorter duration and lower temperature.

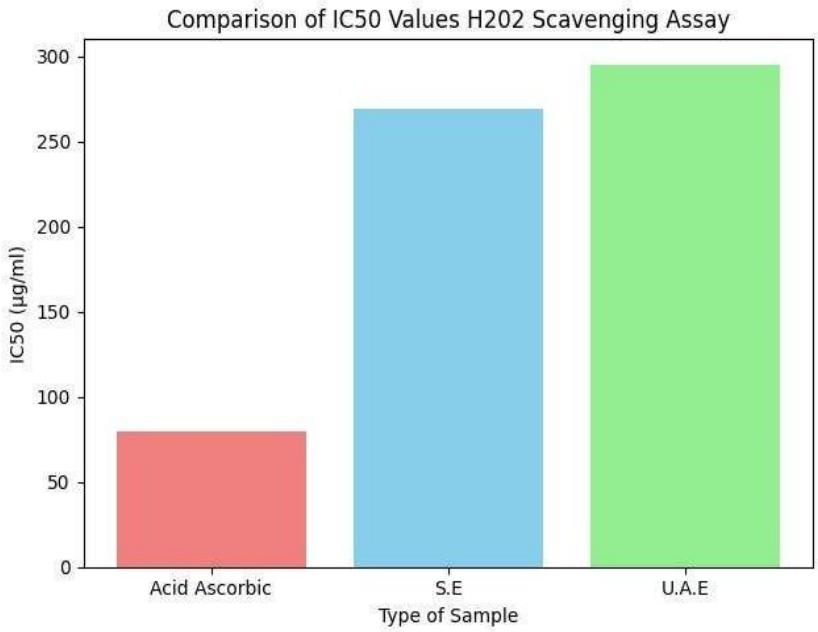

Figure 4 IC50 of anti-oxidant activities

3.3 Anti-inflammatory activities of *Curcuma Longa*

The quantities of sodium diclofenac and two distinct *Curcuma longa* extracts required to suppress inflammation were assessed using the same BSA denaturation test as the standard diclofenac. As

depicted in Figure 5, the half-maximal inhibitory concentration (IC50) indicates the amount of each sample needed to prevent protein conformational changes, a crucial step in inflammation. In the BSA denaturation test, sodium diclofenac inhibits protein damage at 179.88 micrograms per milliliter.

The tests revealed that the U.A.E. extract was the most potent, with an IC50 value of 213.79 µg/mL, followed by the S.E. extract at 301.99 µg/mL. Standard sodium diclofenac exhibited the lowest IC50 value of 179.88 µg/mL. The lower IC50 value of the U.A.E. extract explains its enhanced anti-inflammatory effect, attributed to the ultrasound extraction process that extracts more anti-inflammatory compounds. The ability of *Curcuma longa* extracts to inhibit BSA denaturation is due to the presence of bioactive compounds, especially curcumin and related curcuminoids, which stabilize proteins and prevent their denaturation [22]. The U.A.E. extraction method, with its shorter processing time and reduced solvent usage, preserved more bioactive compounds compared to the S.E. method, resulting in greater anti-inflammatory activity.

These results underscore the significant anti-inflammatory potential of *Curcuma longa* extracts, particularly those obtained through U.A.E., suggesting their potential as natural anti-inflammatory agents to replace synthetic drugs in pharmaceutical formulations.

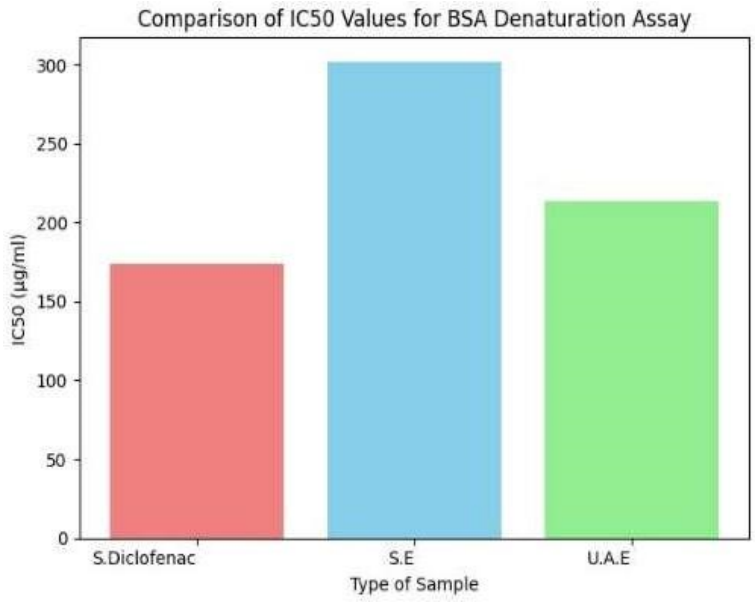

Figure 5 IC50 of anti-inflammatory activity

3.4 Anti-bacterial properties of *Curcuma Longa*

As shown in Table 3, erythromycin, the positive control, illustrates the efficacy of standard antibiotics when compared to *Curcuma Longa* extract. Meanwhile, the negative control, deionized water, confirms that the solvent is not responsible for any observed anti-bacterial activity. Both extraction samples, S.E. and U.A.E., exhibit antibacterial effects against *S. aureus*, with inhibition zones measuring 4.5 mm and 10.0 mm, respectively. However, neither extraction sample shows an inhibition zone against *E. coli*. When comparing *Curcuma Longa* extract to the positive control, erythromycin demonstrates greater anti-bacterial activity, with inhibition zones of 13.5 mm for *S. aureus* and 18.5 mm for *E. coli*. The negative control, deionized water, shows no inhibition zones for either bacterial strain, confirming that the antibacterial effects are solely due to the active

compounds in *Curcuma Longa* extract, not the solvent. The limited suppression of *S. aureus* by *Curcuma Longa* extract, with a 4.5 mm inhibition zone, is less effective compared to erythromycin. This finding underscores the superior efficacy of antibiotics over 100% concentrated *Curcuma Longa* extract in inhibiting bacterial growth. This is further supported by the lack of an inhibition zone for *E. coli* with *Curcuma Longa* extract, while erythromycin presents a 7 mm inhibition zone. Research by Khatun et al. (2021) indicated that turmeric extract was effective against various bacterial strains, including *E. coli* and *S. aureus*, with curcuminoids, particularly curcumin, being potent anti-bacterial agents that can damage bacterial membranes, reduce microbial motility, and disrupt quorum sensing. However, Chetan et al. (2022) reported that curcumin exhibits higher anti-bacterial activity against Gram-positive bacteria than Gram-negative strains. This is because Gram-positive bacteria have a thick but porous peptidoglycan layer and lack an outer membrane, making them more susceptible to bioactive compounds. In contrast, Gram-negative bacteria possess an outer membrane composed of lipopolysaccharides, phospholipids, and proteins, which acts as a barrier, providing greater resistance to many antibiotics, including plant extracts. Consistent with previous research, turmeric extract showed significant antibacterial activity against Gram-positive *S. aureus*, while no inhibition zones were observed for Gram-negative *E. coli*, indicating their higher resistance to *Curcuma Longa* extract.

Table 3 Diameter of Inhibition zone (mm) on *Curcuma Longa*

Sample	Extraction Method	Bacteria Strain	Diameter of Inhibition zone (mm)
Curcuma longa	Soxhlet	E. Coli	-
		S. Aureus	4.5
Curcuma longa	Ultrasound-	E. Coli	-
	Assisted	S. Aureus	10.0
Erythromycin	-	E. Coli	18.5
		S. Aureus	13.5
Deionized water	-	E. Coli	-
		S. Aureus	-

4. CONCLUSION

The current study explored the preliminary phytochemical analysis of the extraction process and evaluated the potential of *Curcuma Longa* extracts as anti-bacterial, anti-inflammatory, and anti-oxidant agents. When comparing extraction yields, Ultrasonic-assisted extraction (U.A.E.) resulted in a higher yield of *Curcuma Longa* extract (8.84%) compared to Soxhlet extraction (6.84%). This is attributed to the cavitation effect of the U.A.E. method, which enhances cell wall disruption and mass transfer, while also reducing extraction time and preserving heat-sensitive bioactive compounds. LC-MS QTOF analysis is crucial for studying and understanding the bioactive compounds in *Curcuma longa* that contribute to its anti-oxidant, anti-inflammatory and anti-bacterial properties. In terms of antibacterial activity against *S. aureus*, the U.A.E. extract demonstrated a larger zone of inhibition (10.0 mm) compared to the S.E. extract (4.5 mm), while neither extract showed an inhibition zone against *E. coli*. The S.E. extract exhibited stronger antioxidant activity with an IC50 value of 269.15 (µg/ml) compared to the U.A.E. extract's 295.12 (µg/ml). However, regarding anti-inflammatory activity, the U.A.E. extract had a better IC50 value (213.79 (µg/ml)) for inhibiting protein denaturation than the S.E. extract (301.99 (µg/ml)). In conclusion, *Curcuma Longa* demonstrated potential as an anti-oxidant, anti-inflammatory, and

anti-microbial agent across various extraction methods. As this research serves only as a preliminary study of *Curcuma Longa*'s properties, further investigation is needed to explore the metabolic pathways of its bioactive substances. Additionally, future studies should examine different bacterial strains and a broader range of testing parameters, such as time and temperature, to optimize extraction and minimize analysis errors, thereby enhancing outcomes.

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