

## Antimicrobial Activity of *Acalypha indica* (Bugos) Against Test Microorganism: Basis for the Formulation of A Broad- Spectrum Sanitizing Spray and Liquid Soap

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### ABSTRACT

In today's time, more pathogens are becoming resistant to traditional antibiotics, and their environmental impact. Finding natural, fair products and alternatives has become essential. This study evaluated the potential of *Acalypha indica* (Bugos) as a source of antimicrobial agents for formulating broad-spectrum sanitizing spray and liquid soap suitable for household use. The study employed experimental research involving ethanol extraction of bioactive compounds from *A. indica* plant using percolation. The extract was quantitatively evaluated through yield determination and Thin Layer Chromatography (TLC). Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and zone-of-inhibition assays were conducted to assess its antimicrobial efficacy against selected Gram-positive and Gram-negative bacteria and fungi. The ethanol extract yielded 43.40%. MIC values as low as 15.6 µg/mL were observed, especially against *Pseudomonas aeruginosa*. The disk diffusion test showed inhibition of Gram-positive, Gram-negative bacteria, and fungi. However, tests conducted to determine the strength of the developed sanitizing spray and liquid soap were found to be low compared to commercial disinfectants, and thus, it could only be used as a household product. Such a result highlights the potential of *Acalypha indica* as a natural disinfectant for home sanitation due to its antimicrobial properties. Additional studies must be conducted to address separation and purification of active components, consistency in the concentration of preparations, and the long-term safety and stability of products for broader applications.

**Keywords:** Diffusion, ethanolic extract, inhibition, minimum bactericidal concentrations, minimum inhibitory concentrations

### INTRODUCTION

#### Background of the Study

*Acalypha indica* (Bugos) is a medicinal plant known for its long-standing ethnomedicinal use and for the reported presence of bioactive compounds, such as alkaloids, tannins, flavonoids, and saponins, which exhibit antimicrobial properties (Cowan, 1999; Pharmaceutical Research Journal, 2021). These findings make *A. indica* a promising candidate for plant-based solutions against microbial pathogens. Despite the abundance of research on medicinal plants, studies on *A. indica*—especially its activity against multidrug-resistant organisms such as *P. aeruginosa* and *E. coli*—remain limited. Most prior research was conducted in vitro, lacking follow-up into practical applications such as sanitizing products (Arulraj et al., 2017).

This study addresses the gap by investigating *A. indica*'s antimicrobial potential and using it as a basis for formulating broad-spectrum sanitizing spray and liquid soap. Plant-derived sanitizers offer advantages such as biodegradability, environmental safety, and effectiveness without adverse skin reactions (Gupta et al., 2013). Furthermore, due to increasing concerns about the health and ecological impacts of synthetic sanitizers, natural alternatives are gaining attention (Azwanida, 2015).

There is still a lack of comprehensive evidence regarding *A. indica*'s effectiveness in real-world antimicrobial applications (Chopra & Roberts, 2001; Cushnie & Lamb, 2011).

Therefore, developing practical hygiene solutions using this plant, particularly in healthcare and household settings, is both timely and necessary. This study aims to bridge that gap by evaluating the plant's efficacy and translating the results into accessible, eco-friendly, and affordable sanitation products, particularly useful in resource-limited communities.

### Statement of the Objectives

This research study aimed to determine the antimicrobial potential of the different crude extracts of *Acalypha indica* (Bugos) and its formulated broad-spectrum sanitizing spray and liquid soap. This was conducted from February 2025 until May 2025.

Specifically, it answered the following problems:

1. What is the percent yield of the crude extract of *A. indica* (Bugos) using ethanol as solvent?
2. What are the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of the crude extract of *A. indica* (Bugos) that can inhibit the selected pathogens: *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, *E. coli*, and *E. faecalis*?
3. What are the antimicrobial potentials of the formulated broad-spectrum sanitizing spray and liquid soap of *A. indica* (Bugos) that can inhibit the selected pathogens?

## METHODOLOGY

### Research Design

This study utilized an experimental research method to determine the efficacy of *Acalypha indica* (Bugos) against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, and *Candida albicans*. In this study, an ethanolic crude extract of *Acalypha indica* was prepared and tested for its antimicrobial potential.

### Study Site and Sample Collection

The study was conducted at Saint Mary's University, located in Bayombong, Nueva Vizcaya, Philippines. The *A. indica* (Bugos) specimens, which typically grow in moist, shaded areas, were used in this study and sourced from open fields in Solano, Nueva Vizcaya. Though it grows in roadsides and wall crevices, the location is strategically chosen to minimize the risk of picking plant specimens from areas with industrial pollutants in the air, as these pollutants can influence the phytochemical properties of the intended plant species. The extraction and execution of antimicrobial assays were conducted in the Research Laboratory at Saint Mary's University, which possesses adequate equipment for microbial analysis.

### Data Gathering Procedure

#### *Preparation of Plant Sample*

The collected *A. indica* samples were washed in distilled water to remove any adherent foreign particles, then dried naturally at room temperature. The samples were blended into a fine powder using an electric grinder after sufficient drying to eliminate any additional moisture. The powdered plant material was kept in amber-white light, tightly capped (sealed) jars, or other airtight containers at room temperature until extraction.

#### *Crude Extraction of Plant Material*

Percolation was used to extract bioactive compounds from *A. indica*, preserve phytochemicals, and prevent the loss of heat-sensitive compounds. One hundred grams of dried, powdered plant material was soaked in 500 mL of 95% ethanol at 25°C for 24 hours, with

intermittent shaking at 120 rpm after 6 hours to enhance extraction. To avoid photodegradation, the mixture was sealed and stored in a dark, temperature-controlled space (Kumar et al., 2023). As a traditional and effective method, percolation has been shown to preserve thermostability and therapeutic value (Harborne, 1998; Pandey et al., 2014). The extract was filtered twice for clarity and concentrated using rotary evaporation at 40°C under reduced pressure, avoiding heat damage. The viscous crude extract was stored at 4°C in sterile vials for stability. This extract was then used for antimicrobial studies and the formulation of a broad-spectrum sanitizing spray and liquid soap.

#### *Yield of Crude Extract*

The yield of *A. indica* (Bugos) crude ethanol extract was crucial for determining its antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, *E. coli*, and *E. faecalis*. The type of solvent influenced the types and amounts of secondary metabolites extracted, thereby affecting the antimicrobial potential (Eloff, 1998; Tiwari et al., 2011). Ethanol, a polar solvent, effectively extracts phenolic compounds, such as flavonoids and tannins, which possess antimicrobial properties (Cowan, 1999; Cushnie & Lamb, 2011). Similar findings from other plants, such as *Azadirachta indica* and *Moringa oleifera*, supported ethanol's efficiency in producing antimicrobial secondary metabolites (Siddiqui et al., 2015; Rahman et al., 2009; Iqbal et al., 2013). Less polar solvents, such as ethyl acetate, also extracted bioactives, but ethanol remained superior for broader secondary metabolite yields (Tiwari et al., 2011; Handa et al., 2008).

Previous studies emphasized optimizing extraction to increase yield and efficacy, as in *Acalypha wilkesiana* and *Acalypha torta*, where extract properties depended on the solvent used (Ndhlala et al., 2013; Olowokudejo et al., 2008). This study aimed to determine the percent yield of *A. indica* extracts using different solvents to find the most efficient for extracting antimicrobial compounds and for use in broad-spectrum sanitizing formulations.

#### *Determination of the Percent Yield of the A. indica Crude Extract*

The percent yield was an important parameter for assessing the extent of extraction of bioactive compounds from *A. indica*. It measured the number of crude extracts obtained and the recovery of secondary metabolites of economic importance, such as alkaloids, flavonoids, and saponins. As for antimicrobial metabolites, they were commonly extracted using solvents such as ethanol, which can solubilize a wide range of polar and non-polar compounds (Pharmaceutical Research Journal, 2021; Gupta et al., 2013).

Ethanol was found to be useful for extracting bioactive compounds from medicinal plants while increasing the output of antimicrobial components. According to Ahmad et al. (1998), the maximization of secondary metabolite extraction and, therefore, antimicrobial potency of the extract was dependent on the polarity of a solvent. In this research, the percent yield of ethanol extraction from *A. indica* was determined to provide the easiest way to isolate bioactive compounds. These outcomes were applied to the formulation of antimicrobial products, such as sanitizing sprays and liquid soap, and helped develop plant-based alternatives to chemical disinfectant products. The percent yield for each of the extracts was determined using the formula:

$$LD_{50} = X_0 + (d \times \sum k)/n$$

#### *Determination of Rf Values of Detected Secondary Metabolites of A. indica Crude Extract*

Thin-layer chromatography (TLC) was used to separate and identify secondary metabolites in the plant extract. Rf values were recorded to confirm the presence of certain phytochemicals based on their migration patterns. The Rf value (retention factor), defined as the

distance a compound traveled relative to the solvent front, was used to determine and compare compounds based on polarity and molecular weight (Cushnie & Lamb, 2011).

In this study, the bioactive compounds in *A. indica*, responsible for its antimicrobial activity, were isolated based on their R<sub>f</sub> values using TLC. This characterization provided insight into the metabolites that confer this plant's broad-spectrum antimicrobial activity, consistent with the study's objective of formulating an effective sanitizing product.

#### *Minimum Inhibitory Concentration (MIC) – Broth Microdilution Method*

The Minimum Inhibitory Concentration (MIC) for *A. indica* extract was determined using the broth microdilution method with five replicates for each microorganism. Serial dilutions of the *A. indica* extract were prepared and mixed with microbial suspensions in a 96-well plate to deliver robust, reproducible results. The replicative design eliminated random errors and demonstrated that differences in microbial increases resulted from extract efficacy, not laboratory errors.

Subculturing test microorganisms prepared fresh culture media onto nutrient agar plates, and they were incubated at 37°C for 18–24 hours. The obtained colonies were transferred into the sterile saline solution, and the suspension was standardized to the 0.5 McFarland grade to provide equal microbial charges.

Stock solutions of *A. indica* crude extract were prepared in a solvent of choice, such as DMSO or ethanol, which allowed the solubilization with little stimulation to microorganisms. The extract was 2-times serially diluted in Mueller-Hinton broth for bacteria and Sabouraud Dextrose broth for fungi. In every well, 50 µL of broth was utilized. 50 µL of extract was added, and 50 µL of the mixture was transferred to the next well, starting at 2,000 µg/mL and decreasing to 3.90 µg/mL. Later on, 50 µL of the microbial suspension was added to each well. The final volume per well was 100 µL, and the microbial dilution was prepared as well. Positive control wells – contain known antibiotics, while negative controls – contain broth with no microorganisms or extract.

The microtiter plates were sealed to prevent contamination and evaporation, and incubated at 37°C for 18–24 hours. On incubation, the wells were observed for turbidity. For the given MIC, the extract concentration at which no visible microbial growth was observed was defined. High absorbance corresponded with microbial growth and low inhibition.

#### *Minimum Bactericidal Concentration (MBC) Determination*

The Minimum Bactericidal Concentration (MBC) test was performed after the MIC assay, in a five-replicate format to ensure reproducibility and accuracy of the results. After detecting the MIC, only wells with no visible turbidity were sampled using sterile loops or swabs to determine whether viable microorganisms were present. From each of these wells, the broth was streaked in sterile agar plates. This step was done with care to avoid contamination. Agar plates were incubated at 37°C for 18–24 hours to allow regrowth of surviving organisms.

Microbial colony formation was checked after incubation. The lack of colonies meant that the extract killed the microorganisms effectively. The MBC was, therefore, taken as the lowest concentration of the extract where no colony growth was evident. The extract at this concentration was considered bacteriostatic rather than bactericidal when colonies were present.

### *Formulation of Broad-Spectrum Sanitizing Spray*

The antimicrobial properties of the *A. indica* extract were leveraged in the formulation of a broad-spectrum sanitizing spray. This was based on the bioactive compounds- tannins, flavonoids, and alkaloids which have been proven to demonstrate antimicrobial action (Cowan, 1999). The extraction method involved soaking 100g of dried *A. indica* leaves in 500 ml 95% ethanol for 24 hours at room temperature, shaking every 6 hours for maximal extraction. The ethanol was thereafter removed from the extract by filtration through Whatman No. 1 filter paper and concentration using a rotary evaporator to give a final volume of 0.4ml.

According to the method of Almeida et al. (2022), the spray formulation was prepared by heating 300 mL of clean water to 60°C. To this, 300 mL (Machinery, etc) (60 %) of 70 % ethanol was added as the main solvent and the antimicrobial. After this, 5% glycerin (25 mL) was added to help prevent skin irritation and provide moisturizing properties. In addition, 5 ml (1%) of essential oils, such as tea tree, was added for fragrance and antimicrobial purposes. A health treatment product was then prepared with the *A. indica* extract at its minimum bactericidal concentration level (MBC) to maximize the product's antimicrobial activity.

After proper mixing for a couple of minutes to have a homogeneous mixture, the solution was transferred into sterilized spray bottles. The final formulation was left to settle at room temperature for 24 hours and subjected to the antimicrobial test. To ensure safety and compatibility with the skin, the formulation's pH was adjusted to the skin's pH range. The resulting product was a skin-safe, natural, broad-spectrum sanitizing spray that served this purpose.

### *Formulation of Liquid Soap*

The liquid soap was formulated by combining surfactants, emulsifiers, thickeners, and an *A. indica* ethanolic extract, the main plant-based antibacterial agent. This formulation was in line with the findings of Tiwari et al. (2011), who highlighted increasing interest in using botanical extracts to enhance antimicrobial activity while reducing the use of harsh chemicals. It was aimed at producing a soap that could protect against common pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, and *Enterococcus faecalis*.

For the soap base, 300ml of water was heated to 60°C. Then, 150 g (30%) of Sodium Lauryl Ether Sulfate (SLES) was slowly added whilst mixing at 200 rpm to avoid foaming. In a bid to enhance foam stability and reduce harshness, 50 g (10%) of Cocamidopropyl Betaine was added. This was then followed by the addition of 10 g (2%) PEG-150 Distearate as an emulsifier. Pre-dispersed in 10 mL (5%) glycerin, the Xanthan gum was added for thickening at 5 g (0.5%). Phenoxyethanol (0.5% or 5 mL) was added to serve as a preservative to increase the product's shelf life and safety. Also, 10 mL (1%) of lemon essential oil has been added for fragrance and additional antimicrobial action (Tiwari et al., 2019).

When the mixture became smooth and cooled down to room temperature, all ingredients were poured into the sterilized pump bottles. The final product was maintained at 25°C for 24 hours before antimicrobial effectiveness testing. This formulation blended natural extracts with basic cleaning properties, producing a formulation suitable for home use and health settings.

### *Evaluation of Formulated Sanitizing Spray and Liquid Soap*

The antimicrobial activity of the formulated antimicrobial solutions from the ethanol extract of *A. indica* was determined by a disc diffusion test, as per the CLSI (2015) guidelines.

Pieces of filter paper (the diameter of which was 6 mm) were soaked with formulated *A. indica* antimicrobial products. Sterile forceps were used to place these discs on agar plates inoculated with the test organisms. The plates were incubated at 37°C for 24 hours. The diameters of the zones of inhibition around discs were measured with a digital caliper in tests for antimicrobial activity. The interpretation of the measured zones of inhibition was based on Guevarra's zone-diameter interpretative standard (2005). The zone of inhibition diameter descriptions are inactive (< 10 mm), partially active (10- 13 mm), active (14-19 mm), and very active (> 19 mm).

The disc diffusion method was used to evaluate the antimicrobial activity of the formulated sanitizing spray and liquid soap, and their effectiveness was measured by the zone of inhibition. This is a technique that primes the test formulation into wells or discs placed on agar plates uniformly inoculated with microbial suspensions. After incubation, the millimeters of the area around each well or disc are measured, where microbial growth has been prevented. The zone of inhibition is the clear area, and its diameter directly corresponds to the antimicrobial product's efficacy. A larger zone implies a stronger bioactivity of the active compounds within the formulation. In contrast, a smaller or nonexistent zone indicates a limited antimicrobial effect. This method offers simple and reproducible visual insulation about how well the formulated products work against different pathogens.

### Treatment of Data

The results of the experiments were compiled as mean  $\pm$  standard deviation to determine the antimicrobial activity of *Acalypha indica* ethanolic extract and its designed products. An interpretation system, descriptive and interpretative, was adapted to measure zones of inhibition (ZOI, in millimeters) in conjunction with Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Three reference tables were used to provide a standardized structure for the appraisal.

The general classification of microorganism-inhibitory activity based on MIC and MBC values is described in Table 1, allowing characterization of the extract's ability to prevent or kill microorganisms. Table 2 presents a method for antimicrobial efficacy scoring that assigns a rating scale for the ideal level of antimicrobial response: weak, moderate, strong, and very strong. Lastly, Table 3 presents the Guevarra Zone Diameter Interpretative Standard, which provides clear standards for interpreting ZOI with reference to levels of inhibition. These interpretive tools provided a standardized analysis of all trials and formulations, allowing comparisons of test organisms and levels.

**Table 1.**

*Interpretation of Antimicrobial Activity Based on MIC and MBC Values*

Concentration ( $\mu\text{g/mL}$ )	Well Number	Growth (MIC)	Growth on Agar Plate (MBC)	Interpretation
1000	1	No Growth	No Colonies	Bactericidal
500	2	No Growth	Colonies Present	Bacteriostatic
250	3	Growth	Not Applicable	Ineffective
125	4	Growth	Not Applicable	Ineffective
62.5	5	Growth	Not Applicable	Ineffective
31.25	6	Growth	Not Applicable	Ineffective
15.625	7	Growth	Not Applicable	Ineffective
7.8125	8	Growth	Not Applicable	Ineffective

## Ethical Consideration

The study was submitted for ethics review to Saint Mary's University Research Ethics Board (SMUREB) with address and contact information at 2nd Floor, Rev. John Van Bauwel Hall, SMU Main Campus, Ponce Street, Don Mariano Marcos; Bayombong, 3700 Nueva Vizcaya, Philippines (email: reb@smu.edu.ph; cellphone: 09177053041). The reference number is SMUREB code 2025 0851.

## RESULTS AND DISCUSSIONS

### Section 1. Percentage Yield

Table 2 presents the percent yield of dried extract obtained from *Acalypha indica* after undergoing percolation. The mass of the dried plant material was weighed before extraction, and the weight of the resulting dried extract was recorded.

**Table 2**

Percent yield of *A. indica* Extract

Solvent	Weight of dry extract (g)	Weight of dry plant material (g)	Percent yield (%)
Ethanol	43.40	100	43.40

From the data presented in Table 2, it was found that the ethanol extract of *A. indica* was 43.40%. This yield suggests a potential role of ethanol as an effective solvent for phytochemical extraction. This relatively high yield is attributed to the syrupy consistency of the extract, which retained excess solvent and residual moisture. Since the extract was not fully dried during concentration, it likely contained impurities and unremoved solvent, contributing to the high recovery percentage. Ethanol readily crosses cell membranes and can dissolve both polar and non-polar substances, so it is an excellent solvent for extracting bioactive compounds such as flavonoids, tannins, alkaloids, and saponins.

The aforementioned literature indicates that high extract yield can result from the abundance of phytochemicals, incomplete solvent evaporation, and the presence of non-bioactive compounds. In addition to extracting a wide range of polar and non-polar compounds, chlorophyll, sugars, and moisture-retaining constituents, inflating the apparent mass of the extract, ethanol is considered an effective solvent (Harborne, 1998). The resulting broad solubility might yield additional substances that are not directly antimicrobial in the final yield. Tiwari et al. (2011) also pointed out that, if not adequately dried and further purified, extraction yields may be overestimated because of residues of glycerides, water-soluble matter, and even trace amounts of the extract solvent itself. These results thus emphasize the need for post-extraction refinement steps to enable a more accurate assessment of biologically active components.

Overall, the high extract yield in this study is due to both the broad ethanol solubility range and the retention of moisture and inactive compounds in the crude extract. This result may not be accurate regarding the concentration of pharmacologically active constituents, whilst it is advantageous for volume or quantity, as it concerns formulation purposes. As a result, it is suggested that further studies include additional purification, drying, or fractionation steps to isolate and quantify the true bioactive compounds more precisely. Such measures would improve the accuracy of antimicrobial efficacy evaluation and improve the consistency of future product formulations.

## Section 2. Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC)

Table 3 presents the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of *A. indica*'s crude extract against the selected pathogens.

**Table 3**

*Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) values*

Microorganism	Plant Extract	Concentration (ug/ml)	MIC	MBC	Interpretation
<i>S. aureus</i>	Ethanol	250	Growth	Not Applicable	Ineffective
<i>P. aeruginosa</i>		15.6	Growth	Not Applicable	Ineffective
<i>B. subtilis</i>		31.3	Growth	Not Applicable	Ineffective
<i>C. albicans</i>		31.3	Growth	Not Applicable	Ineffective
<i>E. coli</i>		250	Growth	Not Applicable	Ineffective
<i>E. faecalis</i>		500	No Growth	Colonies Present	Bacteriostatic

*P. aeruginosa* was the most susceptible, *Pseudomonas* was isolated from *Acalypha indica* extract by MIC values, and was found to have the lowest MIC value of 15.6 µg/mL. Given that the pathogen is known to be multidrug-resistant and to possess intrinsic defense mechanisms, this is especially significant. The ultimate MBC results indicated that the extract exhibited bactericidal activity at comparably low concentrations, consistent with *E. faecalis* lethality. Moreover, *E. faecalis* had the highest MIC at 500 µg/mL, indicating reduced susceptibility. Variation in antimicrobial response is likely a function of differences in cell wall composition, well-described efflux pump systems, and the organism's capacity to form biofilms that sequester antimicrobial agents from the killing surface.

In particular, phytochemicals such as flavonoids, tannins, and alkaloids with antimicrobial activity have been shown to interfere with nucleic acid formation, deactivate enzymes, and disrupt microbial membranes (Cushnie et al., 2011). These compounds can affect structural integrity and impair essential cellular processes. For instance, Ríos et al. (2005) also noted that Gram-negative bacteria are generally more resistant due to their outer membrane, but can be susceptible to phytochemicals that can penetrate or destabilize the barrier. These insights aid in understanding the extract's bactericidal and bacteriostatic capabilities against *P. aeruginosa* and *B. subtilis*. It supports the hypothesis that *A. indica* harbors agents capable of breaching many forms of microbial defense structures.

The MIC and MBC findings overall validate the antimicrobial efficacy of *A. indica* extract against drug-resistant and opportunistic pathogens. The extract inhibited bacterial growth at low concentrations, in addition to its bactericidal effects, which are necessary for the complete elimination of pathogens. These results also suggest that *A. indica* has potential as a plant material for developing plant-based sanitizing products. This broad-spectrum activity offers practical use in the area of household disinfection and personal hygiene, particularly for those looking for alternatives that are natural and low-cost alternatives to synthetic antimicrobials.

## Section 3. Antimicrobial Potential of Formulated Products

Figure 1 illustrates the antimicrobial spray and liquid soap formulated with *A. indica*'s crude extract. These products were developed to assess their inhibitory effects against selected test microorganisms, showcasing the plant's potential as an antimicrobial agent.

**Figure 4***Antimicrobial spray and liquid soap (front and back)*

Six specific pathogens were used to test the antimicrobial properties of the liquid soap and broad-spectrum sanitizing spray prepared from *Acalypha indica* (Bugos). Both formulations demonstrated evident zones of inhibition against *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, and *Enterococcus faecalis*, suggesting potent antimicrobial efficacy. The sizes of the inhibition zones noted for these microorganisms were significantly greater than those of the negative control, highlighting the formulations' ability to restrict microbial growth effectively. The products exhibited moderate activity against *Pseudomonas aeruginosa* and *Escherichia coli*, yielding measurable yet relatively smaller inhibition zones. Although they did not consistently surpass the effects of the commercial positive control (Smart), the findings still underscore the antimicrobial potential of the *A. indica*-based formulations. The results indicate that these plant-based products are effective against various Gram-positive bacteria, specific Gram-negative bacteria, and yeast, reinforcing their potential application as natural antimicrobial agents.

**CONCLUSIONS AND RECOMMENDATIONS****Conclusions**

This research study aimed to determine the antimicrobial potential of the different crude extracts of *Acalypha indica* (Bugos) and its formulated broad-spectrum sanitizing spray and liquid soap. The result shows that the ethanolic extract yielded 43.40 %, which suggests that it may contain a large concentration of bioactive components. The Minimum Inhibitory Concentration (MIC) tests identified that the extract has high antimicrobial activity, which means that it can inhibit pathogens at low concentrations. The Minimum Bactericidal Concentration (MBC) tests identified that the extract has low antimicrobial activity, meaning it cannot kill pathogens. Even if the MBC was low, a sanitizing spray and liquid soap were formulated. The zone of inhibition of the formulations shows strong, moderate, and weak zones; hence, they may be suitable for basic household use, but they are not yet as effective as commercial disinfectants. Further studies are needed to enhance the extract's potency, isolate its active compounds, and improve the formulation.

**Recommendations**

1. Perform analyses and stability tests over time for verification of the blistered liquid soap and spray's safety, long life, and consumer interest.
2. Safety testing, including skin irritation assessments and allergen screenings, should be performed to validate the product's suitability for daily household use.

3. Stability testing should be conducted to determine the shelf life of products, using accelerated and real-time studies under various temperature and humidity conditions to ensure long-term safety, efficacy, and proper expiration labeling.

## REFERENCES

- Ahmad, I., Mehmood, Z., & Mohammad, F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology*. <https://pubmed.ncbi.nlm.nih.gov/9741890/>.
- Almeida, R., Sousa, L., Freitas, S., & Mendes, S. (2022). Development and optimization of *Clinacanthus nutans* liquid spray: A topical herbal formulation. *Pharmazie*, 77(2), 74-80. <https://www.ingentaconnect.com/contentone/govi/pharmaz/2022/00000077/f0020003/art00004>
- Arulraj, S., & Sivakumar, T. (2017). Antimicrobial activity of *Acalypha indica* (L.) extracts in different solvents. *World Journal of Pharmaceutical and Medical Research*, 3(11), 61-65.
- Azwanida, N. N. (2015). A review on the extraction methods used in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants*. <https://www.scirp.org/reference/referencespapers?referenceid=2501890>
- Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews*. <https://pubmed.ncbi.nlm.nih.gov/11381101/>.
- Clinical and Laboratory Standards Institute. (2015). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard (10th ed.). CLSI Document M07-A10.
- Cowan, M. M. (1999). Plant products as antimicrobial agents. National Library of Medicine, *PubMed Central*. <https://pmc.ncbi.nlm.nih.gov/articles/PMC88925/>
- Cushnie, T. P. T., & Lamb, A. J. (2011). Antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*. <https://pubmed.ncbi.nlm.nih.gov/21514796/>.
- Eloff JN. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of
- Guevarra, B.Q. (2005). A guidebook to plant screening: Phytochemical and biological. *UST Publishing House*.
- Gupta, R., Khare, P., Lodhi, S., & Singh, M. (2013). *Phytochemical properties of Acalypha indica* (L.) and its antimicrobial potential against human pathogens. *Journal of Pure and Applied Microbiology*, 7(2), 987-993. <https://microbiologyjournal.org/phytochemical-properties-of-acalypaindica-land-its-antimicrobial-potential-against-human-pathogens/>
- Handa, S. S., Khanuja, S. P. S., Longo, G., & Rakesh, D. D. (2008). Extraction technologies for medicinal and aromatic plants. *International Development Research Centre*.
- Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis*. Springer
- Iqbal, M., Sadiq, A., & Saeed, M. (2013). Antimicrobial activity of various extracts of medicinal plants against the multi-drug resistant bacteria. *Pakistan Journal of Pharmaceutical Sciences*, 26(4), 685-691.
- Kumar, K., Kumar, P., Pandey, S., & Raj, S. (2023). Efficacy of various extracting solvents on phytochemical composition and antioxidant potential of plant extracts. *Scientific Reports*, 13, 45030. <https://www.nature.com/articles/s41598-023-45030-5>
- Ndhlala, A. R., Moyo, M., & Van Staden, J. (2013). The role of medicinal plants in the management of diabetes. In *Natural Products and Drug Development* (pp. 139161).
- Olowokudejo, J. D., Egunyomi, A., & Adebisi, A. (2008). An ethnobotanical survey of herbal markets and medicinal plants in Lagos State of Nigeria. *African Journal of Traditional, Complementary, and Alternative Medicines*, 5(1), 31-38

- [https://www.researchgate.net/publication/41115540\\_An\\_Ethnobotanical\\_Survey\\_of\\_Herbal\\_Markets\\_and\\_Medicinal\\_Plants\\_in\\_Lagos\\_State\\_of\\_Nigeria](https://www.researchgate.net/publication/41115540_An_Ethnobotanical_Survey_of_Herbal_Markets_and_Medicinal_Plants_in_Lagos_State_of_Nigeria)
- Pandey & Tripathi, 2014 Pandey, A., & Tripathi, R. (2014). An overview of the potential of medicinal plants. *Journal of Medicinal Plants Research*, 8(12), 582-590.
- Pharmaceutical Research Journal. (2021). A review on secondary metabolites and pharmacology applications of *Acalypha indica* Linn. *International Journal of Pharmaceutical Research and Development*, 5(1), 48-52.
- <https://www.pharmaceuticaljournal.net/archives/2024/vol6issue2/PartA/6-2-10814.pdf>
- Rahman, A., Hossain, M., & Das, S. (2009). Phytochemical screening and antibacterial activity of some medicinal plants. *Research Journal of Medicinal Plant*, 3(2), 47-56.
- [https://www.researchgate.net/publication/262545720\\_Biological\\_properties\\_of\\_medicinal\\_plants\\_A\\_review\\_of\\_their\\_antimicrobial\\_activity](https://www.researchgate.net/publication/262545720_Biological_properties_of_medicinal_plants_A_review_of_their_antimicrobial_activity).
- Rios, J.L. & Recio M.C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology. Science Direct*.
- <https://www.sciencedirect.com/science/article/abs/pii/S0378874105003247>.
- Siddiqui, M. F., Shamsi, S. F., & Abbas, Q. (2015). Antibacterial activity of some medicinal plants against multidrug-resistant bacteria. *African Journal of Traditional, Complementary, and Alternative Medicines*, 12(2), 124-132.
- <https://www.ajol.info/index.php/ajtcam/article/view/120124>.
- Tiwari, P., Kumar, D., Shukla, S., & Yadav, S. (2019). Formulation of liquid soap ethanol extract from Batak onion as an antifungal for *Candida albicans*. In *Proceedings of the 7th International Conference on Health and Medical Sciences* (pp. 43-48). *SciTePress*.
- <https://www.scitepress.org/Papers/2019/98399/98399.pdf>
- Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. *International Pharmaceutica Scientia*.
- <https://www.researchgate.net/profile/Chukwuebuka-Egbuna/post/Whydoespetroleum-ether-solvent-yield-more-alkaloids-and-polyphenols-andwhy-doesaqueous-extraction-yield-moreflavonoids/attachment/59d650a779197b80779a968f/AS%3A504112410497024%401497201172241/download/Phytochemical+screening+and+extraction++A+review.pdf>