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Antibacterial activity of a fraction of *Senna alata L.* leaves against *Staphylococcus aureus*: A comparative study of solvent types and concentrations

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ABSTRACT

Skin infections occur when microorganisms enter the bloodstream, spread to other organs, multiply, and cause severe illness. One of the common causative agents is *Staphylococcus aureus*. Thank to their phytochemical content, the *Senna alata L.* leaf have been used traditionally for antibacterial properties. Herein, we report the utilize of *Senna alata L.* leaf extract as an antibacterial agent against the *Staphylococcus aureus* grow up using a two-solvent fractionation method i.e. water and 96% alcohol at concentrations of 40%, 50%, 60%, and 70%. The phytochemical screening and disc diffusion tests were adopted to determine the phytochemical compounds and antibacterial efficacy of the fraction, respectively. Further, the statistical analysis including normality and homogeneity tests followed by a two-way ANOVA to evaluate the effects of solvent and concentration. The phytochemical screening detected the presence of alkaloids, flavonoids, tannins, and saponins in both fractions. The antibacterial efficacy results shows that the water and alcohol fractinations at a concentration of 70% produced the greatest antibacterial effect, with an inhibition zone diameter of 18 ± 0.18 mm and 18.90 ± 0.32 mm, respectively. A two-way ANOVA revealed a significant interaction (p = 0) between solvent type and concentration, indicating that antibacterial potency depends on both factors. These results confirm that the *Senna alata L.* leaf extract has a good potential for use as an antimicrobial agent.

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1. INTRODUCTION

The global burden of bacterial disease remains a serious public health concern, with infections caused by the opportunistic pathogen *Staphylococcus aureus* posing a particularly significant challenge. As a Gram-positive pathogen, *Staphylococcus aureus* is implicated in a wide range of clinical conditions, ranging from localized skin and soft tissue infections to severe invasive syndromes, including bloodstream infections and pulmonary involvement [1]. The World Health Organization (WHO) has classified antimicrobial resistance (AMR) as one of the top 10 global health threats. *Staphylococcus aureus* is of particular concern due to its ability to develop complex resistance mechanisms, such as the expression of the mecA gene, which alters Penicillin-Binding Protein 2a (PBP2a), thereby leading to the emergence of Methicillin-Resistant *Staphylococcus aureus* strains [2].

Nationwide, the prevalence of MRSA in Indonesia is showing an alarming trend. Surveillance data from 2024 indicates a prevalence rate of 44% in certain clinical isolates [3]. This urgency is particularly evident in West Sumatra Province, where Dr. M. Djamil General Hospital in Padang has recorded similar resistance rates in joint fluid specimens [3]. In addition to clinical settings, the risk of *Staphylococcus aureus* contamination in West Sumatra also extends to the public health sector, with reports of bacterial counts ranging from 2.5×10^6 to 4.9×10^6 CFU per sample in street food in the

Padang area. This situation has created an urgent need for the discovery of effective and safe alternative antibacterial agents [4].

Staphylococcus aureus is a human pathobiont which frequently colonises the anterior nares and predisposes to infection, ranging from localised skin infection to systemic, life-threatening complications of bloodstream infection. Host- and pathogen-related factors affecting human colonisation dynamics have been reviewed recently [5] and are key to developing interventions for minimising or preventing Staphylococcus aureus infection risk, especially in higher-risk groups or populations [6]. *Staphylococcus aureus* is a leading cause of infection-related mortality worldwide, representing the only bacterial pathogen other than *Mycobacterium tuberculosis* with an attributed number of deaths that consistently exceeds one million annually [7]. Most severe *Staphylococcus aureus* infections occur in the hospital setting, often in predisposed patients, such as those with immune deficiencies or those undergoing surgery [8, 9]. Most of these infections are lower respiratory tract, blood, and peritoneal or intra-abdominal infections. Furthermore, *Staphylococcus aureus* is a common source of mild to moderate skin infections in the community.In some individuals, *S aureus* skin infections can be chronic or recurring. Moreover, *Staphylococcus aureus* has been implicated in atopic dermatitis (eczema) [10, 11].

Senna alata L. is a tropical plant that thrives in West Sumatra and has long been used in traditional medicine to treat skin infections [12]. The phytochemicals in *Senna alata L.* leaves contain abundant secondary metabolites, including flavonoids (kaempferol), anthraquinones, tannins, and saponins, which work synergistically to disrupt the integrity of bacterial cell membranes and inactivate vital enzymes [13]. A recent study conducted in 2026 confirmed that extraction using polar solvents such as alcohol yields superior antibacterial efficacy against pathogenic bacteria [14].

Although research on the antibacterial activity of *Senna alata L.* leaf extract has been widely reported, most studies are still limited to laboratory-scale in vitro testing without standardization of extract parameters in relation to specific local isolates from regions with high resistance prevalence, such as West Sumatra. There is a data gap regarding the efficacy of this extract against *Staphylococcus aureus* strains directly isolated from public and clinical environments in West Sumatra, as well as how the standardization of its minimum inhibitory concentration (MIC) can be applied in the development of pharmaceutical formulations that are stable in terms of bioavailability. Given these problems, in this study, we evaluate of *Senna alata L.* leaf extract as an antibacterial agent against the *Staphylococcus aureus* grow up using a two-solvent fractionation method i.e. water and 96% alcohol at concentrations of 40%, 50%, 60%, and 70%. The phytochemical compounds and antibacterial efficacy of the fraction were evaluate using phytochemical screening and disc diffusion tests, respectively. From the phytochemical screening detected the existence of alkaloids, flavonoids, tannins, and saponins in both fractions. The antibacterial efficacy results shows that the water and alcohol fractinations at a concentration of 70% produced the greatest antibacterial effect, with an inhibition zone diameter of 18 ± 0.18 mm and 18.90 ± 0.32 mm, respectively. The results are expected to provide a strong scientific basis for the standardization of local herbal plants as candidates for modern antibacterial formulations capable of addressing the challenges of antibiotic resistance in the future.

2. RESEARCH METHODS

2.1. Materials

The raw material used is Senna alata L. leaves as an antibacterial agent was collected from Padang Pariaman Regency, West Sumatra, Indonesia. The analytical grade (p.a.) chemicals include 96% alcohol, ethyl acetate, chloroform, distilled water, sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), and Mayer's reagent were purchase from several market place. For microbiological testing, satellite agar media, test bacterial suspensions (bacteria from the umbilical area), disinfectants, antiseptics, and Gram stains (gentian violet, iodine, and safranin) were used. The filter paper, aluminum foil, plastic wrap, paper disks, thin layer chromatography (TLC) plates, and lean beef as a test substrate were used as the supporting materials.

2.2. Sample Preparation and Extraction Fractionation of *Senna alata L.* leaf

2.2.1. 96% Alcohol Fraction

The 2 kg of *Senna alata L.* leaf were thoroughly washed, chopped, and dried using the air-drying method, followed by oven drying at 40°C until a constant moisture content was achieved. The dried leaf was then ground into a powder using a blender. The 0.5 kg of the dried leaf powder was macerated in a dark glass container using 1500 ml of 96% alcohol for 3 × 24 hours, with manual stirring every 24 hours. The filtrate was then filtered using filter paper and concentrated using a water bath (or rotary evaporator) to obtain a concentrated extract of *Senna alata L.* leaf.

2.2.2. Water Fraction

The 2 g of concentrated extract of *Senna alata L.* leaf was dissolved in 62.5 ml of hot distilled water in a beaker. The solution was then transferred to a separatory funnel, and an equal volume of chloroform with solution volume ratio (v/v) of 1:1 was added. The mixture was homogenized manually and allowed to stand for 10 – 15 minutes until two completely separate phases formed. The aqueous phase (polar phase) that formed was separated, then evaporated using a water bath to obtain a concentrated water fraction extract of *Senna alata L.* leaf.

2.3. Phytochemical Screening

Phytochemical screening was performed using thin-layer chromatography (TLC). The stationary phase used was a 9 × 4 cm TLC plate, with the lower and upper boundaries set at 1 cm each.

2.3.1. Alkaloid Analysis

Water and 96% alcohol fractions were spotted at the starting line of the TLC plate, then eluted using a chloroform:ethyl acetate solvent system (7:3 v/v). Compounds were detected by spraying with Mayer's reagent. Visualization of the spots was observed under ultraviolet (UV) light, and the retention factor (Rf) values were calculated based on the migration distance of the solute relative to the mobile phase.

2.3.2. Flavonoid Analysis

The samples were spotted on TLC plates and eluted using a mixture of ethyl acetate and 96% alcohol (3:2 v/v). Flavonoids were identified by spraying with a NaOH solution. The resulting color changes were observed under a UV light, after which the Rf values were calculated.

2.3.3. Tannin Analysis

The separation was performed using a 96% alcohol:distilled water eluent (6:4 v/v). The presence of tannins was identified by spraying with FeCl3 reagent. The chromatogram results were observed under a UV lamp to determine the Rf value for each fraction.

2.3.4. Saponin Analysis

The presence of saponins test was conducted in a test tube; 1 ml of the water and 96% alcohol fraction was placed in a test tube, then 8 ml of distilled water and 2 ml of HCl were added. The mixture was shaken vigorously to observe the formation of persistent foam, which serves as a positive indicator of the presence of saponins.

2.4 Preparation of Bacterial Inoculum

2.4.1. Preparation of Nutrient Agar (NA) Medium

Nutrient Agar medium is prepared by extracting 250 g of lean beef into 1000 ml of distilled water through a boiling process until the remaining filtrate volume is approximately 500 ml. The filtrate is then filtered using filter paper into an Erlenmeyer flask. To this filtrate, 7 g of agar and 1 g of sucrose (sugar) are added, then heated on an electric hot plate while stirring constantly with a stirring rod until boiling. The homogenized medium is then aseptically poured into sterile Petri dishes and allowed to solidify at room temperature.

2.4.2. Bacterial Inoculation and Incubation

The inoculation procedure was performed using the swab method. A sterile cotton swab was first moistened with a NaCl solution, then swabbed over the inner area of the navel using uniform circular motions. The swabbed material was immediately inoculated onto the surface of Nutrient Agar in a petri dish using the streaking technique. After inoculation, the petri dish is tightly covered with plastic wrap to maintain humidity and prevent contamination, then incubated in an incubator at 37°C (or room temperature depending on the research objective) for 24 hours.

2.4.3. The Gram Stain

The Gram staining procedure is performed by aseptically collecting bacterial isolates using a loop, placing them in the center of a glass slide to form a smear, and allowing them to air-dry. The smear is then immersed in a crystal violet (gentian violet) solution for 3 – 5 minutes, rinsed under running water for 10 – 15 seconds, and dried. Next, the glass slide is dipped into a Lugol's solution for 3 – 5 minutes as a mordant fixation step. The decolorization step is performed by dipping the smear into alcohol, followed by drying for 3 – 5 minutes. As a final staining step, the specimen is soaked in a safranin solution for 3 – 5 minutes, rinsed with distilled water, and dried again before observing the morphological structure of the bacteria under a microscope.

2.4.4. Preparation and Dilution of the Extract

The dilution procedure was performed on the water fraction and the 96% alcohol fraction of the concentrated extract of *Senna alata L.* leaf. Each fraction was accurately weighed using an analytical balance and placed in a glass beaker to prepare a series of concentrations in a total volume of 4 ml. The concentration variations prepared included 40%, 50%, 60%, and 70% (w/v), obtained by weighing 1.6 grams, 2.0 grams, 2.4 grams, and 2.8 grams of extract, respectively. After the solutions were homogeneous, sterile paper disks were soaked in each concentration series of both fractions for further testing.

2.4.5. Culture and Dilution of *Staphylococcus aureus*

A *Staphylococcus aureus* inoculum was aseptically collected using a loop and placed into a test tube containing 10 ml of Nutrient Broth (NB) as the initial suspension (dilution factor 10^{-0}). The mixture was homogenized and incubated at 37°C for 24 hours. Then, serial dilutions were performed by taking 1 ml of suspension from the first tube using a volumetric pipette, then transferring it into a second test tube containing 9 ml of NaCl solution to obtain a 10^{-1} dilution. This procedure was continued systematically until dilutions of 10^{-2} and 10^{-3} was reached by transferring 1 ml of suspension from the previous tube into 9 ml of NaCl, accompanied by homogenization at each dilution stage.

2.5 Antibacterial Activity Test

Antibacterial activity testing was conducted using the disk diffusion method (Kirby-Bauer) with the pour plate technique. A 10^{-3} dilution of *Staphylococcus aureus* bacterial suspension was inoculated into petri dishes containing Nutrient Agar (NA) medium. The dishes were divided into four quadrants to test various concentrations. Paper disks saturated with *Senna alata L.* leaf extracts in water and 96% alcohol fractions at concentrations of 40%, 50%, 60%, and 70% (w/v) were aseptically placed on the surface of the medium. All plates were incubated at 37°C for 24 hours. Antibacterial activity was determined by measuring the diameter of the inhibition zone formed around the paper disks using a Vernier caliper with a precision of 0.02 mm.

2.6 Data Analysis

Antibacterial activity was quantified by measuring the diameter of the inhibition zones (clear zones) formed around the paper discs using a digital caliper. The measurement aimed to compare the efficacy of the water fraction and the 96% alcohol fraction of *Senna alata L.* leaf extract. To ensure data validity and reliability, all experimental treatments were performed in four replicates (n = 4).

The quantitative data are expressed as mean ± standard deviation (SD). Statistical significance was determined using Two-Way Analysis of Variance (ANOVA) to evaluate the simultaneous effects of two independent variables-fraction type and extract concentration–on the inhibition zone diameter.

Following the ANOVA, Tukey's Post–Hoc Test was applied to identify specific differences between treatment groups. All statistical analyses were performed using OriginPro 8.5.1.

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical Analysis

Phytochemical screening is a crucial step in the exploration of natural products to identify classes of secondary metabolites that may possess biological activity [15]. A phytochemical qualitative analysis of the water and 96% alcohol fractions of *Senna alata L.* leaf was conducted to identify the presence of secondary metabolites. The phytochemical compounds the water and 96% alcohol fractions of *Senna alata L.* leaf are summarized in Table 1.

Table 1. The phytochemical compounds of the the water and 96% alcohol fractions of *Senna alata L.* leaf.

Phytochemical compounds	Reagents	Water fraction	96% alcohol fraction	Results
Alkaloids	Mayer's reagent + HCl	Yellow precipitate	Yellow precipitate	(+)
Flavonoids	NaOH	Dark greenish-brown to dark green color	Greenish to blackish-brown color	(+)
Tannins	$FeCl_3$	Blackish-green color	Brownish-green color	(+)
Saponins	HCl (Froth test)	Stable foam formation (1 – 3 cm)	Stable foam formation (1 – 3 cm)	(+)

These observational data formed the basis for a comparative test of two solvent fractions with different polarities: the water fraction (highly polar) and the 96% alcohol fraction (polar to semi–polar). Qualitative screening detected alkaloids, flavonoids, tannins, and saponins in both fractions, indicating that the collected plant material contains various bioactive constituents soluble in polar media. The consistent identification of these four classes of compounds across all solvents suggests a chemical profile that may contribute to the antimicrobial effect. Furthermore, the co–presence of these phytochemicals is expected to result in synergistic interactions that enhance the overall antimicrobial potential [16]. The flavonoids work by forming complexes with extracellular proteins, a process that disrupts the structural integrity of the bacterial cytoplasmic membrane [17]. Tannins accomplish this by inactivating microbial enzymes and transport proteins [6]. Saponins act as natural detergents that increase cell membrane permeability, facilitating the entry of alkaloids into bacterial cells to disrupt DNA replication [18].

3.1.1. Alkaloid Analysis

The identification of alkaloids was confirmed in both fractions via the Mayer test, as indicated by the formation of an insoluble yellow precipitate. Mechanistically, Mayer's reagent (mercury potassium iodide) reacts with the nitrogen atoms of the alkaloids to form a slightly soluble mercury–alkaloid salt complex [15]. Alkaloid compounds are often associated with various biological activities, particularly analgesic, anti–inflammatory, and antimicrobial properties [19]. Both the aqueous and alcoholic extracts yielded similar results, suggesting that the alkaloids in the sample are widely soluble in various solvents. This pattern is consistent with the presence of a mixture of polar alkaloid salts and less polar free bases, which together account for their solubility in both aqueous and alcoholic media.

3.1.2. Flavonoid Analysis

The addition of NaOH to the sample produced a distinct dark green color, consistent with a positive flavonoid reaction. Chemically, exposure to a strong base causes deprotonation of the phenolic OH group in the flavonoid, which extends conjugation within the chromophore and thus produces an intense bathochromic color shift [20]. Flavonoids function as potent antioxidants that scavenge reactive free radicals and mitigate oxidative injury to cellular components. Their redox–active phenolic structures donate electrons or hydrogen atoms to unstable radical species, thereby interrupting radical chain reactions and preserving biomolecular integrity [16].

3.1.3. Tannin Analysis

Interaction with ferric chloride causes a color change from greenish-black to greenish-brown, a characteristic diagnostic response in polyphenolic compounds and tannins. This color change reflects the chelation process between Fe^{3+} ions and phenolic groups; the dark green color observed indicates the presence of hydrolyzable or conjugated tannin species forming iron–phenolate complexes [21]. Tannins contribute to health through their protein-precipitating activity, which underlies their astringent effects and supports uses as antidiarrheal and wound-healing agents. Their capacity to form insoluble protein complexes reduces tissue exudation, diminishes intestinal secretions, and promotes surface contraction that aids repair [19].

3.1.4 Saponin Analysis

Following the addition of hydrochloric acid, a stable, long–lasting foam with a thickness of between 1 cm and 3 cm forms, indicating the presence of saponins. This is due to the amphipathic structure of saponins–hydrophilic glycosidic groups bound to a hydrophobic sapogenin core–which facilitates the formation of a stable air-liquid interface. Functionally, the detection of saponins suggests that these botanical materials may possess hypocholesterolemic and immunomodulatory activity [19].

3.2. Antibacterial Activity Test Analysis

The antibacterial activity test of the water and 96% alcohol fractions of the *Senna alata L.* leaf at various concentrations are illustrated in Figures 1 and 2, respectively.

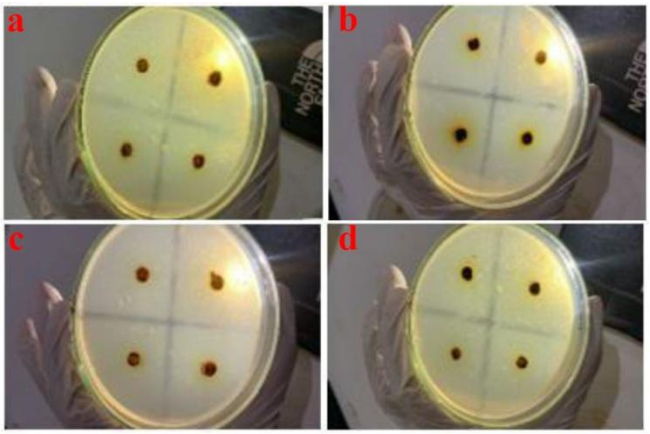

Figure 1. Antibacterial activity test of the water fraction of *Senna alata L.* Leaf at various concentrations (a) 40%, (b) 50%, (c) 60%, and (e) 70%.

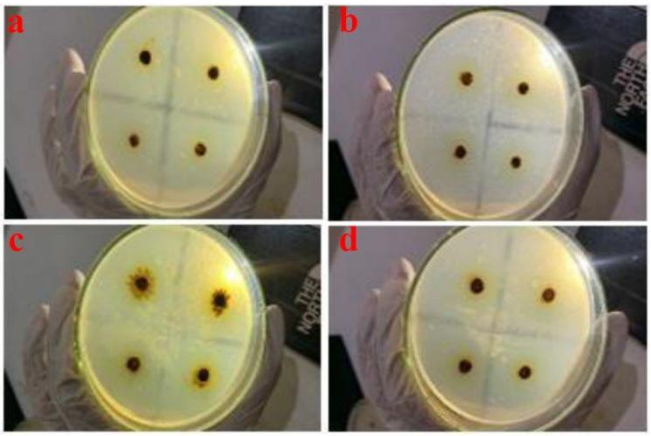

Figure 2. Antibacterial activity test of the 96% alcohol fraction of *Senna alata L.* Leaf at various concentrations (a) 40%, (b) 50%, (c) 60%, and (e) 70%.

All experiments were carried out in four replicates, with each Petri dish containing four spots of the same concentration. For the water fraction (Figure 1 (a) – (d)), the average diameter of the inhibition zone was 10.5 mm, 10.8 mm, 17.8 mm, and 18.0 mm at a concentration of 40%, 50%, 60%, and 70%, respectively. Meanwhile, the 96% alcohol fraction (Figure 2 (a) – (d)) showed an average inhibition zone of 10.2 mm at a concentration of 40%, 14.9 mm at 50%, 16.1 mm at 60%, and 18.9 mm at 70%. Figure 3 shows the correlation of the concentration and mean inhibition zone of water and 96% alcohol fractions of the *Senna alata L.* leaf. From the Figure 3, it can be concluded that the concentration affect the inhibition zone diameter of water and 96% alcohol fractions of the *Senna alata L.* Leaf. The higher concentration produce the higher of inhibition zone diameter.

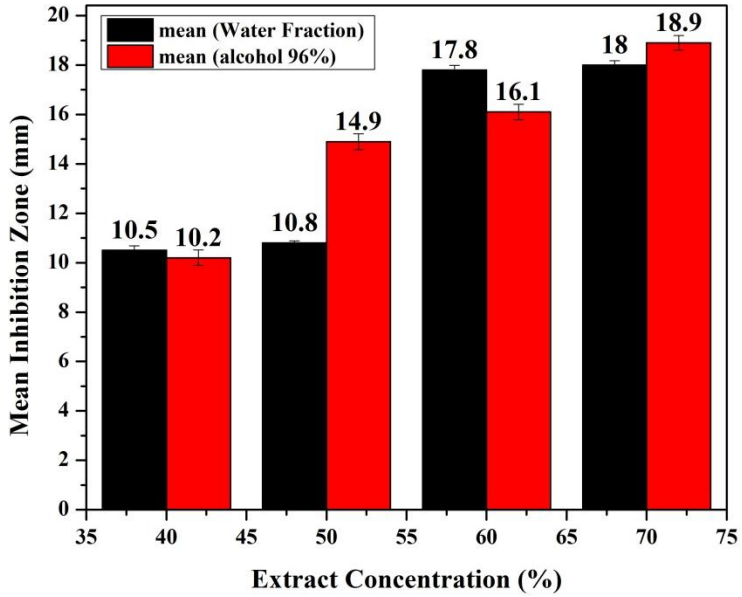

Figure 3. The correlation of the concentration and mean inhibition zone of water and 96% alcohol fractions of the *Senna alata L.* leaf.

3.3. Statistical Analysis

The data analysis carried out included statistical tests using normality tests, homogeneity, and two-way ANOVA to assess the effects of fraction type and concentration.

3.3.1. Normality Test on Water and 96% Alcohol Fractions

In experimental research, particularly when testing the effectiveness of plant extracts at various concentrations, validating data through tests of basic assumptions is a crucial step. One of the most fundamental assumption tests is the normality test. The primary purpose of this test is to determine whether the data obtained from the sample follow a normal (Gaussian) distribution.

Table 2. Shapiro-wilk normality test results.

Treatment group	Concentration (%)	Statistic	df	Sig. (p-value)	Result
Water fraction	40	0.945	4	0.683	Normal
	50	0.845	4	0.212	Normal
	60	0.945	4	0.683	Normal
	70	0.945	4	0.683	Normal
96% alcohol fraction	40	0.821	4	0.141	Normal
	50	0.845	4	0.212	Normal
	60	0.845	4	0.212	Normal
	70	0.723	4	0.054	Normal

Normality assessments were carried out separately for both treatment groups (water fraction and 96% alcohol fraction) at four concentration levels (40%, 50%, 60% and 70%). As each group

yielded a small number of observations (df = 4; n < 50), the Shapiro–Wilk test was selected as the normality test and the result is listed in Table 2. The Shapiro–Wilk procedure is preferred for small sample datasets due to its relatively high sensitivity and statistical power for detecting deviations from the Gaussian distribution [22]. The decision criteria for a normality test are based on the significance level, or p-value. If the p-value is greater than 0.05, then H0 is accepted, meaning the data are normally distributed. Conversely, if the p-value is less than 0.05, then the data are not normally distributed.

In the water fraction group, all concentrations (40% to 70%) showed significance values well above the 0.05 threshold. The 40%, 60%, and 70% concentrations had identical p-values of 0.683, while the 50% concentration had a p-value of 0.212. These high p-values indicate that the data variation in the water fraction is highly consistent and follows a symmetrical bell-shaped distribution pattern. Biologically and chemically, this suggests that the response generated by the water solvent at various concentration levels to the measured parameters (e.g., bacterial inhibition zone or antioxidant activity) is stable and uniform across all experimental units [23].

The 96% alcohol fraction group also showed similar results, with all data classified as "Normal." The p-value for the 40% concentration was 0.141, while the 50% and 60% concentrations had a p-value of 0.212. However, there is an important note regarding the 70% concentration, which has a p-value of 0.054. Although this value is very close to the critical threshold (0.05), the data is still statistically categorized as normal because it remains above the alpha level ($p > \alpha$) Values approaching this threshold often reflect slightly higher variability or minor outliers in the experimental data at high concentrations, but are not strong enough to undermine the overall normality of the data [24].

3.3.2. Homogeneity Test

Antimicrobial activity testing measured using the inhibitory zone diameter parameter. The assumption of homogeneity of variances is a key requirement that must be met following a normality test. Homogeneity of variances assumes that the compared data groups (in this case, various concentrations of water and 96% alcohol) originate from populations with the same variability or data distribution. Based on the presented data, the test was conducted using Levene's Test and the result is given in Table 3. A robust statistical procedure for testing the equality of variances across groups without relying too heavily on the assumption of perfect normality [25].

Table 3. Levene's test for equality of variances.

Variable	Levene Statistic	df1	df2	Sig.	Result
Inhibition Zone Diameter	1,284	7	24	0.301	Homogeneous

Based on the Levene's test results, the inhibitory zone diameter variable shows a Levene's statistic of 1.284, with degrees of freedom 1 (df1) equal to 7 and degrees of freedom 2 (df2) equal to 24. The most crucial parameter in this table is the significance value (Sig.) or p-value of 0.301. The Sig. value of 0.301, which is significantly greater than 0.05, allows us to conclude with certainty that the data on inhibition zone diameters exhibit homogeneous variance. This indicates that the differences in inhibition zone diameters observed in each treatment group are truly influenced by differences in concentration or fraction type, rather than being caused by the magnitude of error or uneven random fluctuations among samples [26].

Based on phytochemical testing, the diameter of the inhibition zone typically increases with rising concentrations of bioactive compounds such as flavonoids or tannins, which were previously identified in the water and 96% alcohol fractions. Homogeneous data at df1 = 7 (representing 8 treatment groups: e.g., 4 water concentrations + 4 alcohol concentrations) indicates that the increase in inhibition zone diameter follows a regular and stable pattern across all groups. This provides confidence that the extract possesses mathematically predictable efficacy [25].

3.3.3. Tukey's HSD Test

To explain the differences in effectiveness among all treatment groups, a Tukey post-hoc test was conducted. This analysis classified groups based on significance levels to determine different letter designations for each mean inhibition zone diameter. Through this procedure, the antibacterial

potential of both types of fractions across a concentration range of 40% to 70% could be objectively ranked. The mean comparison of inhibition zone diameter by Tukey's HSD test is summarized in Table 4.

Table 4. Mean comparison of inhibition zone diameter by Tukey's HSD test.

Extract concentration (%)	Inhibition zone diameter (mm) (Mean ± SD)*		Growth inhibition intensity**
	Water fraction	96% Alcohol fraction	
40%	10.5 ± 0.18^{e}	10.20 ± 0.32^{e}	Strong
50%	10.8 ± 0.08^{e}	14.90 ± 0.32^{d}	Strong
60%	17.8 ± 0.18^{b}	16.10 ± 0.32^{c}	Strong
70%	18.0 ± 0.18^{b}	18.90 ± 0.32^{a}	Strong

According to Table 4, the water fraction showed the most significant increase when the concentration was raised from 50% (10.8 ± 0.08 mm) to 60% (17.8 ± 0.18 mm). This indicates that the threshold for optimal effectiveness of polar compounds in the water fraction is around 60%. At a concentration of 70%, the diameter of the inhibition zone reached 18.0 ± 0.18 mm, indicating that the inhibitory effect began to enter a plateau phase, where further increases in concentration no longer drastically increased the area of the inhibition zone [21]. The 96% alcohol fraction showed a more consistent and linear increase. At the highest concentration (70%), the 96% alcohol fraction produced the largest inhibition zone among all treatments, namely 18.90 ± 0.32 mm. This statistically (indicated by the notation 'a') demonstrates that the 96% alcohol fraction at a 70% concentration possesses the most superior antibacterial potential compared to the other groups.

The very small standard deviation (SD) values (0.08 to 0.32) across all data sets indicate a very high level of precision in the replication of the experiment. Scientifically, this indicates that the data is highly reliable and that the observed variation is not due to random error. The letter codes (a, b, c, d, e) indicate significant differences between groups based on post-hoc tests (e.g., Duncan or Tukey). It is evident that at a 40% concentration, both fractions exhibit similar efficacy (notation 'e'), but at a high concentration (70%), the 96% alcohol fraction (notation 'a') significantly outperforms the water fraction (notation 'b') [22].

3.5. Two-Way ANOVA Analysis

Anti-bacterial activity testing showed that all concentrations in both fractions exhibited strong inhibitory activity. This is demonstrated by the results of the two-way ANOVA in Table 5.

Table 5. Two-way ANOVA summary.

Source of variation	DF	Sum of squares	Mean square	F-value	P-value
Fraction	1	4.50	4.50	72.97	9.69259×10^{-9}
Concentration (%)	3	331.68	110.56	1,792.86	0
Interaction	3	36.70	12.23	198.38	0
Model	7	372.88	53.27	863.81	0
Error	24	1.48	0.06	--	--
Corrected total	31	374.36	--	--	--

According to Table 5, the P-value for the "Fraction" variable is 9.69×10^{-9} (well below 0.05). This indicates that the choice of solvent (water vs. 96% alcohol) makes a highly significant difference in antibacterial efficacy. Theoretically, 96% alcohol has an advantage over water in dissolving more complex diterpenoid and flavonoid compounds, which explains why, in the previous data, the 70% alcohol fraction achieved the highest inhibition zone (18.90 ± 0.32 mm). The very low Mean Square Error (0.06) indicates that this statistical model is capable of explaining 99.6% of the data variation (a very high R^2). This low error value demonstrates that factors outside the scope of the study (such as incubation temperature or technical errors) were successfully controlled very well [16]. The P-value for the interaction was 0. This indicates a significant interaction between concentration and the inhibition zone, depending on the type of solvent used.

4. CONCLUSION

In summary, we have successfully reported the utilization of of *Senna alata L.* leaf extract as an antibacterial agent against the *Staphylococcus aureus* grow up using a two-solvent fractionation method i.e. water and 96% alcohol at concentrations of 40%, 50%, 60%, and 70%. From the qualitative testing using various specific reagents found that both the water fraction and the 96% alcohol fraction consistently contained groups of alkaloids, flavonoids, tannins, and saponins. The antibacterial efficacy results shows that the water and alcohol fractinations at a concentration of 70% produced the greatest antibacterial effect, with an inhibition zone diameter of 18 ± 0.18 mm and 18.90 ± 0.32 mm, respectively. Normality tests for each concentration group (40% to 70%) showed a significance value (p-value) greater than 0.05, so the data were declared to be normally distributed. This indicates that the variation observed in the experiment remains within the reasonable range of a Gaussian distribution. A test for homogeneity of variances using Levene's test yielded a significance value of 0.301 ($p > 0.05$). These results demonstrate that the variability of the data across treatment groups is homogeneous. The fulfillment of these two prerequisites provides justification for proceeding with the analysis using statistical methods. The 96% alcohol fraction exhibited the most superior performance at a concentration of 70%, with an inhibition zone diameter of 18.90 ± 0.32 mm, outperforming the water fraction at the same concentration, reinforced by the Two-way ANOVA results, which showed a very high calculated F-value for the Concentration factor (1,792.86) and the presence of a significant interaction ($p = 0$) between fraction type and concentration. This indicates that bacterial inhibitory activity is not only influenced by the concentration of the extract administered but also heavily depends on the type of solvent used. 96% alcohol proved to be a more efficient extraction solvent than water in extracting the bioactive compounds responsible for this antibacterial activity. These findings confirm that the *Senna alata L.* leaf extract is a promising candidate for antimicrobial applications.

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