

Antimicrobial activity of three medicinal plants against acne-inducing bacteria *Propionibacterium acnes*

S. E. Abiya^{1,*}, B. O. Odiyi¹, L. R. Falarunu¹ and N. U. Abiya²

¹Biology Department. Federal University of Technology. Akure.

*Email: esabiya@futa.edu.ng.

²Department of Pharmacology and Therapeutics. University of Ibadan.

Abstract. The antimicrobial activities of three medicinal plants (*Senna alata* L., *Azadirachta indica* A. Juss, and *Aloe vera* (L.) Burm.f.) against pimples causing bacterium *Propionibacterium acnes* was studied using disc diffusion method. Extracts from each plant were used on the bacterium at three different dosage concentrations (0.1 mL, 0.15 mL and 0.2 mL). Their Zone of inhibition was measured in millimeter (mm) and compared against a known synthetic standard (Gentamycin). Results indicate that the plants differ significantly in their activity against the studied microorganism. *S. alata* had the highest inhibitory effect of all the plants used (26.00 mm, 30.67 mm and 36.00 mm, for 0.1 mL, 0.15 mL and 0.2 mL dosage concentration, respectively). This was followed by *A. indica* with 9.33 mm, 15.67 mm and 16.67 mm zone of inhibition for 0.1 mL, 0.15 mL and 0.2 mL dosage concentration respectively. *A. vera* had no effect (0.0 mm zone of inhibition) at 0.1 mL and 0.15 mL dosage concentrations, but at 2.0 mL dosage concentration, 4.0 mm zone of inhibition was achieved. Gentamycin showed zones of inhibition of 17.33 mm, 26.67 mm and 22.67 mm, for 0.1 mL, 0.15 mL and 0.2 mL dosage concentration, respectively. A comparison of all result obtained from the three plant extracts and gentamycin shows that *S. alata* have a significantly higher ($p < 0.05$) inhibitory effect against the pimples causing bacterium; *Propionibacterium acnes* than all the other treatments. The trend follows *S. alata* > Gentamycin > *A. indica* > *A. vera*, respectively, in terms of their inhibitory effect. Therefore, *S. alata* is more active and is the most appropriate plant to be used for treating of acne vulgaris among the three plant species selected for this experiment.

Keywords: *Propionibacterium acnes*; Antimicrobial; Zone of inhibition; Medicinal plants.

Introduction

The use of herbs and medicinal plants is a universal phenomenon. Every culture on earth has relied on the huge

variety of natural chemistry found in healing plants for their therapeutic properties. As reported by Serrentino (1991), the World Health Organization (WHO) observed that about 80% of the

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ORCID

- 0000-0002-9155-2307
S. E. Abiya
- 0000-0002-4079-9895
B. O. Odiyi
- 0000-0002-2601-5839
L. R. Falarunu
- 0000-0001-8972-6374
N. U. Abiya

world population use medicinal plants to treat human disease. Plants have been reported to be antimicrobial and as such, some of them are capable of inhibiting the growth of microorganism (Heinrich et al., 2004).

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous follicles that affects more than 85% of adolescents and young adults (Hanna et al., 2003). Acne vulgaris is a common chronic skin disease involving blockage and/or inflammation of pilosebaceous units (hair follicles and their accompanying sebaceous gland). Acne can occur as non-inflammatory lesions, inflammatory lesions, or a mixture of both, affecting mostly the face but also the back and chest (Dawson and Dellavalle, 2013). According to Thiboutot et al. (2009), acne develops as a result of interplay of the following four factors: follicular epidermal hyperproliferation with subsequent plugging of the follicle, excess sebum production, the presence and activity of the commensal bacteria *Propionibacterium acnes*, and subsequently inflammation. *P. acnes* is an anaerobic organism present in acne lesions. The presence of *P. acnes* promotes inflammation through a variety of mechanisms. *P. acnes* stimulate inflammation by producing pro-inflammatory mediators that diffuse through the follicle wall (Kim et al., 2002).

Most teenagers suffer from this common skin problem. In a Nigerian survey reported by Husain (2009), a 90.7% prevalence of acne among teenagers was observed. In the survey, a total of 539 (274 males, 265 females) randomly selected teenagers between the ages of 11-19 years, 379 out of 418 teenagers examined had acne.

For many years, antibiotics have been used to treat acne vulgaris, however, antibiotic resistance has been increasing in prevalence within the dermatologic setting (Swanson, 2003).

The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics, and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases.

This research aims at determining the susceptibility of *Propionibacterium acnes* to the ethanolic extracts of *Azadirachta indica* A. Juss, *Aloe vera* and *Senna alata* L. and to compare the antibacterial activity between each active plant material against a known synthetic anti-acne agent as standard (Gentamycin).

This may lead to the discovery of an alternative form of treatment other than antibiotics being used at present, to which many of the bacteria are developing resistance.

Materials and method

Plant materials and sample collection

Leaves of *Azadirachta indica* were collected from The Federal University of Technology, Akure (FUTA) Campus. The pulp of *Aloe vera* was collected from Isikan in Akure and was planted in a pot for three months before use. *Senna alata* leaves were collected from Ijare Town near Akure. Specimens were prepared and brought to the Department of Biology Laboratory, the Federal University of Technology Akure for further processing.

Aloe vera gel extraction

A. vera plant was collected from the pot where they were planted. The extract was prepared by cutting the aloe leaves and collecting the yellow liquid that came from the open part of the leave. The gel was also collected and mixed with the liquid. The mixture was subjected to phytochemical analysis.

***Azadirachta indica* and *Senna alata* leaf extraction**

A slightly modified method by Hubert et al. (2012) was adopted for the extraction of *Azadirachta indica* and *Senna alata* leaves. Collected leaf samples of both plants were air dried and pulverized using Marlex blender. 100 g of each pulverized leaves were weighed using JA 3003 Electronic Balance. The weighed samples were soaked in 400 mL of 99.9% ethanol and the mixture swirled intermittently. After 24 h, the ethanolic extracts were separated using rotary evaporator (Resona Labo Rota 300, Type: SW 200). A portion of each extract obtained was subjected to phytochemical analysis and the remainder stored in the refrigerator until further use.

Phytochemical screening of different extracts

The presence of various phytochemical constituents in the extracts was evaluated qualitatively (alkaloids, flavonoids, phenol, saponins, steroids, tannins, terpenoids, glycosides and fixed oils). The test for alkaloid was conducted according to Siddiqui and Ali (1997). The production of a white yellowish precipitate at the addition of few drops of Mayer's reagent indicates presence of alkaloids. Shindo's test was used for the determination of flavonoids. Phenol and Tannin was tested for by the addition of ferric chloride solution. Libermann-Burchard Test was used to test for Steroids and Terpenoids as described by Harborne (1996). Saponin was determined by Froth test. The test solution mixed with distilled water is shaken thoroughly. Copious lather formation indicates the presence of saponin. Shindo's test is used to determine the presence of flavonoids. To 2 mL the test solution, a few magnesium turnings and a few drops of concentrate hydrochloric acid were added and boiled for 5 min. Appearance of red or orange red colour indicates the presence of flavonoid. Glycoside is determined by

mixing the extract with a little anthrone on a watch glass. One drop of concentrate sulphuric acid was added and made into a paste and warmed gently over the water bath. Dark green colouration indicates the presence of glycosides. Spot test was used in determining fixed oil. A small quantity of powder/extract was pressed between the filter papers. Formation of grease spot indicates the presence of fixed oils and fats.

Isolation of *P. acnes*

Following the standard microbiological procedure, *P. acnes* were collected from acne patients using swab stick. A total of 10 patients were randomly selected from a population of acne patients and from them; the bacteria were isolated from mature pimple papules without blood contamination from around their nose region.

Biochemical test and bacterium identification

Masamichi et al. (1980), stated the biochemical and physical tests needed for the identification of *Propionibacterium acnes*. The following tests were conducted to ensure that it was *P. acnes* that grew on the culture medium.

Catalase test

Using a sterile wooden applicator stick, a small amount of organism from a well-isolated 78 to 96 h colony was collected and placed on a microscopic slide. Using a dropper, a drop of 3% H₂O₂ was added onto the organism on the microscopic slide without mixing. This was immediately covered and observed for immediate bubble formation (O₂ + water = bubbles). The production of bubbles indicates that the organism is positive for the test.

Litmus milk test

This test is used to determine the ability of the bacterium to ferment

lactose. A Lactose broth was used. The broth was inoculated with inoculating loop. This is followed by the addition of Phenol red indicator. A yellow colouration after the addition of the indicator indicates a positive test (indicating lactose fermentation).

Gram staining

A heat fixed bacterial smear was taken and flooded with Crystal Violet and allowed to stand for 1 min and then washed with running water. The slide containing the heat fixed and the stained smear was again flooded with Iodine solution and allowed to stand for 1 min, then washed with water. Ethanol-acetone was then added and quickly washed off with water to discolourise the smear. A drop of Safranin red was added and allowed to stay for 1 min, then washed off with water. The smear was blotted; air dried and observed under 100x objective lens. Pink coloured cells indicate a positive test.

Inoculation of *P. acnes*

The microorganism was grown in the laboratory following Masamichi et al. (1980) method with slight modification using Tryptic Soy Agar (TSA) instead of his proposed medium. The medium formulated contained 30 g tryptic soy broth, 20 g agar agar, 10 g NaCl, 10 mL glycerol (4 M) and 10 g yeast. All was dissolved in 1,000 mL of distilled water. The medium was sterilized in an autoclave and used to culture the microorganism. For the primary isolation, the culture was incubated at 36 °C ± 4 for 4 days, while subsequent incubation was for 3 days.

Antibacterial assay

Agar Well Diffusion Method was adopted for the evaluation of antimicrobial activity. From the pure culture plate of *P. acnes* already cultivated, a sub-culture was done using pure plate technique. A total of 36 plates were cultivated and a well approximately 9 mm in diameter bored on the surface of the agar medium using a sterile cork borer. Extracts of *A. vera*, *A. indica* and *Salata*, and 10 µg/mL Gentamycin (serving as the standard) were introduced into the well at 3 different concentrations (0.1 mL, 0.15 mL and 0.2 mL, respectively). The whole set up was replicated thrice. All the plates were incubated at 36 °C ± 4 for 4 days.

At the end of the incubation, the plates were observed for growth and inhibition. The diameter of the zones of inhibition was measured in millimeter (mm) and the records taken.

Statistical analysis

All data were expressed as mean ± standard deviation. Analysis of variance was performed by ANOVA using the SPSS software (version 21.0 for windows). Significant differences between means were determined by Tukey new multiple-range test. A significant difference was considered at the level of P < 0.05.

Result

The result of the phytochemical screening of the test plants (*Aloe vera*, *Azadiratcha indica* and *Senna alata*) indicate the presence of glycosides, saponins, tannins, terpenoids, phenols and flavonoids in all three plants. This is shown in Table 1.

Table 1. Phytochemical analysis of the three nigerian plants used.

Plant species		<i>A. vera</i>	<i>A. indica</i>	<i>S. alata</i>
Phytochemical Constituents	Glycosides	+	+	+
	Alkaloids	+	-	+
	Saponins	+	+	+
	Steroids	+	+	-
	Tannins	+	+	+
	Resins	-	-	-
	Volatile Oil	-	-	+
	Terpenoids	+	+	+
	Phenols	+	+	+
	Flavonoids	+	+	+

+ = Present - = Absent

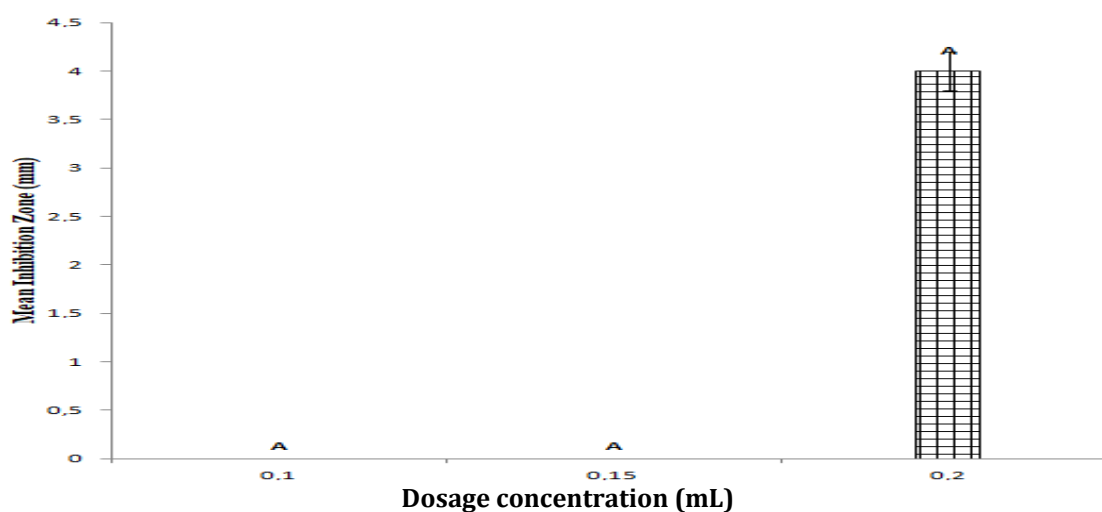
The result of the biochemical and physical observation of the bacteria colony is shown in Table 2. It indicates a bacillus that test positive to gram staining, catalase test and litmus test.

Table 2. Biochemical test and physical observation of the bacterium colony.

Test	Observation
Gram's staining	Positive (+)
Catalase	Positive (+)
Litmus milk	Positive (+)
Shape	Rod-Like (<i>Bacillus</i>)
Colour on agar	Glistening milk

The result of the inhibitory effect of *Aloe vera* against the growth of *Propionibacterium acnes* shows that at 0.1 mL and 0.15 mL dosage concentration, no inhibition was attained but at 0.2 mL a 4.0 mm zone of inhibition was achieved. This is shown in Figure 1.

The result of the inhibitory effect of *A. indica* leaf extract as an antibacterial agent on the growth of *P. acnes* is presented in Figure 2. It shows that leaf extracts of *A. indica* applied at 0.15 mL and 0.2 mL had a significantly higher zone of inhibition when compared 0.1 mL dosage concentration (16.2 mm, 15.5 mm and 9.6 mm, respectively) at $p < 0.05$.

**Figure 1.** Inhibitory effect of *Aloe vera* on *Propionibacterium acnes*.

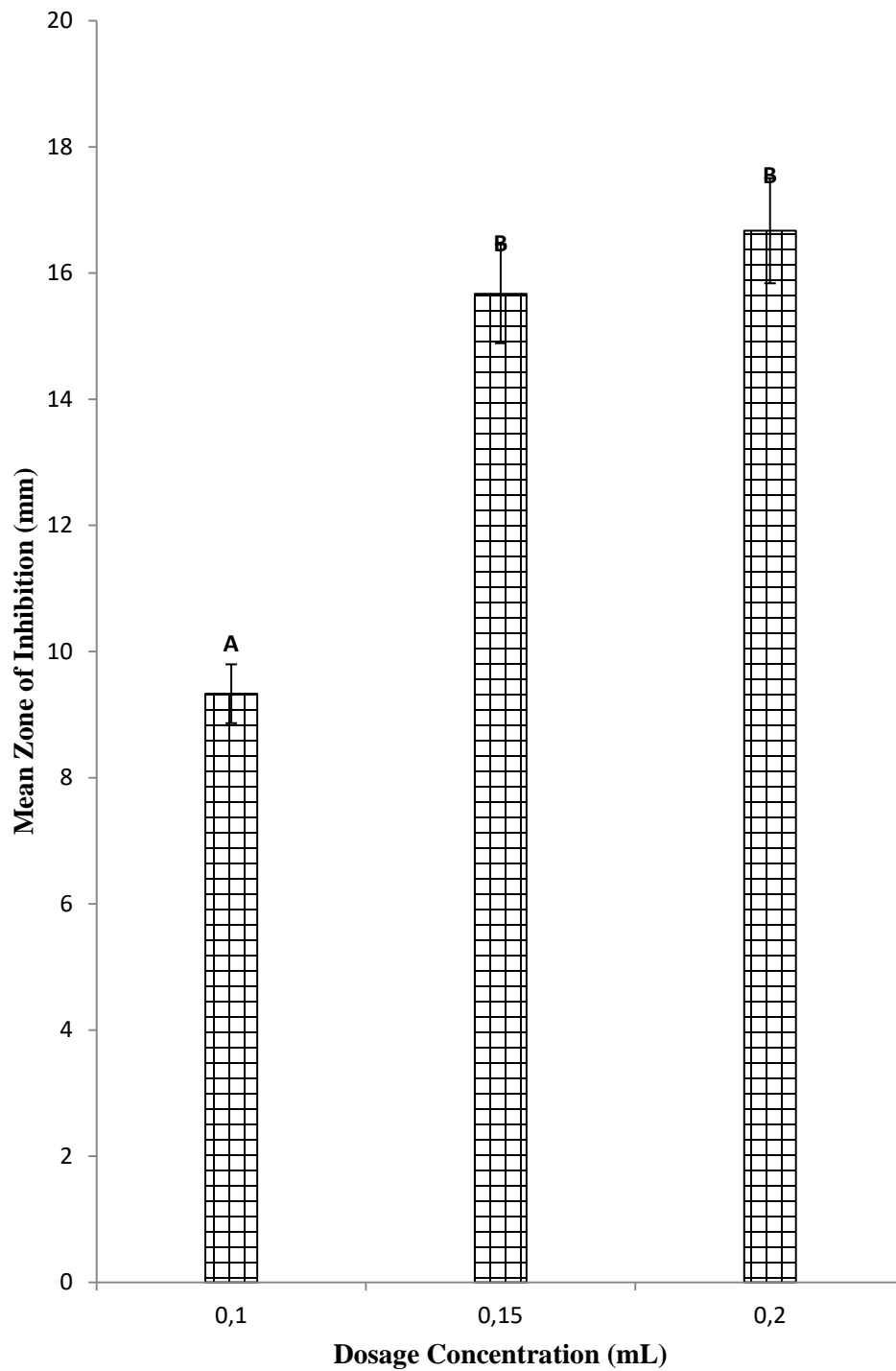


Figure 2. Inhibitory effect of *Azadirachta indica* on *Propionibacterium acnes*.

The result of the inhibitory effect of synthetic Gentamycin as an antibiotic

on the growth of *P. acnes* is presented in Figure 3.

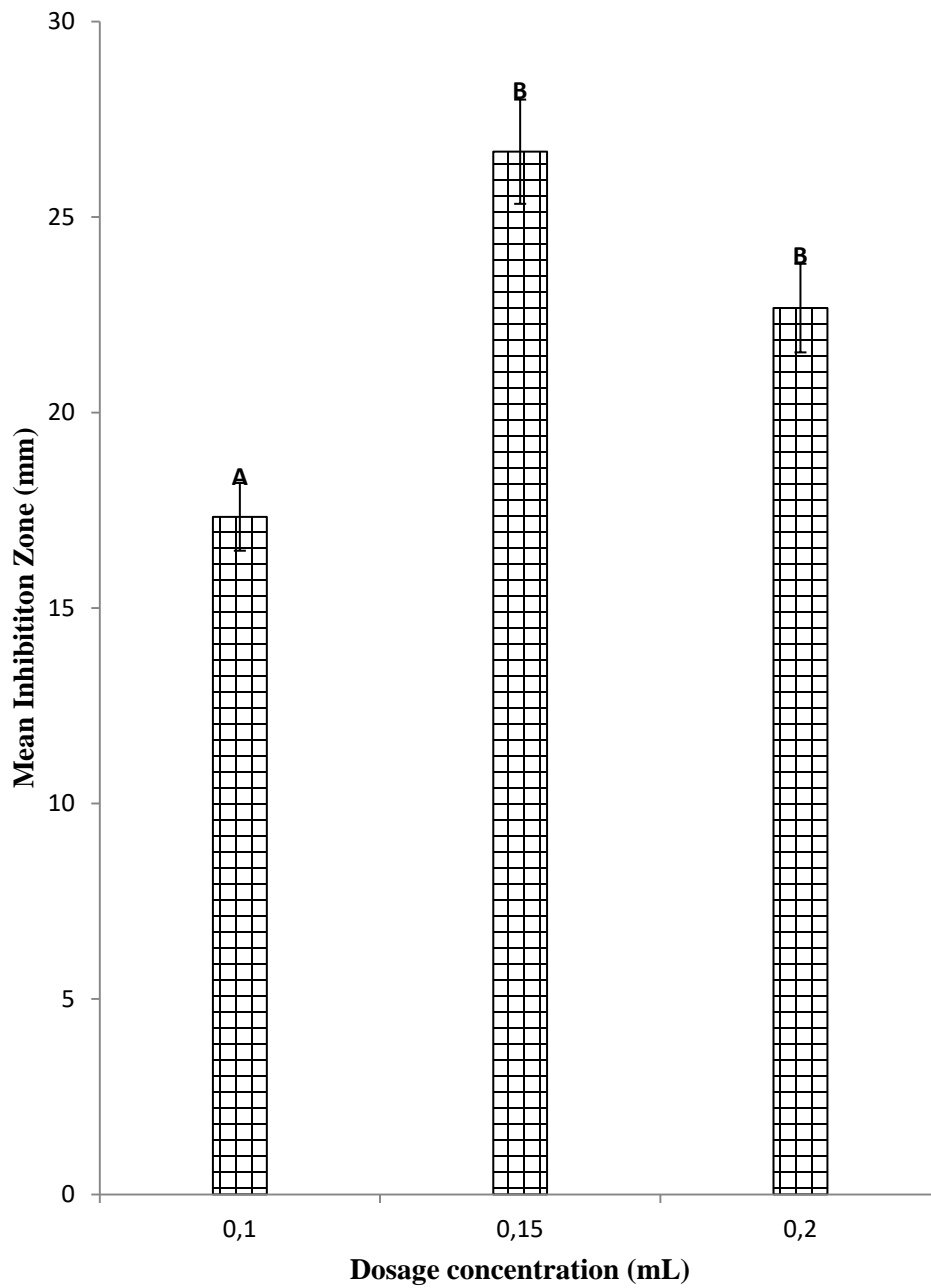


Figure 3. Inhibitory effect of Gentamycin on *Propionibacterium acnes*.

Highest inhibition was attained when 0.15 mL dosage concentration (27 mm). This was significantly higher than that obtained at 0.1 mL (17 mm) at $p < 0.05$.

The result of the inhibitory effect of synthetic Gentamycin as an antibiotic on the growth of *P. acnes* is presented in Figure 4.

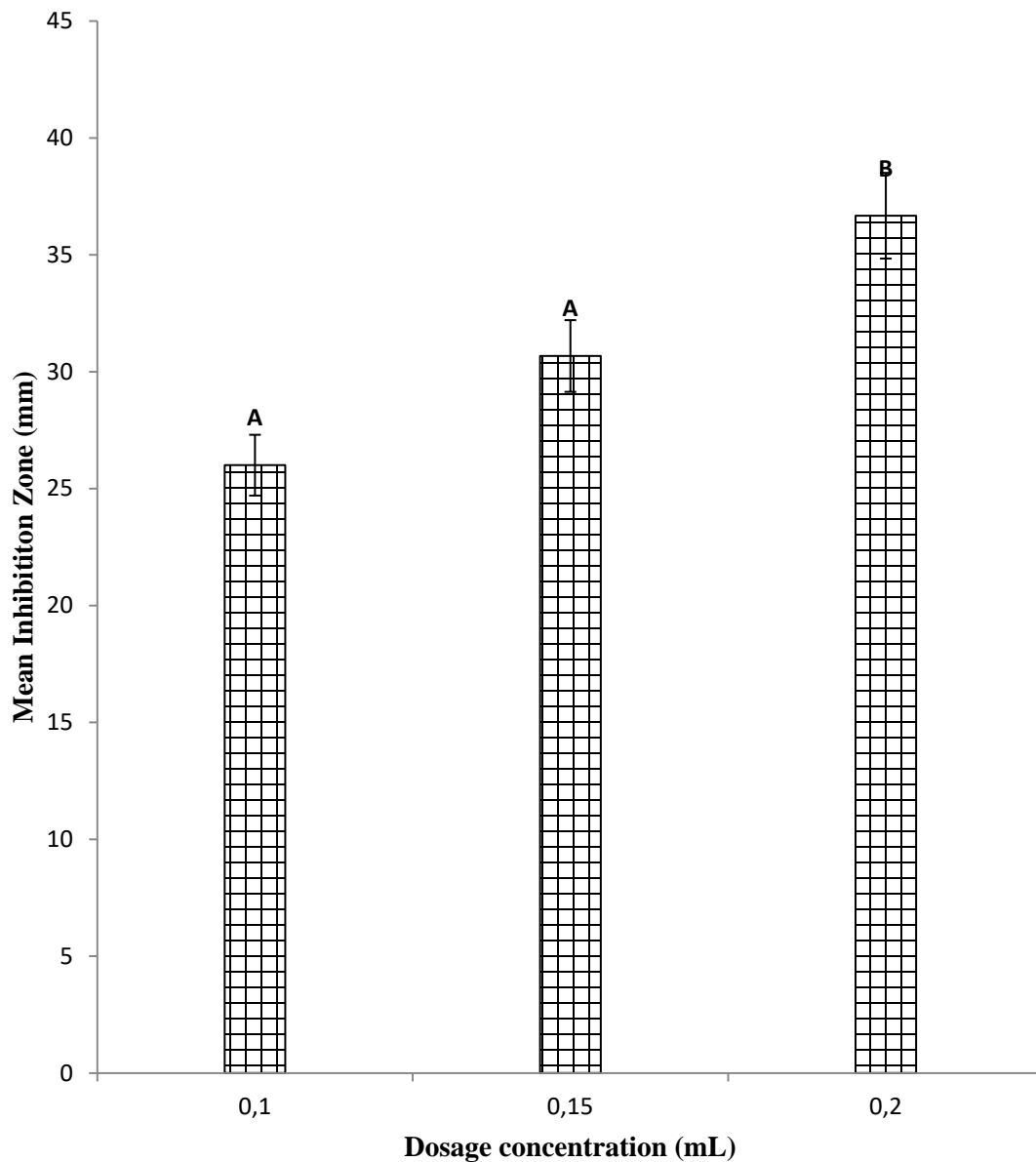


Figure 4. Inhibitory effect of *Senna alata* on *Propionibacterium acnes*.

Using the conventional antibacterial drug, highest inhibition was achieved at 0.2 mL dosage concentration (31 mm). This was significantly higher than inhibition attained at 0.15 mL

(30 mm) and 0.1 mL (26 mm) at $p < 0.05$.

The result of the comparative inhibitory capacity of the four treatments against *P. acnes* is presented in Figure 5.

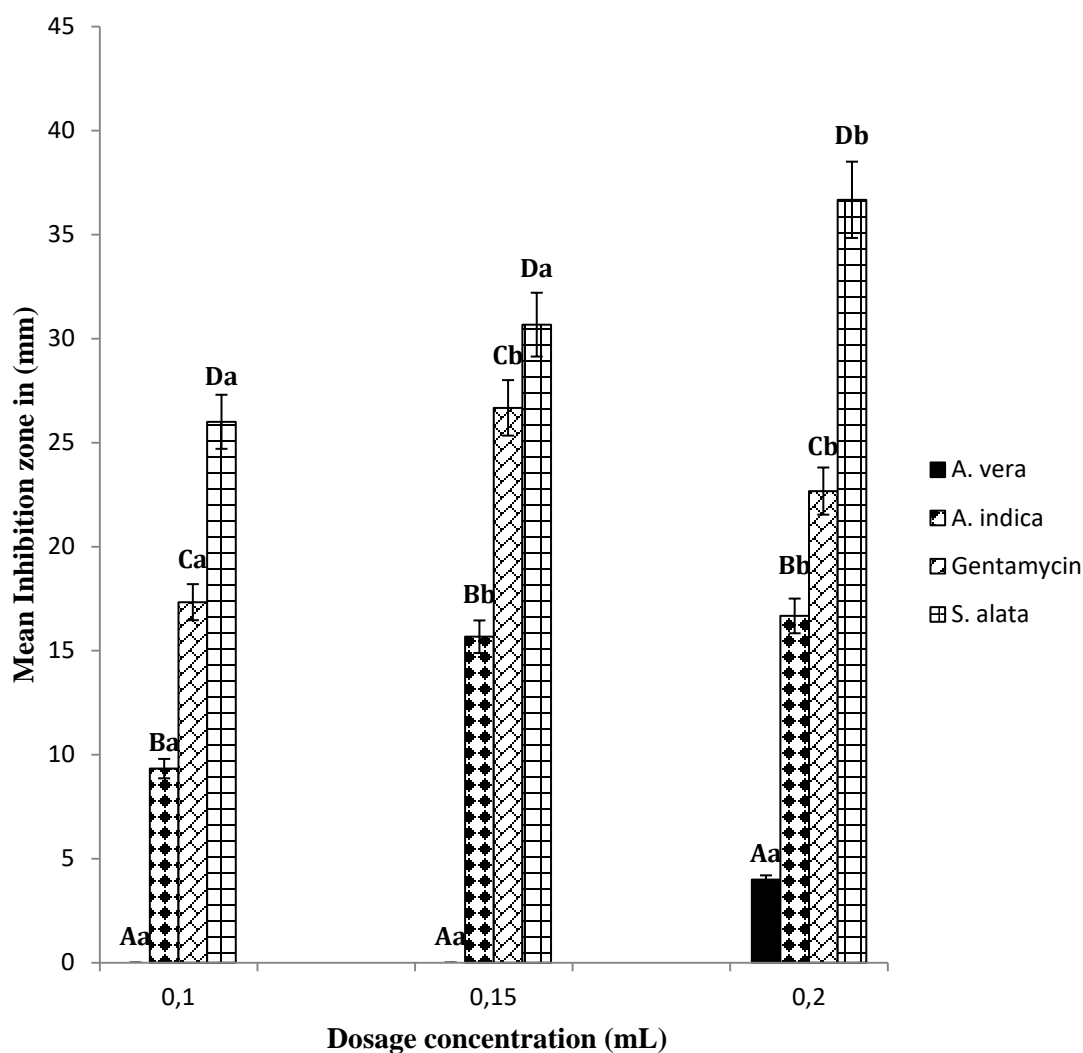


Figure 5. Inhibitory effects of the three plants on *Propionibacterium acnes*. The capital letter shows the significant difference between the four treatments at the same dosage concentration, while the small letter shows the significant difference between each treatment at different dosage concentration (different letters show significant difference $p < 0.05$).

Comparing the four treatments at the three dosage concentration (0.1 mL, 0.15 mL and 0.2 mL) shows that *S. alata* had a significantly higher inhibitory ability than the other treatments across all levels of dosage concentration at $p < 0.05$. The trend was *S. alata* > Gentamycin > *A. indica* > *A. vera*.

Discussion

Plants are known to be rich source of antimicrobials. They produce

secondary metabolites (phytochemicals), which have demonstrated their potential as antimicrobials when used alone and as synergist or potentiators of other antimicrobial agents (Crozier et al., 2006, Abreu et al., 2012). Phytochemicals like flavonoids, terpenoids and alkaloids have been reported to have antibacterial properties and as such their presence in a plant indicates potential antibacterial capacity of such plant (Cowan, 1999; Herbon, 1999; Pichersky and Gershenzon, 2002). The result of the

phytochemical analysis of the three plants showed that all of them contain at least one of these phytochemicals which makes them antimicrobial in nature.

Masamichi et al. (1980) stated the characteristics necessary for the positive identification of *Propionibacterium acnes* is. *P. acnes* was described as a relatively slow growing bacterium (takes up to 3-4 days before a visible colony can be seen) that is aerotolerant and anaerobic, named after its ability to generate propionic acid. It is a gram positive bacterium characterized by its rod-like shape and always tests positive to the following biochemical tests; catalase, indole, nitrate, gelatin hydrolysis, gram staining and litmus milk. It also forms a glistening milk colour on Chocolate Blood Agar (CBA) and Tryptic Soy Agar (TSA). The result of the biochemical and physical test of the cultured micro-organism satisfies all the stated conditions of Masamichi et al. (1980) needed for the identification of *Propionibacterium acnes*.

From the result, *A. vera* showed little to no inhibition especially at lower dosage concentrations. This might be due to the low quantity of the antimicrobial compound in its crude gel. At a higher dosage concentration, the plant gel might be effective as seen at 0.2 mL which produced 4.0 mm diameter zone of inhibition. Lalla et al. (2001) noted that extracts of *A. vera* have anti-bacterial and anti-inflammatory properties. This has also been confirmed by Sampath et al. (2010) who reported that *A. vera* gel has anti-inflammatory property, good burn and wound healing property. He also reported that the bark extract have minimal anti-acne effect on *P. acnes*.

Ethanol extract of *Azadirachta indica* has been reported by Nasri et al. (2015) to have potential for inhibiting acne when used on an anti-acne formulation. Results from this study showed that *A. indica* has anti-acne capability. The inhibitory effect of the plant showed significant increase

($p < 0.05$) with each increase in dosage concentration.

Senna alata is a good source of antimicrobials. It has strong antibacterial properties which may be due to the possession of the three plant chemicals that indicates antimicrobial ability. The anti-microbial ability of this plant had been reported by (Chomnawang et al., 2005; Owoyale et al., 2005; Pukumpuang et al., 2012). There was progressive increase in the amount of inhibition of *P. acnes* achieved as the dosage concentration increased from 0.1 mL to 0.2 mL. This increase was statistically significant ($p < 0.05$). The plants showed better anti-acne property than any of the other plants used. It even showed better inhibition than the synthetic antibiotic used (Gentamycin).

Gentamycin is a synthetic antibiotic. It is known to be one of the most popular over the counter (OTC) antibiotics used in the treatment of acne. It is made of synthetic chemical that are antimicrobial. Its anti-acne properties were reported by (Ericsson and Sherris, 1971; Hoeffler et al., 1976). The result of this study revealed that the drug is effective in the inhibition of the growth of *P. acnes*.

A comparison of the three plant extract used shows that *S. alata*, has the highest inhibitory property, followed by *A. indica*. Crude extract of *A. vera* seem to have a negligible to no effect at low dosage concentration (0.1 mL and 0.15 mL) contrary to the popular opinion in Nigeria that *A. vera* gel can be used in the treatment of acnes. The comparison of the antimicrobial properties of the three plant extracts to a synthetic antibiotics (Gentamycin 10 µg), showed that *S. alata* was significantly more effective than all, even to Gentamycin at all dosage concentrations. *A. indica* and *A. vera* on the other hand had lesser inhibitory effect on the growth of *P. acnes* when compared to Gentamycin (Figure 4). *S. alata* and *A. indica* have been reported to inhibit the growth of *P. acnes* *in vitro* (Pukumpuang et al., 2012), but

no literature has been able to show that *S. alata* has a higher inhibitory property than *A. indica*.

Conclusion

Azadirachta indica and *Senna alata* are profoundly anti-microbial; capable of inhibiting the growth of *Propionibacterium acnes*, with both extensive folklore as well as literature supporting their use. This research has been able to confirm the effectiveness of *S. alata* and *A. indica* in inhibiting the growth of *P. acnes*. *A. vera* has also been shown not to be effective in the inhibition of the growth of *P. acnes*.

Conflict of interest

The authors declare that they have no conflict of interest in the publication.

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