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COMPARATIVE ANALYSIS OF THE ANTIBACTERIAL EFFECTS OF N-HEXANE AND AQUEOUS LEAF EXTRACTS OF ANACARDIUM OCCIDENTALE ON *BACILLUS SPP.* AND *PSEUDOMONAS SPP.*

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ABSTRACT

The antibacterial properties of *Anacardium occidentale* (cashew) leaf extracts were evaluated using n-hexane and distilled water as extracting solvents. This study aimed to compare the effectiveness of these extraction methods against *Bacillus spp.* and *Pseudomonas spp.* The extracts were prepared by washing, air-drying, and grinding the cashew leaves, followed by extraction with the respective solvents. The agar well diffusion method was used to evaluate the antibacterial activity and estimate the minimum inhibitory concentrations (MICs). Results indicated that both n-hexane and aqueous extracts exhibited antibacterial activity. The n-hexane extract showed significant inhibition zones for *Bacillus spp.* (15 mm at 400 mg/ml and 11 mm at 200 mg/ml) and *Pseudomonas spp.* (14 mm at 400 mg/ml and 10 mm at 200 mg/ml). Conversely, the aqueous extract was effective only at the highest tested concentration (400 mg/ml), showing inhibition zones of 11 mm for *Bacillus spp.* and 10 mm for *Pseudomonas spp.* The n-hexane extract demonstrated an MIC of 400 mg/ml for both bacteria, whereas the aqueous extract did not reach the MIC for *Pseudomonas spp.* at the tested concentrations. Statistical analysis, including ANOVA and Tukey HSD tests, confirmed significant differences in antibacterial activity between the various concentrations of the n-hexane extract. The results highlight the superior efficacy of n-hexane in extracting antibacterial compounds from cashew leaves, likely due to its nonpolar nature. This study underscores the importance of solvent choice in optimizing the extraction of bioactive compounds with potential applications in developing antibacterial agents. Key findings of this research suggest that n-hexane extracts are more effective at lower concentrations compared to aqueous extracts. Future research should focus on identifying the specific antibacterial compounds within these extracts and exploring their applications in medicine and agriculture. Additionally, investigating the synergistic effects of combining different solvents may enhance the overall extraction efficiency and antibacterial potency of *Anacardium occidentale* extracts.

INTRODUCTION

Medicinal plants have long been recognized for their ability to combat microbial agents and treat a variety of illnesses. Through various preparation methods, these plants can be highly effective against numerous pathogens. The extraction and formulation of numerous medications from these plants, as well as traditional rural herbal remedies, have led to an increased reliance on medicinal plants in many industrialized societies. Significant efforts have been made to uncover new bioactive compounds with therapeutic potential to combat pathogenic bacteria through systematic screening of plant materials (Vamshi *et al.*, 2010). The medicinal value of these plants is derived from various chemical constituents, including alkaloids, flavonoids, tannins, and phenolic compounds, which have significant physiological effects on humans (Edeoga *et al.*, 2005).

Anacardium occidentale is one of the medicinal plants that possess antimicrobial properties. Their fruits, leaf, and shoot powder, and other components feature abundant secondary metabolites that have been demonstrated to have a variety of uses (Salehi *et al.*, 2019). Ros (2010) illustrates in its case study the potential health benefits of cashews, including the prevention of heart disease. Studies consistently show that nut consumption decreases cholesterol when combined with a healthy diet. Furthermore, there is growing proof that nuts may be beneficial for vascular reactivity, inflammation, and oxidative stress. Cashews decrease low-density lipoproteins (LDL) and increase high-density lipoproteins (HDL). The HDL is in control of extracting cholesterol from the heart and transferring it to the liver, where it will be oxidized (Ros, 2010; Rajaram, 2010).

Regularly consuming cashews in moderation may help prevent blood problems. Copper, which is essential for the body's elimination of free radicals, is plentiful in cashews. Copper deficiency can lead to iron deficits, including anemia. As a result, we should consume the appropriate amounts of copper in our diet, and cashews are a great source (Kapur, 2022). Our eyes frequently get numerous illnesses in metropolitan and urban environments due to excessive pollution. Zea Xanthin, a powerful antioxidant pigment found in cashews, is quickly and readily absorbed by the retina. Thus, our retina is subsequently protected from harmful ultraviolet (UV) light by this barrier (UV) radiation (Murillo, 2019).

These effects on pathogens and microbial matter are a result of biologically active substances present in different parts of *Anacardium occidentale*. To be used in medicine and the pharmaceutical industry, these biologically active compounds must be extracted from the cashew leaf, nut, bark, or stem. One way to extract these compounds is by using solvents, and the product of these extractions is called plant extract. According to Fotsing *et al.* (2022) and Varma (2016), a material or active ingredient with desired qualities that has been extracted from a plant's tissues—often by subjecting it to a solvent treatment—for a specific use is known as a plant extract. Although they have not been proven to be necessary nutrients, "bioactive compounds" are typically referred to as biologically significant chemicals. Biesalski *et al.* (2009) also posit that bioactive substances are substances that are found in nature, form a component of the food chain, and have an impact on human health. Examples of these

substances include vitamins and non-essential substances like polyphenols and alkaloids. These bioactive compounds are derived from plants, animals, and microorganisms (Swamy & Akhtar, 2019). Every component of the plant, including the leaves, roots, barks, tubers, woods, gums, or exudates of oleoresin, as well as the fruits, figs, flowers, rhizomes, berries, twigs, and the plant itself, synthesizes active chemicals in different amounts and concentrations (Fotsing *et al., 2022;* El-Shemy, 2022). After extraction, additional procedures could be needed to separate or purify the target chemicals (Patel *et al.*, 2019; Zhang & Li, 2010).

However, as shown by Jones and Kinghorn (2006), some challenges have to be addressed throughout the solvent extraction process, so the solvent extraction procedure must be carried out carefully. It is advisable to use fresh plant tissues and to submerge the specimen in boiling alcohol right away after collection in order to prepare the plant material for extraction (Fotsing *et al.*, 2022). An alternative is to dry the plants before extracting them. Since dried materials have a more extended conservation period than fresh samples, they are typically selected in reported cases (Harborne, 1998).

Truong *et al.* (2019) conducted a study that demonstrated that the effects of extraction solvents significantly impact the biological activity of the extract on the extraction yield and the quantity of bioactive components. This was also supported by Turkmen *et al.* (2006), McDonald *et al.* (2001), and Ngo *et al.* (2017). Truong *et al.* (2019) particularly discovered that the extraction yield was higher for highly polar solvents. In their study using *Severinia buxifolia* branches, they used Principal Component Analysis (PCA), which showed that the extraction solvents' effects significantly impact the biological activity of the extract on the extraction yield and the quantity of bioactive components. They also used Tukey's test at $p<0.05$, which showed that the differences observed in the extraction efficiencies of the various solvents for the phytochemical components in *S. buxifolia* branches were statistically significant. These studies indicate that the solvent has an impact on the yield and phytochemical content of extracts.
Relating to *Anacardium occidentale*, Sinlapapanya *et al.* (2022) conducted a study to check the antimicrobial activity of ethanolic cashew leaf extracts, which showed that cashew leaf extract is effective against *P. aeruginosa* and *Shewanella sp.* at concentrations of 200, 400, and 600 ppm. Similarly, Salehi *et al.* (2020) conducted a study that also confirmed that *Anacardium occidentale* extracts were effective against microbial agents. Before those, Varghese *et al.* (2013) also conducted a study that confirmed that *Anacardium occidentale* leaf extract was effective against pathogens causing periodontal disease.

This particular study, “Comparative Analysis of the Different Effects of N-Hexane and Aqueous *Anacardium occidentale* Leaf Extracts on Bacteria," investigates the efficacy of these two extraction methods in isolating bioactive compounds with antimicrobial activity. The research offers a significant understanding of the relative potency of aqueous extracts and n-hexane in stopping the growth of bacteria. The findings are expected to guide future research in optimizing extraction techniques for maximizing the antibacterial potential of *Anacardium occidentale* leaf extracts, with potential applications in medicine, agriculture, and related fields. The plant material (*Anacardium occidentale* leaves) used in the different solvent extractions was subjected to the same conditions—pH, temperature, technique, extraction time,

agitation, pressure, and material-to-solvent ratio—with the only variable being the solvent used.

The purpose of this research is to assess the effectiveness of the extraction methods used for *Anacardium occidentale* leaf extracts, explicitly focusing on n-hexane and distilled water as solvents. The study aims to determine which method is more effective in isolating bioactive compounds with antibacterial properties, thereby providing insights into optimizing extraction processes for potential applications in medicine, pharmacy, agriculture, and related fields.

MATERIALS AND METHODS

The materials used in this study included fresh leaves of *Anacardium occidentale*(cashew) sourced from cashew trees at the University of Nigeria Nsukka, Enugu State, and various laboratory equipment and reagents. The equipment and reagents comprised test tubes, cotton wool, a Bunsen burner, normal saline, nutrient broth, Petri dishes, aluminum foil, a micropipette, an incubator, measuring cylinders, Dimethyl sulfoxide (DMSO), storing bottles, a cork borer, a spatula, a pipette, hand gloves, an inoculating loop, distilled water, filter paper, Mueller Hinton agar (MHA), a cotton swab, masking tape, an autoclave, and the McFarland standard. The organisms used for the study were *Bacillus spp.* and *Pseudomonas spp.*, with the isolates obtained from the Medical Microbiology Laboratory, Department of Microbiology, University of Nigeria Nsukka, Enugu State.

Extraction Process Using Distilled Water and n-Hexane

The cashew leaves were washed with distilled water and were air-dried at room temperature for five days. They were then further dried and ground in a mortar before being blended into a fine powder using an electric blender. The resulting powder was stored in sterile bottles for various extraction processes. For the aqueous extraction, 25 grams of the powdered cashew leaves were placed into a bottle and labeled. Sterile distilled water (500 ml) was added to the bottle. The mixture was manually shaken every three hours for 72 hours. The mixture was strained and filtered through a very fine nylon sieve to prevent tannin absorption by paper filters. The filtrate was then concentrated by evaporating to a semi-solid state using a water bath at 100°C. This semi-solid extract was further concentrated with a rotary evaporator at 80°C. The final extract was stored in sterile sample bottles and refrigerated at 4°C. For the n-hexane extraction, 25 grams of the powdered cashew leaves were placed into a bottle and labeled. Analytical n-hexane (500 ml) was added to the bottle. Similar to the aqueous extraction, the mixture was manually shaken every three hours for 72 hours. It was then strained and filtered through a fine nylon sieve to avoid tannin absorption by paper filters. The clear filtrate was concentrated by evaporating to a semi-solid state in a water bath at 100°C. This semi-solid extract was further concentrated using a rotary evaporator at 70°C. The final extract was stored in sterile sample bottles and refrigerated at 4°C.

Evaluation of antibacterial activities against *Bacillus spp.* and *Pseudomonas spp.*

Bacillus spp. and *Pseudomonas spp.* were isolated for the study. Petri dishes were subsequently washed, air-dried, wrapped in aluminum foil, and sterilized via autoclaving at 121°C for 15 minutes, as per the method described by Ajiboye (2021). Post-sterilization, the dishes were

cooled and filled with Mueller Hinton agar (MHA). After mixing 38 grams of MHA powder with one liter of distilled water for 15 minutes at 121°C, the agar was autoclaved, following the protocols by Ahmadi & Aarabi (2019) and Mohamed (2009). Following the agar's placement in the Petri dishes and allowing it to solidify, 2 ml of normal saline was inoculated with the test organisms, *Bacillus spp.* and *Pseudomonas spp.* (Balouiri *et al.*, 2016). The culture was changed to correspond with the turbidity of a 0.5 McFarland standard, equivalent to approximately 1.5×10^8 colony-forming units (CFU)/ml, as specified in the US Patent for Antifungal Agents (US Patent No. 8,722,727, 2014), and corroborated by Mhawesh *et al.* (2018) to ensure consistency. Using the test tube dilution method described by Chigurupati *et al.* (2019), different concentrations of the leaf extracts were prepared with distilled water. The concentrations used were 400 mg/ml, 200 mg/ml, 100 mg/ml, and 50 mg/ml, as per Tishin *et al.* (2017). Each extract concentration (0.1 ml) was added to 2 ml of sterile nutrient broth, followed by 0.1 ml of the test bacteria diluted with distilled water. The test tubes were incubated aerobically for 24 hours at 37°C, as outlined by Alves-Silva *et al.* (2016). The MIC was identified as the lowest extract concentration that showed no bacterial growth after 24 hours, according to the method described by Tangjitjaroenkun (2018). The antibacterial activities of aqueous and n-hexane cashew leaf extracts against test organisms were evaluated using the agar well diffusion method, following the protocol described by Rao *et al.* (2014). Nutrient plates were inoculated with the test organisms to examine if the leaf extracts had any antibacterial properties. Duplicate plates were prepared for each test organism by flooding each plate with 0.1 ml of an 18-hour-old culture. A sterile cork borer (6 mm diameter) was used to create four wells on each plate. To make the initial concentration of the leaf extracts, 2 grams of the extracts were dissolved in 4.5 milliliters of distilled water and 0.5 milliliters of DMSO, yielding a 400 mg/ml concentration.

Using the formula C1V1 = C2V2, where:

- C1 = initial concentration
- V1 = initial volume
- C2 = final concentration
- V2 = final volume

The following concentrations were prepared:

- **200 mg/ml**: C1 = 400 mg/ml, V1 = 1 ml, V2 = 2 ml. Thus, C2 = (400 * 1) / 2 = 200 mg/ml.
- **100 mg/ml**: C1 = 200 mg/ml, V1 = 1 ml, V2 = 2 ml. Thus, C2 = (200 * 1) / 2 = 100 mg/ml.
- **50 mg/ml**: C1 = 100 mg/ml, V1 = 1 ml, V2 = 2 ml. Thus, C2 = (100 * 1) / 2 = 50 mg/ml.

Using a micropipette, each well was filled with 0.1 ml of the corresponding extract concentration. As a control, an antibiotic (Streptomycin at 30 µg/ml) was placed in the center of the four wells. The inoculated plates were left on the laminar bench for 1 hour to allow the leaf extracts to diffuse into the agar. A 37°C incubator was then used for the plates in an aerobic environment. Using a meter rule, the zones of inhibition were measured in millimeters following incubation.

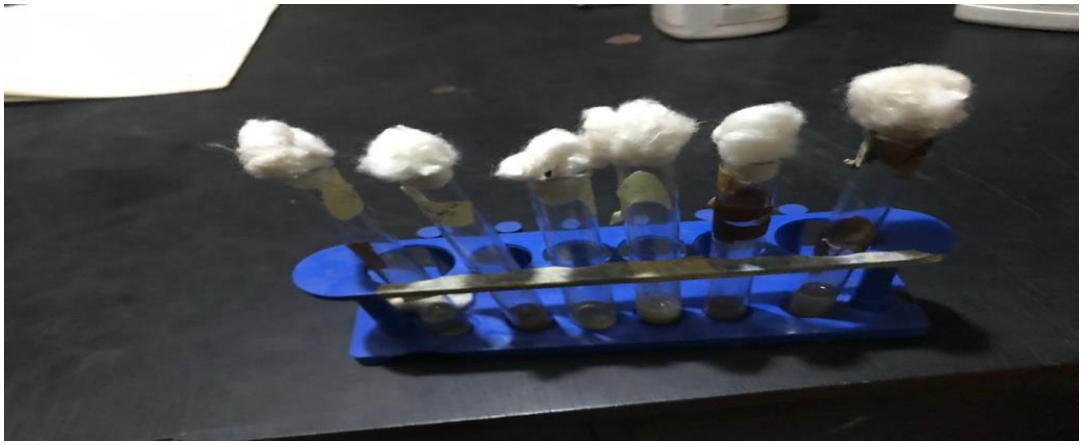

Figure 1. *Preparation of Bacterial Cultures for Determining the Minimum Inhibitory Concentration (MIC) of Cashew Leaf Extracts against Bacillus spp. and Pseudomonas spp.*

Statistical Analysis

Several statistical tests were run using version 22 of SPSS (Statistical Package for the Social Sciences). The treatments' level of significance was assessed using analysis of variance (ANOVA) at the 0.05 confidence level. Additionally, post hoc tests, including Tukey HSD, were conducted to determine significant differences between specific concentration pairs. Descriptive statistics were also calculated to summarize the data.

RESULTS

Comparison of antimicrobial activities between different extraction methods

The antibacterial activities of the n-hexane and aqueous extracts against *Bacillus spp.* and *Pseudomonas spp.* are summarized in the tables below Both extracts exhibited antibacterial properties against these organisms The n-hexane leaf extract of *Anacardium occidentale* demonstrated antibacterial activity against *Bacillus spp.* and *Pseudomonas spp.* at concentrations of 200 mg/ml and 400 mg/ml. The aqueous extract showed antibacterial activity against *Bacillus spp.* and *Pseudomonas spp.* only at a concentration of 400 mg/ml.

Table 1 Antibacterial activity of n-hexane leaf extract of Anacardium occidentale against Bacillus spp. and Pseudomonas spp.

Test Organisms	**Concentration (mg/ml)**			
	400	**200**	**100**	**50**
Bacillus spp.	15 mm ± 00	11 mm ± 0.711	-	-
Pseudomonas spp.	14 mm ± 00	10 mm ± 00	-	-
Positive Control				
Streptomycin	25 mm	30 µg/ml		

The mean difference is significant at the 0.05 confidence level.

Table 2 Antibacterial activity of aqueous leaf extract of Anacardium occidentale against Bacillus spp. and Pseudomonas spp.

Test Organisms	**Concentration (mg/ml)**			
	400	**200**	**100**	**50**
Bacillus spp.	11 mm ± 00	-	-	-
Pseudomonas spp.	10 mm ±00	-	-	-
Positive Control				
Streptomycin 26 mm	30 µg/ml			

The minimum inhibitory concentrations (MICs) of n-hexane and aqueous leaf extracts of *Anacardium occidentale* were found for both Bacillus species and Pseudomonas species. For both species, the n-hexane extract showed an MIC of 400 mg/ml. For Pseudomonas species, the aqueous extract failed to reach MIC at the tested doses.

Table 3 Minimum inhibitory concentration (MIC) of n-hexane and aqueous leaf extracts of Anacardium occidentale on Bacillus spp. and Pseudomonas spp.

Test extracts	***Bacillus spp.***	***Pseudomonas spp.***
N-hexane	400 mg/ml	400 mg/ml
Aqueous	-	-

For the agar well diffusion assay, the presence of inhibition zones in the aqueous leaf extract showed inhibitory effects on *Bacillus spp.*; the n-hexane leaf extract of *Anacardium occidentale* demonstrated clear inhibitory zones against both *Bacillus spp.* and *Pseudomonas spp.*, indicating significant antibacterial activity It did not, however, exhibit any discernible suppression against Pseudomonas species at the tested dosages.

The n-hexane and aqueous extracts of *Anacardium occidentale* have varied antibacterial properties, as seen in Figures 2, 3, 4, and 5. These results are visually portrayed in these figures.

Figure 2 *N-hexane leaf extracts in Bacillus spp.*

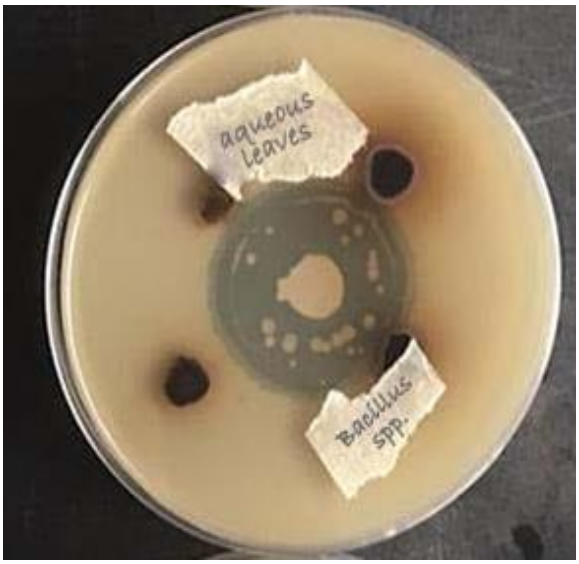

Figure 6 *Aqueous leaf extracts in Bacillus spp.*

Figure 5 *N-hexane leaf extracts in Pseudomonas spp.*

Figure 7 *Aqueous leaf extracts in Pseudomonas spp.*

Statistical analysis of the data

To compare the effectiveness of the n-hexane and aqueous extraction methods on *Bacillus spp.* and *Pseudomonas spp.*, several statistical tests were conducted.

Descriptive Statistics

As observed in Table 4, for *Bacillus spp.*, the n-hexane extract showed mean inhibition zone diameters of 15 mm at 400 mg/ml (± 0), 11 mm at 200 mg/ml (± 0.711), and no inhibition at 100 mg/ml and 50 mg/ml concentrations For *Pseudomonas spp.*, the mean inhibition zone diameters were 14 mm at 400 mg/ml (± 0), 10 mm at 200 mg/ml (± 0), and no inhibition at 100 mg/ml and 50 mg/ml concentrations On the other hand; Table 5 reveals that for *Bacillus spp.*, the aqueous extract showed a mean inhibition zone diameter of 11 mm at 400 mg/ml (± 0),

with no inhibition at lower concentrations For *Pseudomonas spp.*, the mean inhibition zone diameter was 10 mm at 400 mg/ml (± 0), with no inhibition at lower concentrations.

Table 4 Descriptive Statistics for N-Hexane Extract Activity on Bacillus spp. And Pseudomonas spp.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Bacillus spp.	400	2	15.0000	.00000	.00000	15.0000	15.0000	15.00	15.00
	200	2	10.5000	.70711	.50000	4.1469	16.8531	10.00	11.00
	100	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	50	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	8	6.3750	7.02928	2.48522	.4984	12.2516	.00	15.00
Pseudomonas spp.	400	2	14.0000	.00000	.00000	14.0000	14.0000	14.00	14.00
	200	2	10.0000	.00000	.00000	10.0000	10.0000	10.00	10.00
	100	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	50	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	8	6.0000	6.59004	2.32993	.4906	11.5094	.00	14.00

Table 5 Descriptive Statistics for Aqueous Extract Activity on Bacillus spp And Pseudomonas spp

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Bacillus spp.	400	2	11.0000	.00000	.00000	11.0000	11.0000	11.00	11.00
	200	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	100	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	50	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	8	2.7500	5.09201	1.80030	-1.5070	7.0070	.00	11.00
Pseudomonas spp	400	2	10.0000	.00000	.00000	10.0000	10.0000	10.00	10.00
	200	2	.0000	.00000	.00000	.0000	.0000	.00	.00

	100	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	50	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	8	2.5000	4.62910	1.63663	-1.3700	6.3700	.00	10.00

ANOVA

The ANOVA test results for the n-hexane extract activity on Bacillus spp., as shown in Table 6, indicated a significant difference between groups ($F = 921.000$, $p < 0.001$) In contrast, for *Pseudomonas spp.*, the F and p-values were not provided but indicated differences as well For the aqueous extract activity, as shown in Table 7, the ANOVA test indicated differences in inhibition zones for *Bacillus spp.* and *Pseudomonas spp.*, but specific F and p-values were not provided.

Table 6. ANOVA for N-Hexane Extract Activity on Bacillus spp. And Pseudomonas spp.

		Sum of Squares	df	Mean Square	F	Sig.
Bacillus spp.	Between Groups	345.375	3	115.125	921.000	.000
	Within Groups	.500	4	.125		
	Total	345.875	7			
Pseudomonas spp.	Between Groups	304.000	3	101.333	.	.
	Within Groups	.000	4	.000		
	Total	304.000	7			

Table 7 ANOVA for Aqueous Extract Activity on Bacillus spp. And Pseudomonas spp.

		Sum of Squares	df	Mean Square	F	Sig.
Bacillus spp.	Between Groups	181.500	3	60.500	.	.
	Within Groups	.000	4	.000		
	Total	181.500	7			
Pseudomonas spp.	Between Groups	150.000	3	50.000	.	.
	Within Groups	.000	4	.000		
	Total	150.000	7			

Post Hoc Tests

Tukey HSD tests for *Bacillus spp.*, as shown in Table 8, with n-hexane extract revealed significant differences between most concentration pairs, with 400 mg/ml showing the highest mean difference compared to lower concentrations. No significant differences were observed between 100 mg/ml and 50 mg/ml, as both showed no inhibition. For the aqueous extracts, Tukey HSD tests were not explicitly provided by the SPSS software. However, based on the data, it can be inferred that the 400 mg/ml concentration showed significant antibacterial activity, while the lower concentrations did not.

Table 8 Multiple Comparison - Tukey HSD for N-Hexane Extract Activity on Bacillus spp. And Pseudomonas spp.

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bacillus spp.	400	200	4.50000*	.35355	.001	3.0607	5.9393
		100	15.00000*	.35355	.000	13.5607	16.4393
		50	15.00000*	.35355	.000	13.5607	16.4393
	200	400	-4.50000*	.35355	.001	-5.9393	-3.0607
		100	10.50000*	.35355	.000	9.0607	11.9393
		50	10.50000*	.35355	.000	9.0607	11.9393
	100	400	-15.00000*	.35355	.000	-16.4393	-13.5607
		200	-10.50000*	.35355	.000	-11.9393	-9.0607
		50	.00000	.35355	1.000	-1.4393	1.4393
	50	400	-15.00000*	.35355	.000	-16.4393	-13.5607
		200	-10.50000*	.35355	.000	-11.9393	-9.0607
		100	.00000	.35355	1.000	-1.4393	1.4393

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Homogeneous subset analysis for *Bacillus spp.*, as shown in Table 9, indicated distinct groups for 100 mg/ml and 50 mg/ml (no inhibition), 200 mg/ml, and 400 mg/ml with increasing mean inhibition zones This pattern was consistent with the descriptive statistics and ANOVA results On the other hand, the SPSS software did not provide explicit analysis for the Homogeneous subset However, the data suggests no inhibition at 200 mg/ml, 100 mg/ml, and 50 mg/ml, with significant inhibition at 400 mg/ml.

Table 9 Bacillus spp - Tukey HSD[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
100	2	.0000		
50	2	.0000		
200	2		10.5000	
400	2			15.0000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 2.000.

The statistical analysis confirmed that both n-hexane and aqueous extracts of *Anacardium occidentale* exhibited antibacterial activity against *Bacillus spp.* and *Pseudomonas spp.*, with

the n-hexane extract being more effective, especially at lower concentrations. The n-hexane extract demonstrated significant antibacterial activity at 200 mg/ml and 400 mg/ml, while the aqueous extract was effective only at 400 mg/ml. The ANOVA and post hoc tests further validated these findings, showing significant differences in inhibition zones across different concentrations.

DISCUSSIONS

The results of this study indicate that both n-hexane and aqueous extracts of *Anacardium occidentale* exhibit antibacterial activity against *Bacillus spp.* and *Pseudomonas spp.* However, the n-hexane extract demonstrated a more potent antibacterial effect compared to the aqueous extract. This disparity in efficacy can be attributed to the differences in solvent polarity, which affects the extraction of bioactive compounds from the cashew leaves.

The n-hexane extract showed significant antibacterial activity for both *Bacillus spp.* and *Pseudomonas spp.* at concentrations of 200 mg/ml and 400 mg/ml. The aqueous extract, on the other hand, was effective only at the highest tested concentration of 400 mg/ml. This suggests that nonpolar solvents like n-hexane are more efficient in extracting nonpolar compounds with antibacterial properties from cashew leaves.

The statistical analysis, including ANOVA and Tukey HSD tests, confirmed the significant differences in antibacterial activity between the various concentrations of the n-hexane extract. The homogeneous subset analysis for *Bacillus spp.* indicated distinct groups with increasing mean inhibition zones, further validating the higher efficacy of the n-hexane extract at lower concentrations.

These findings highlight the role of solvent polarity in the extraction efficiency and biological activity of plant extracts. Previous studies, such as those by Truong *et al.* (2019) and Turkmen *et al.* (2006), demonstrated that highly polar solvents generally yield higher extraction efficiencies for specific bioactive components. However, this is only sometimes true for all types of bioactive compounds. In the case of the current study, the n-hexane extract, which is a nonpolar solvent, was more effective in isolating antibacterial compounds from *Anacardium occidentale* leaves. From this, it seems that different solvents are effective for extracting different types of bioactive compounds based on their polarity, which implies that nonpolar solvents like n-hexane are better at extracting nonpolar bioactive compounds, which may include some antibacterial agents present in *Anacardium occidentale* leaves On the other hand, polar solvents are more effective for extracting polar compounds.

The significant antibacterial activity of the n-hexane extract at lower concentrations suggests its potential for use in developing antibacterial agents. This is particularly relevant for treating infections caused by *Bacillus spp.* and *Pseudomonas spp.*, which are known for their resistance to conventional antibiotics. The ability of the n-hexane extract to inhibit bacterial growth at lower concentrations could lead to more efficient and cost-effective antibacterial treatments.

CONCLUSIONS

This study provides a comparative analysis of the antibacterial activities of n-hexane and aqueous extracts of *Anacardium occidentale* leaves against *Bacillus spp.* and *Pseudomonas*

spp. The n-hexane extract demonstrated superior antibacterial activity at lower concentrations compared to the aqueous extract, highlighting the importance of solvent choice in the extraction of bioactive compounds.

Key findings of the study include:

- The n-hexane extract exhibited significant antibacterial activity against *Bacillus spp.* and *Pseudomonas spp.* at concentrations of 200 mg/ml and 400 mg/ml.
- The aqueous extract was effective only at 400 mg/ml, indicating lower extraction efficiency for antibacterial compounds.
- Statistical analysis confirmed the significant differences in antibacterial activity between the different concentrations of the extracts.

Based on these findings, it is recommended that extraction methods be optimized by selecting appropriate solvents to maximize the yield of bioactive compounds with antibacterial properties. Further research should focus on identifying the specific compounds responsible for the antibacterial activity and exploring their potential applications in medicine and agriculture.

Future studies could also investigate the synergistic effects of combining different solvents and extraction techniques to enhance the antibacterial efficacy of *Anacardium occidentale* extracts. Additionally, exploring the antimicrobial activity of other parts of the cashew plant, such as the bark and nuts, could provide a broader understanding of its medicinal potential.

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