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ANTIBACTERIAL ACTIVITY OF WATER HYACINTH ROOT EXTRACT (EICHHORNIA CRASSIPES) AGAINST ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

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ABSTRACT

Water hyacinth roots contain flavonoids, phenolic compounds, and tannins with potential antibacterial activity. This study assessed the effects of sonication duration on extract yield, bioactive constituents, and antibacterial activity of Eichhornia crassipes root extracts against Escherichia coli and Staphylococcus aureus. Roots were extracted with 96% ethanol and treated by sonication for 0, 20, 40, or 60 min at 20°C and 42 kHz. A completely randomised design with three replicates was employed. Extract yield, phytochemical content, and bacterial inhibition zones were assessed. Total measured bioactive content at 0, 20, 40, and 60 min was 0.06, 0.24, 0.36, and 0.23 mg/g extract, respectively. The highest reported tannin concentration was 0.16 mg TAE/g extract. At 1,000 ppm, inhibition zones against E. coli were 0.57 ± 0.2, 1.25 ± 0.1, 1.77 ± 0.2, and 0.93 ± 0.1 mm, respectively, whereas those against S. aureus were 0.77 ± 0.2, 1.40 ± 0.1, 1.97 ± 0.1, and 0.90 ± 0.1 mm. Sonication influenced phytochemical recovery, but antibacterial activity remained weak under the investigated conditions.

KEYWORDS: Water hyacinth roots; sonication; antibacterial activity; Escherichia coli; Staphylococcus aureus.

1. INTRODUCTION

Water hyacinth (Eichhornia crassipes) is an aquatic plant commonly found in wetlands, rivers, rice-field margins, and reservoirs. Its rapid growth makes it an invasive aquatic weed. Water hyacinth roots contain secondary metabolites that remain underutilised. Tyagi and Agarwal (2017) reported that phytochemical screening of the roots detected alkaloids, glycosides, flavonoids, quinones, anthraquinones, sterols, polyphenols, anthocyanins, carotenoids, proteins, and essential oils. Indrayati and Hikmah (2018) reported that water hyacinth covered approximately 731.50 ha, equivalent to 48.23% of Rawa Pening, in 2011. Its rapid growth contributes to sediment accumulation and shallowing of the lake.

Water hyacinth is capable of rapid vegetative propagation and can form extensive floating mats, making its management a persistent environmental concern. Nevertheless, its roots represent a potentially useful biomass for recovering plant-derived compounds. The phytochemical groups identified in earlier screening studies justify investigating extraction conditions; their presence alone, however, does not establish an effective antimicrobial formulation.

Antibacterial compounds interfere with or inhibit bacterial growth by disrupting cellular metabolism. Such compounds may exhibit selective toxicity against pathogenic bacteria. Escherichia coli and Staphylococcus aureus are important food-contaminating bacteria that may cause foodborne illness under certain conditions. The Indonesian National Standardization Agency (BSN) establishes microbiological limits for specified foods to protect food safety.

In this context, a plant extract's antibacterial potential should be interpreted with respect to the test organism, extract concentration, assay format, and measured inhibition zone. The two bacterial species used here provide contrasting Gram-negative and Gram-positive models for screening activity, although a disc-diffusion assay alone cannot establish a minimum inhibitory concentration or safety for use in food.

Maceration is a common and straightforward extraction technique involving immersion of the sample in a solvent, but it typically requires substantial solvent quantities and long extraction periods. Ultrasonication disrupts cellular structures through acoustic waves and promotes solvent penetration, potentially improving the recovery of secondary metabolites. Sari and Triyasmono (2017) stated that ultrasonication facilitates the release of bioactive constituents from plant matrices. This study therefore examined the effects of sonication duration on extract yield, bioactive compounds, and antibacterial activity of water hyacinth root extract against E. coli and S. aureus.

The duration of ultrasound exposure is a relevant process variable because the positive effects of cavitation on solvent penetration and mass transfer may eventually be offset by compound degradation or altered extraction conditions. Accordingly, comparisons across a conventional non-sonicated control and three sonication durations can help identify a condition that balances extract recovery with the measured phytochemical and antibacterial responses.

2. MATERIALS AND METHODS

2.1 Study Period and Location

The study was conducted from December 2019 to January 2020. Water hyacinth (Eichhornia crassipes) root extracts were prepared at the Integrated Laboratory of Universitas Diponegoro, Semarang. Phytochemical screening for flavonoids, tannins, and phenolic compounds was carried out at the Analytical Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Diponegoro. Antibacterial testing was performed at the Microbiology Laboratory of the same faculty.

2.2 Materials and Equipment

Materials included water hyacinth roots, 96% ethanol, double-distilled water, $AlCl_3$, NH_4OH, quercetin, Folin–Denis reagent, tannic acid, Na_2CO_3, gallic acid, Staphylococcus aureus FNCC 0047, Escherichia coli FNCC 0091, nutrient broth, distilled water, eosin methylene blue agar, Baird–Parker agar, and alcohol.

Equipment included an ultrasonic homogeniser (Branson 2510), analytical balance, rotary evaporator, glass stirring rods, graduated cylinders, spectrophotometer, volumetric pipettes, pipette fillers, incubator, laminar-flow cabinet, autoclave, hot plate, vortex mixer, Bunsen burner, Petri dishes, Erlenmeyer flasks, test-tube racks, and magnetic stirrer.

2.3 Sample Preparation

Water hyacinth roots were collected from Rawa Pening in Rowoboni Village, Banyubiru District, Semarang Regency, Central Java. Roots were washed with lake water, transferred to an insulated box containing ice at an ice-to-root ratio of 5:1, air-dried indoors, and cut into small pieces.

The material was subsequently prepared for ethanol extraction. These collection and transport procedures are reported as conducted; the original records do not specify independent measurement of initial root moisture content.

2.4 Root Extraction

Dried samples were extracted using 96% ethanol at a sample-to-solvent ratio of 1:25. Sonication treatments were conducted for 20, 40, and 60 min at 20°C and 42 kHz. The

resulting extracts were concentrated using a rotary evaporator at 70°C. Extraction yield was calculated using the formula given below.

A non-sonicated extraction served as the control (0 min). The design therefore comprised four sonication-duration conditions (0, 20, 40, and 60 min). The sample-to-solvent ratio is expressed as reported in the original protocol, which does not explicitly specify whether it was calculated by mass or volume.

Extraction yield (%) = (mass of dried extract / initial sample mass) × 100. (The original manuscript does not provide the mass basis used; please confirm.)

2.5 Flavonoid Determination

A 5 g sample was dissolved in 100 mL distilled water, and 25 mL of 10% acetic acid in ethanol was added. The mixture was filtered. A 1 mL aliquot of the filtrate was mixed with 2 mL of 5% $AlCl_3$ and diluted to 10 mL using distilled water. Absorbance was measured at 420 nm. Flavonoid content was determined using a quercetin calibration curve and expressed in the units reported in the study.

Flavonoid content was determined from the corresponding standard calibration curve; values are reported as standard equivalents per gram of extract.

Calibration relationship: $x = (y - a) / b$, where x denotes the estimated concentration, y the absorbance, a the intercept, and b the slope.

2.6 Total Phenolic Content

A 5 g sample was dissolved in 100 mL distilled water and filtered. A 1 mL aliquot of filtrate was combined with 0.5 mL Folin–Ciocalteu reagent and 1 mL saturated Na_2CO_3, then diluted with distilled water to 10 mL. Absorbance was read at 730 nm. Total phenolic content was calculated against a gallic acid calibration curve.

Phenolic content was determined from the corresponding standard calibration curve; values are reported as standard equivalents per gram of extract.

Calibration relationship: $x = (y - a) / b$, where x denotes the estimated concentration, y the absorbance, a the intercept, and b the slope.

2.7 Tannin Determination

A 5 g sample was dissolved in 100 mL distilled water and filtered. A 1 mL aliquot of filtrate was mixed with 0.5 mL of Folin–Denis reagent (1:1) and 1 mL saturated Na_2CO_3, then diluted to 10 mL using distilled water. Absorbance was measured at 730 nm. Tannin content was determined using a tannic acid calibration curve.

Tannin content was determined from the corresponding standard calibration curve; values are reported as standard equivalents per gram of extract.

Calibration relationship: x = (y − a) / b, where x denotes the estimated concentration, y the absorbance, a the intercept, and b the slope.

2.8 Antibacterial Activity Assay

Fresh isolates of Staphylococcus aureus and Escherichia coli were cultured by spreading them on Baird–Parker agar (S. aureus) and eosin methylene blue agar (E. coli), respectively. Paper discs measuring 10 mm in diameter were immersed in the extract solution (1,000 ppm) and placed onto the inoculated agar. Ethanol served as the negative control and amoxicillin as the positive control. Plates were incubated for 24 h at 37°C. The resulting inhibition zones were measured using a ruler.

The disc-diffusion procedure provided a comparative screening measurement of inhibition at a single extract concentration. Because the initial protocol does not provide inoculum-density standardisation, measurement conventions for the disc diameter, or replicate-level raw values, the reported inhibition-zone results should not be interpreted as a standardised clinical susceptibility test.

3. RESULTS AND DISCUSSION

3.1 Extraction Yield

Extraction yields of Eichhornia crassipes roots subjected to different sonication durations are presented in Figure 1.

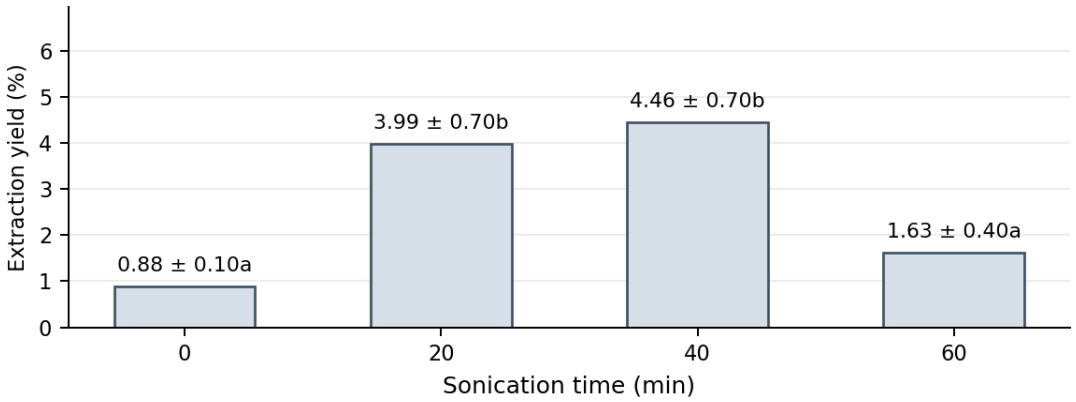

Figure 1. Extraction yield of water hyacinth root extract.

Note: Values are means of three replicates ± standard deviation. Bars with different lowercase superscript letters were reported as significantly different (P < 0.05).

Extraction with 96% ethanol and sonication produced the highest yields after 20 and 40 min, at 3.99% and 4.46%, respectively. These yields exceeded that of maceration without sonication (0.81%). Sondari et al. (2016) attributed improved extraction under ultrasonication

to thinning of the boundary layer between the sample and solvent, which enhances solvent penetration, mass transfer, and dissolution of intracellular bioactive substances.

In the original graphical data, the unsonicated control was labelled 0.88%, whereas the accompanying narrative reported approximately 0.81%. This discrepancy should be checked against the laboratory dataset before publication; the reproduced English-language figure retains the graphical value.

Sonication duration influenced extraction yield. The unsonicated treatment (0 min) produced the lowest yield compared with treatments of 20, 40, and 60 min. Yield increased up to 40 min and subsequently declined, producing an inverted U-shaped response. Prolonged sonication may damage secondary metabolites or alter extraction efficiency. Bintari and Elyani (2017) explained that mass transfer may improve only up to an optimum, after which excessive acoustic energy, temperature, or exposure duration can reduce extraction efficiency.

The response also illustrates the importance of optimising rather than merely maximising extraction time. Increasing exposure can initially improve the release of target compounds, but a higher extraction yield does not necessarily indicate greater biological efficacy. Treatment selection should therefore consider the chemical and antibacterial results jointly, without assuming that all response variables attain their maxima at the same duration.

3.2 Flavonoid Content

Quantitative flavonoid contents of the water hyacinth root extracts are shown in Figure 2.

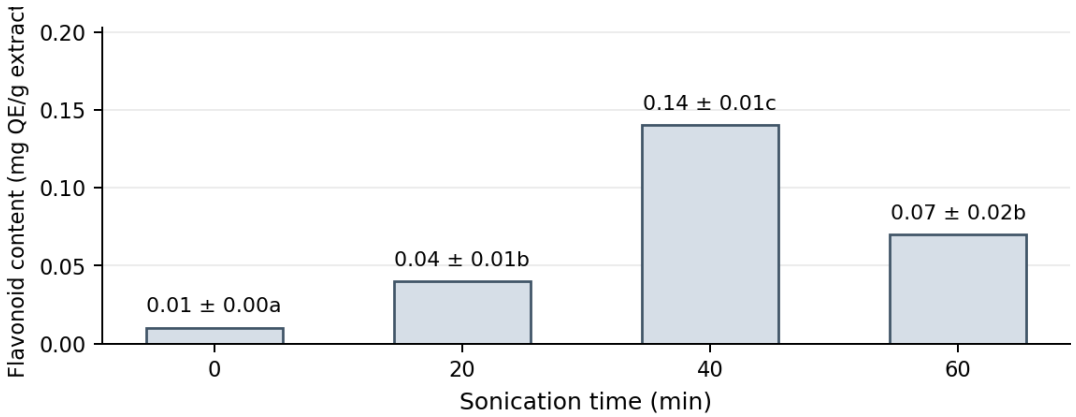

Figure 2. Flavonoid content of water hyacinth root extract.

Note: Values are means of three replicates ± standard deviation. Bars with different lowercase superscript letters were reported as significantly different (P < 0.05).

The lowest flavonoid content (0.01 mg QE/g extract) was obtained without sonication, whereas the highest (0.14 mg QE/g extract) occurred after 40 min of sonication. The response followed an inverted U-shaped pattern. Maceration without sonication extracted fewer flavonoids than ultrasound-assisted extraction. Widyasanti et al. (2018) reported that ultrasonication can extract plant metabolites more effectively than conventional maceration under comparable processing periods, as acoustic disruption facilitates mass transfer and solvent accessibility.

The 40-min condition produced the largest reported flavonoid concentration, corresponding to a marked increase over the unsonicated sample. Following this maximum, the concentration decreased at 60 min, suggesting that extending sonication does not necessarily improve flavonoid recovery. The study did not identify individual flavonoid compounds; the measured values are total flavonoid equivalents based on the quercetin standard curve.

3.3 Total Phenolic Content

Quantitative phenolic contents of water hyacinth root extracts are presented in Figure 3.

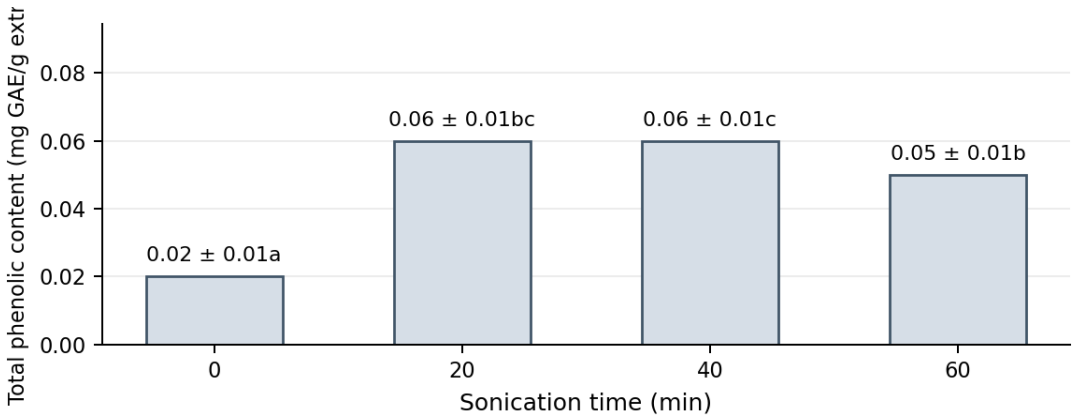

Figure 3. Total phenolic content of water hyacinth root extract.

Note: Values are means of three replicates ± standard deviation. Bars with different lowercase superscript letters were reported as significantly different (P < 0.05).

As shown in Figure 3, the lowest phenolic content was recorded without sonication (0.02 mg GAE/g extract), whereas 20 min of sonication yielded 0.06 mg GAE/g extract. The response was nonlinear, as longer ultrasound exposure facilitated the liberation of metabolites up to an optimum but could subsequently promote degradation. Yuliantari et al. (2017) explained that longer extraction may initially enhance solvent–sample contact, although excessive processing may lead to degradation or loss of bioactive compounds.

Although the values for 20 and 40 min were similar when rounded to two decimals, the graphical annotations show different superscript groupings. Statistical interpretation should therefore follow the reported significance indicators rather than relying solely on the rounded means. No chromatographic identification of phenolic compounds was presented; these results represent assay-based total phenolic estimates.

3.4 Tannin Content

Quantitative tannin contents of the water hyacinth root extract are illustrated in Figure 4.

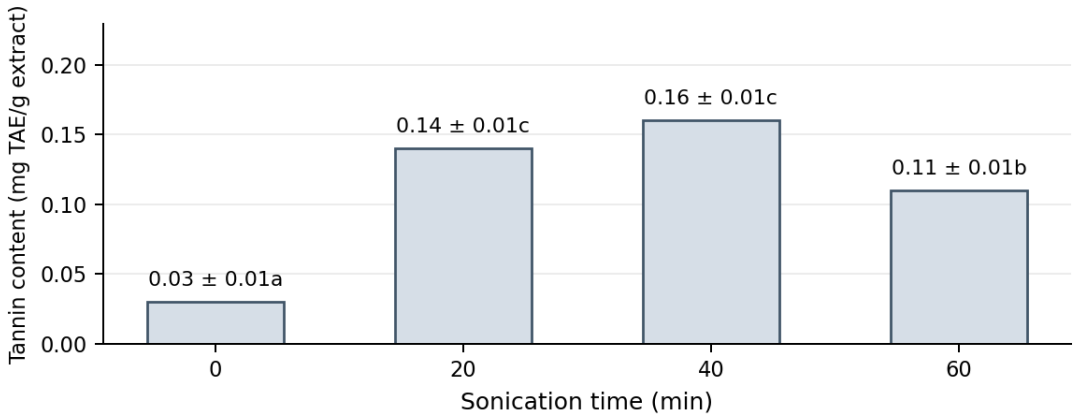

Figure 4. Tannin content of water hyacinth root extract.

Note: Values are means of three replicates ± standard deviation. Bars with different lowercase superscript letters were reported as significantly different (P < 0.05).

The source reports a tannin content of 0.14 mg TAE/g extract after 20 min and a peak of 0.16 ± 0.01 mg TAE/g extract after 40 min, compared with 0.03 mg TAE/g extract without sonication. Tannin content increased up to 40 min and decreased at 60 min, consistent with possible ultrasound-associated degradation during prolonged exposure. Tannins were the predominant measured bioactive group and may contribute to the observed antibacterial effects.

The ranking of tannin concentrations provides a plausible explanation for the increased bacterial growth inhibition at intermediate sonication durations. However, the extract is chemically complex, and the observed inhibition cannot be attributed specifically to tannins without fractionation or another mechanism-oriented experiment.

3.5 Inhibition Zone against Escherichia coli

The antibacterial activity of Eichhornia crassipes root extract against E. coli is presented in Figure 5.

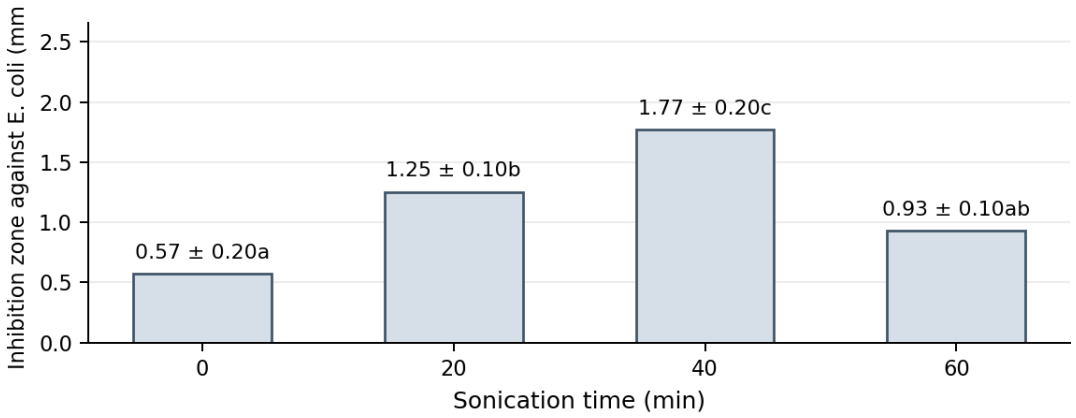

Figure 5. Inhibition zones of water hyacinth root extract against E. coli.

Note: Values are means of three replicates ± standard deviation. Bars with different lowercase superscript letters were reported as significantly different (P < 0.05).

The largest inhibition zone against E. coli occurred after 40 min of sonication (1.77 ± 0.2 mm), whereas extraction without sonication produced the smallest zone (0.57 ± 0.2 mm). Sonication treatments generally produced greater inhibition than maceration without ultrasound, and the response across durations was nonlinear. Inhibition-zone diameter is used as an indicator of antibacterial performance under the assay conditions. Apriyani et al. (2015) associated greater inhibition-zone dimensions with stronger antibacterial effects.

The 40-min extract exhibited the greatest measured antibacterial response within the evaluated conditions, but the absolute inhibition zones were small. It is therefore more precise to describe the observed response as an increase in relative inhibition under the study conditions rather than evidence of strong antibacterial potency.

Tannins present in the water hyacinth root extract (reported at up to 0.16 ± 0.01 mg TAE/g extract) may have contributed to inhibition of E. coli. Tannins can interact with bacterial cell envelopes and proteins, altering membrane permeability and cellular function. Armanda et al. (2017) discussed tannin-induced protein denaturation and potential interference with microbial enzymes, while Carolia and Noventi (2016) described effects of tannins on cell-wall or membrane integrity. These mechanisms are proposed interpretations rather than direct mechanistic measurements in the present study.

These explanations remain hypotheses based on existing literature. The present experiment quantified general phytochemical groups and inhibition zones; it did not test bacterial membrane permeability, enzyme inhibition, or the activity of purified tannin fractions.

3.6 Inhibition Zone against Staphylococcus aureus

The antibacterial activity of Eichhornia crassipes root extract against S. aureus is presented in Figure 6.

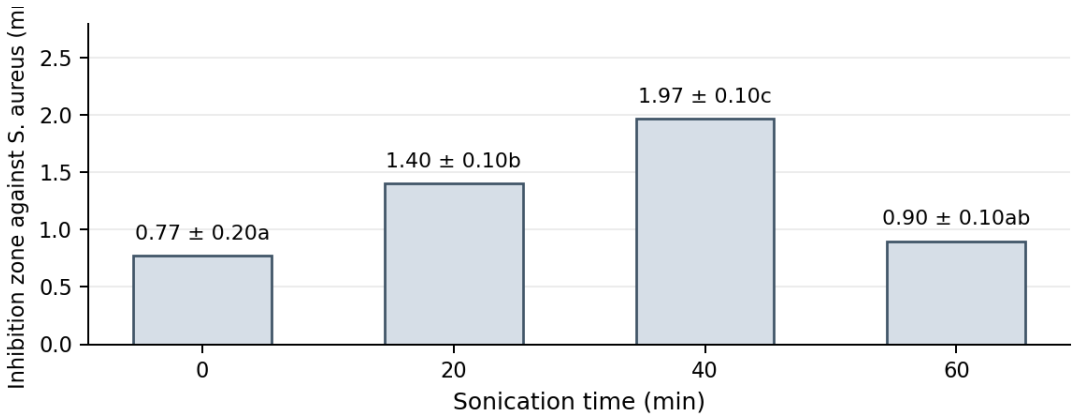

Figure 6. Inhibition zones of water hyacinth root extract against S. aureus.

Note: Values are means of three replicates ± standard deviation. Bars with different lowercase superscript letters were reported as significantly different (P < 0.05).

Sonication for 40 min produced the largest inhibition zone against S. aureus (1.97 ± 0.01 mm, as reported in the results text), whereas the smallest zone was measured without sonication (0.77 ± 0.02 mm). The resulting antibacterial inhibition was classified as weak because all zones were below 5 mm. Yutika et al. (2015) classified zones below 5 mm as weak, 5–10 mm as moderate, 10–20 mm as strong, and above 20 mm as very strong. The response to increasing sonication duration was nonlinear, with the highest reported inhibition at 40 min. Tannins were suggested to contribute to these effects.

The source narrative and graphical annotation differ in the reported uncertainty for the 40-min S. aureus result: the narrative states ±0.01 mm, while the figure shows ±0.1 mm. The English-language figure preserves the graphical value, and this inconsistency requires author verification against the original measurements.

Water hyacinth root extracts produced larger inhibition zones against S. aureus than against E. coli. This difference may be related to the distinct bacterial cell-wall architectures: Gram-positive S. aureus lacks the outer membrane found in Gram-negative E. coli, which can restrict the entry of certain compounds. Fatisa (2013) discussed the different lipid compositions of these bacterial envelopes, noting a relatively low lipid proportion in the S. aureus cell wall compared with the more lipid-rich envelope of E. coli. Such structural differences may influence sensitivity to plant-derived antibacterial compounds.

Differences in inhibition can also be influenced by the diffusion of plant-derived compounds through the agar matrix and by the intrinsic susceptibility of the tested organisms. Therefore, the observed pattern is consistent with, but does not prove, a cell-envelope mechanism.

4. CONCLUSIONS

Sonication duration significantly influenced the extraction yield and measured bioactive constituents of Eichhornia crassipes root extract. The extract exhibited weak antibacterial activity against both Escherichia coli and Staphylococcus aureus at 1,000 ppm, with inhibition zones smaller than 5 mm. Although ultrasound-assisted extraction enhanced several measured responses, the reported data should not be interpreted as evidence of strong antimicrobial efficacy.

Among the tested exposure times, 40 min gave the highest graphically reported extraction yield, flavonoid and tannin contents, and inhibition-zone diameters, whereas the original abstract described 20 min as the best treatment. A single overall optimum therefore cannot be asserted without specifying the primary response or a formal multi-criterion decision rule. Further experiments at additional concentrations, using standardised antibacterial assays and chemical characterisation, would be necessary to establish antimicrobial efficacy and clarify the preferred processing conditions.

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