

A Comparative Study of Antibacterial Activity of Leaf and Root Extracts of *Sida acuta* Burm. F. Against *Staphylococcus sciuri* Isolated From Acne Using Computer-Aided Technique

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ABSTRACT

This study investigated the significant anti-bacteriological efficacy between leaf and root of *Sida acuta* Burm. F. extracts against acne inducing bacteria. Twelve samples were collected from twelve persons with facial acne. Nutrient agar was used for the isolation of the bacteria and 16S rRNA gene sequencing technique was used for molecular identification. Bioinformatics analysis was carried out using Basic Local Alignment Search Tools (BLAST) for similarity, Cluster W software for multiple sequence alignment and MEGA 7 software for the phylogenetic analysis. Antibacterial susceptibility test of the bacterial isolates was carried out using the agar well diffusion method. A total number of six bacterial species were identified as *Staphylococcus sciuri*. The study revealed that control antibiotic- Ciprofloxacin (CFX) inhibited the growth of all the bacterial isolates. *Staphylococcus sciuri* was susceptible to leaf ethanol extract of *Sida acuta* at 75 µg/ml and 100 µg/ml and was susceptible to the aqueous root extract at 25 µg/ml and 100 µg/ml while it was susceptible to the ethanol root extract at 2.5 µg/ml. From this study, it was shown that the aqueous root extracts had more zones of inhibition with the maximum inhibition of 45mm at 100 µg/ml. Hence, *Sida acuta* Burm. F. is a powerful antimicrobial agent.

Keywords: Antibacterial activity, Molecular characterization, Acne, Phylogenetic analysis.

Introduction

The body's most enormous organ is the skin and covers approximately 2 m² (20 ft²) of the adult human (Selly, 2015). The skin being exposed, is easily accessed by environmental microorganisms.

These organisms may live within the skin pores or on the skin as commensals (resident or transient) and varies from one portion of the body to another (Skowron *et al.*, 2021). Microorganisms known to be present in/on the human skin include *Propionibacterium acnes*, *Staphylococcus aureus*,

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Staphylococcus epidermidis, *Streptococcus pyogenes*, *Corynebacteria* sp., *Candida* sp., *Bacillus* sp., *Pseudomonas aeruginosa*, *Malassezia furfur* - *Pityrosporum ovale* and *P. orbiculare* (Cogen *et al.*, 2008; Zeeuwen *et al.*, 2012; Scharschmidt and Fischbach, 2013). *Propioni-bacterium acnes* is a prevalent organism among the different groups of skin microbiota and actively isolated from acne disorder (Zandi *et al.*, 2011; Jonczyk-Matysiak *et al.*, 2017).

Acne vulgaris (or simply acne) is a frequent disorder of the skin diagnosed and treated by dermatologist(s) and non-dermatologist(s) (skin therapist) alike (Rathi, 2011; Knutsen-Larson *et al.*, 2012). It is a multifactorial pleomorphic disorder of the pilosebaceous follicles of the skin characterized by non-inflammatory and inflammatory lesions (Dhillon and Varshney, 2013; Patel *et al.*, 2015; Nelson *et al.*, 2016). Acne is also referred to as a skin disease caused by bacteria induction (Nasri *et al.*, 2015; Polugari *et al.*, 2016). The site of occurrence is usually the face, chest, neck, upper back and upper arm as those portions of the body possess the largest oil glands (Oberemok and Shalita, 2002; Zandi *et al.*, 2011; Polugari *et al.*, 2016).

Antibiotics (both oral and topical) have been used as therapeutics for acne treatment along with the use of benzoyl peroxide, hormonal therapies and topical retinoid (Rathi, 2011; Knutsen-Larson *et al.*, 2012, Lynn *et al.*, 2016). Long term use of antibiotics is proving not effective owing to increasing antibiotic resistance (Zandi *et al.*, 2011; Jonczyk-Matysiak *et al.*, 2017). Resistance to antibiotics is a multifactorial process, which includes host characteristics, the precise nature of the bacteria's association to antibiotics, environmental factors and the antibacterial usage (Kumar *et al.*, 2007).

Medicinal plants have been extensively studied for use as a different treatment for acne and other diseases of the skin, to manage the problem of antibiotic resistance and the unwanted side effects caused harmful chemicals in other topical and systemic therapies (Kumar *et al.*, 2007; Nasri *et al.*,

2015). Plants as medicine have been used as the oldest method of treating diseases and infections. Various plants are used in different parts of the world to treat human diseases and infections especially in Africa and Nigeria as whole (Ekpo and Etim, 2009). *Sida acuta* Burm. F., a member of the Malvaceae family of medicinal plants, the subject of the current study has anthelmintic, suppurative, astringent, antiplasmodial, antioxidant, anti-inflammatory, antibacterial, and cytotoxic characteristics (Konaté *et al.*, 2012).

Sida acuta have antimicrobial and antioxidant properties as well as cytotoxic activities (Ajeet Singh and Navneet, 2018). The plant is known to be used for cases such as malaria, ulcer, fever, eczema, wounds, snake bite, inflammation, headache and cold (Akilandeswari *et al.*, 2010, Nigeria Natural Medicine Development Agency, 2013). Its chemical constituents (phytochemicals) comprises of alkaloids, steroids, phenols, saponins and tannins (Akilandeswari *et al.*, 2010; George *et al.*, 2017).

Materials and Methods

Plant sample collection and identification

Fresh, healthy and disease free, whole *Sida acuta* Burm. F. plant was obtained locally from the campus ground of Samuel Adegboyega University, Ogwa, Edo State. Ogwa is a town in the Edo Central Senatorial District of Esan West Local Government Area, Edo State. It is among the constituent communities of Esan land in Edo State. It lies approximately on latitude 60 30' 0N and longitude 60 12' 0E. The plant was identified at the Department of Biological Sciences, Samuel Adegboyega University by a botanist.

Sample Collection

Samples were collected using sterile cotton swab sticks. The cotton swabs were moistened with normal saline (Excess of the fluid was squeezed out by pressing the swab against the side of the tube). Twelve (12) samples were collected from 12

persons with acne on the face by breaking open the acne pores. The swabs were then aseptically transported to the Microbiology Laboratory, Samuel Adegboyega University, Ogwa, Edo State for microbiological analyses.



Fig 1: An uprooted *Sida acuta* plant.

(Source: Photographed personally on the campus ground of Samuel Adegboyega University, Ogwa, Edo State).

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Bacterial extraction and biochemical test

The samples were inoculated on nutrient agar plates using the streak plate method (Microbiology Society, 2016). The inoculated nutrient agar plates were incubated at 37°C for 24 hrs in an incubator. Visible discrete colonies on inoculated plates after the specified period of incubation were noted. Sub-culturing was done to obtain pure isolates. Pure culture bacterial isolates were characterized and identified macroscopically and microscopically based on colour, elevation, margin, shape, texture, surface, appearance, arrangement, shape (cell shape)

and gram reaction (Leboffe and Pierce, 2008). Biochemical tests such as catalase, oxidase, coagulase, indole, sugar fermentation tests were carried out.

Preparation of *Sida acuta* Burm. F. Extracts

The leaves and roots of the plants collected was separated and washed thoroughly to remove the soil and dust particles; the leaves and roots were exposed to ambient condition (shade dried) at an average temperature of 30°C for seven days. The dried leaves and roots were grinded separately with the aid of an electric mill in coarse powder and stored in an air tight containers before use (Ali *et al.*, 2022).

Aqueous and Ethanol Extraction

The method of Azwanida, (2015) was used for the plant extraction:

Aqueous Extraction: 45g and 11g of the leaves and roots were soaked in 380 ml and 400 ml of distilled water respectively and were left to stand for 72hrs. Thereafter, it was filtered through clean muslin cloth. The filtrate was collected and concentrated in an oven at 45°C.

Ethanol Extraction: 45g and 11g of the grinded leaves and roots were soaked in 500 ml and 350 ml of absolute ethanol and left to stand for 72hrs. The mixture was then filtered using a clean muslin cloth. The filtrate was collected and concentrated in an oven at 45°C.

Antibacterial Assay of *Sida acuta* Burm. F. Extracts

The ability of the leaf and root extracts at different concentrations to inhibit growth of bacterial isolates was determined with the agar well diffusion method. Different concentrations of the leaf and root extracts: 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml were prepared.

Agar well diffusion method

Sterile Mueller-Hinton agar (L:S-Biotech) in a petri dish was seeded with bacteria culture and spread uniformly by streak plate method to ensure even distribution of the microorganisms and then allowed to stand. Five wells were made using a sterile cork borer (6mm) and 25µg/ml, 50µg/ml,

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75µg/ml and 100µg/ml of the extracts was transferred into four of the wells. Ciprofloxacin (CFX) served as positive control. The plates were allowed to stand for an hour for pre-diffusion of the extracts and thereafter, the plates were incubated 37°C for 24 hrs and the zones of inhibition were measured in mm (Saravanan *et al.*, 2012).

Statistical Analysis

Statistical analysis was done using mean and Z statistics at level of significance = 0.05 using Statistical Product and Service Solutions (SPSS) software

DNA extraction

Genomic DNA was extracted from the cultures using the Quick-DNA[®] Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005)

16S rRNA gene amplification

The 16S target region was amplified using OneTaq[®] Quick-Load[™] 2X Master Mix (NEB, Catalogue No. M0486) with the primers presented as

Table 1: 16S Primer sequences for Polymerase Chain Reaction (PCR)

Name of Primer	Target	Sequence (5' to 3')
16S-27F	16S rDNA sequence	AGAGTTTGATCMTG GCTCAG
16S-1492R	16S rDNA sequence	CGGTTACCTTGTTA CGACTT

Amplified product detection by agarose gel electrophoresis

The PCR products were run on a gel and gel extracted with the Zymoclean[™] Gel DNA Recovery Kit (Zymo Research, Catalogue No. D4001). The extracted fragments were sequenced in the forward and reverse direction (Nimagen, Brilliant Dye[™] Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit[™], Catalogue No. D4050). The purified fragments were analyzed on the ABI 3500xI Genetic Analyzer (Applied Bio

systems, Thermo Fisher Scientific) for each reaction for every sample.

Bioinformatics analysis

The 16S rRNA gene sequence was done using the BLAST- NCBI database to evaluate the closely related bacterial strains. The gene sequence was submitted to NCBI database with the accession number (NR) and a total of fourteen sequences were subjected to multiple sequence alignment using Cluster W program. The phylogenetic tree was constructed using the MEGA 7 software.

Result

The colony morphology of bacterial isolates from sample is presented in Table 1. Some of the isolates were circular, low convex, entire with wet and smooth surfaces, small in size, creamy in colour and did not produce any pigment. The biochemical characterization of the isolates is represented in Table 2. Some of the organisms were able to produce the enzyme catalase, some were able to utilize citrate as a sole carbon source, some organisms were able to reduce nitrate. Organisms were able to ferment glucose with the production of gas, fructose, maltose, glucose etc.

The result of the antibiotic susceptibility test for the root and leaf extract of *Sida acuta* on the bacteria is presented in table 3. The extracts showed zones of inhibitions at some specific concentrations.

Table 4 and 5 showed the statistical analysis that was carried out to test the potencies of the extracts using Z-calculated and Z-tabulated. The result showed high values for Z-calculated at 1.96 critical level. Table 6 showed that the two extracts have antibacterial activities.

The phylogenetic analysis was done based on the Tamura-Neil method. The tree was constructed using the MEGA 7 software. 16S rRNA of *Staphylococcus sciuri* from sample is closely related to 16S rRNA of other strains.

Table 1: Cultural and Morphological Characteristics of Bacterial Isolates

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Isolate Code	Colour	Elevation	Margin	Shape	Texture	Surface	Appearance	Gram reaction	Cell Shape	Arrangement
A1	White	Flat	Undulate	Irregular	Viscous	Smooth	Shiny	+ve	Cocci	Clusters
A2	Creamy	Convex	Undulate	Irregular	Moist	Rough	Shiny	+ve	Cocci	Pairs
A3	Creamy	Flat	Entire	Round	Viscous	Smooth	Shiny	+ve	Cocci	Pairs
A4	Creamy	Flat	Entire	Round	Moist	Smooth	Shiny	+ve	Cocci	Pairs
A5	Creamy Yellow	Flat	Undulate	Irregular	Dry	Rough	Dull	+ve	Cocci	Clusters
A6	Creamy Yellow	Flat	Undulate	Irregular	Dry	Rough	Dull	+ve	Cocci	Pairs

Key: A1-A6 = Isolation code for the *Staphylococcus* sp. +ve= positive

Table 2: Biochemical Characteristics Of Bacterial Isolates

Isolate Code	Catalase	Oxidase	Coagulase	Indole	Glucose	Lactose	Maltose	Sucrose	Haemolysis	Mannitol	Probable Organism
A1	+ve	+ve	+ve	-ve	A	-ve	-ve	AG	γ	+ve	<i>Staphylococcus</i> sp.
A2	+ve	+ve	-ve	-ve	A	-ve	-ve	A	γ	+ve	<i>Staphylococcus</i> sp.
A3	+ve	-ve	+ve	-ve	AG	-ve	AG	AG	γ	+ve	<i>Staphylococcus</i> sp.
A4	+ve	-ve	-ve	-ve	AG	-ve	AG	AG	γ	+ve	<i>Staphylococcus</i> sp.
A5	+ve	+ve	+ve	-ve	AG	G	G	G	α	+ve	<i>Staphylococcus</i> sp.
A6	+ve	+ve	+ve	-ve	AG	AG	AG	AG	α	+ve	<i>Staphylococcus</i> sp.

Key: A1-A12 = Isolation code for the bacterial isolates, +ve = Positive, -ve = Negative, A = Acid Production, G = Gas Production, AG = Acid and Gas Production, α -haemolysis = Alpha haemolysis, γ -haemolysis = Gamma haemolysis

Table 3: Antibiotic Susceptibility Test for The Root and Leaf Extract of *Sida acuta* on *Staphylococcus* sp.

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Plant part	Solvents	Conc. (µg/ml)	A1	A2	A3	A4	A5	A12	
Leaf	Aqueous	25	-ve	-ve	-ve	-ve	-ve	-ve	
		50	-ve	-ve	-ve	-ve	-ve	-ve	
		75	-ve	-ve	-ve	-ve	-ve	-ve	
		100	-ve	-ve	-ve	-ve	-ve	-ve	
		CFX	+ve (40)	+ve (40)	+ve (40)	+ve (40)	+ve (40)	+ve (45)	
	Ethanol	25	-ve	-ve	-ve	-ve	-ve	-ve	
		50	-ve	-ve	-ve	-ve	-ve	-ve	
		75	-ve	+ve (12)	-ve	-ve	-ve	-ve	
		100	-ve	-ve	-ve	-ve	-ve	-ve	
		CFX	+ve (40)	+ve (40)	+ve (40)	+ve (40)	+ve (40)	+ve (45)	
	Root	Aqueous	25	-ve	-ve	+ve (25)	-ve	-ve	-ve
			50	-ve	-ve	-ve	-ve	-ve	-ve
			75	-ve	-ve	-ve	-ve	-ve	-ve
			100	-ve	+ve (45)	-ve	-ve	-ve	-ve
		CFX	+ve (45)	+ve (45)	+ve (35)	+ve (40)	+ve (45)	+ve (40)	
Ethanol		25	+ve (25)	-ve	-ve	-ve	-ve	-ve	
		50	-ve	-ve	-ve	-ve	-ve	-ve	
		75	-ve	-ve	-ve	-ve	-ve	-ve	
		100	-ve	-ve	-ve	-ve	-ve	-ve	
		CFX	+ve (25)	+ve (35)	+ve (35)	+ve (35)	+ve (20)	+ve (25)	

Key:-ve = No zone of inhibition; +ve = show zones of inhibition; Conc. – Concentration. A1 – A3, A5, A12 = *Staphylococcus* sp.

Table 4: Determination of the Antibacterial Activity of Leaf Extracts using Statistical Tool

Plant Extract	Mean	z-Calculated	z-Critical	Decision
Leaf	22.5	4.29	1.96	Reject H ₀

Table 5: Determination of the Antibacterial Activity of Root Extracts using Statistical Tool

Plant Extract	Mean	z-Calculated	z-Critical	Decision
Root	20.5	3.47	1.96	Reject H ₀

Table 6: Comparison Between Leaf and Root Extracts

Plant Extract	Mean	z- Calculated	Antibacterial Activity
Leaf	22.5	4.29	YES
Root	20.5	3.47	YES

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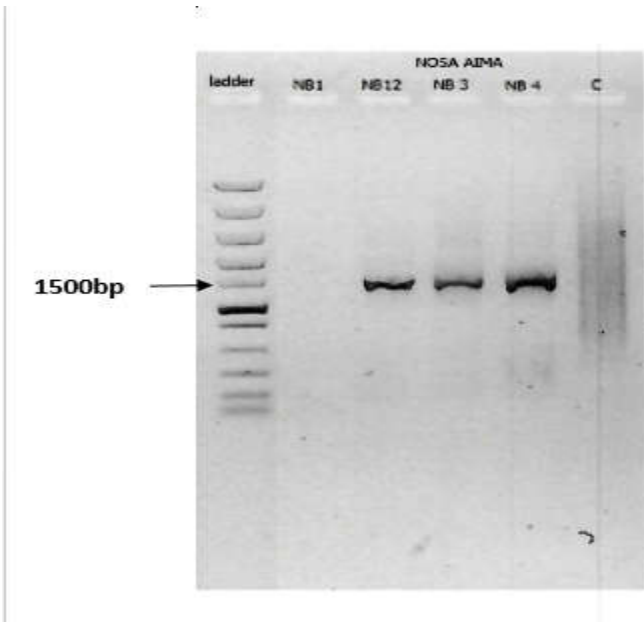


Figure 1: Agarose gel electrophoresis of PCR products with 1500 bp target sequence. DNA ladder, lane 1: amplified DNA of *Staphylococcus sp.* as a positive control. Lanes 2, 3, and 4, show the amplified DNA of *Staphylococcus sp.* while Control is in the last lane.

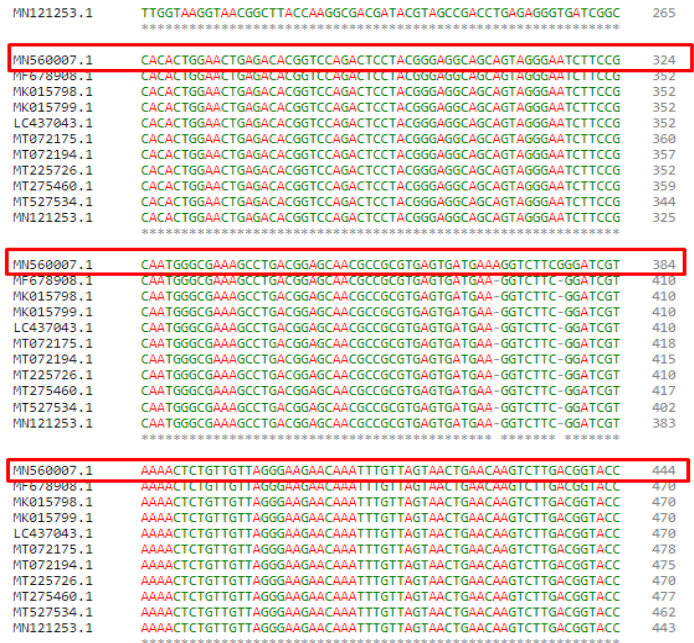


Figure 2: Multiple sequence alignment of 11 databases 16S rRNA genes with 16S rRNA gene of *Staphylococcus sciuri* strain bn1 (MN560007.1)

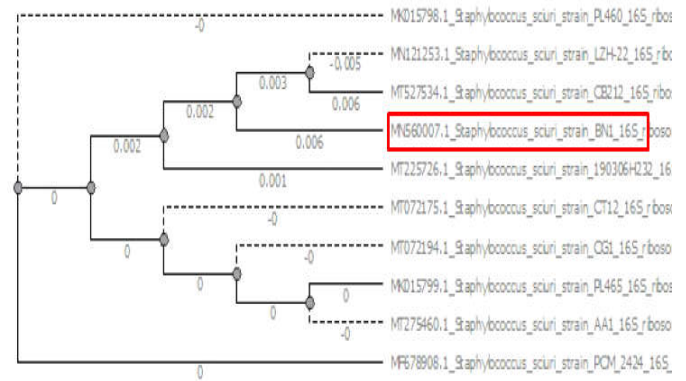


Figure 3: The phylogenetic relationship of *Staphylococcus sciuri* strain bn1 16S ribosomal RNA gene, partial sequence

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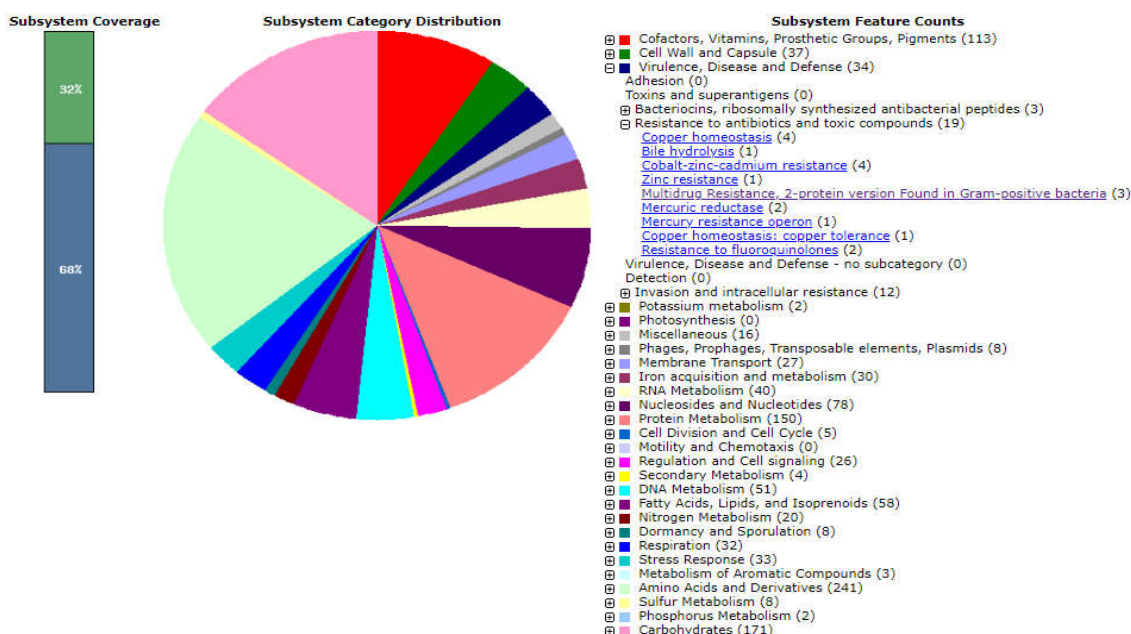


Figure 4: The subsystem features of *Staphylococcus sciuri* strain bn1 indicating its multidrug resistance.

Discussion

The microorganisms obtained from the samples are identified to be normal floral of the human skin (Zeeuwen *et al.*, 2012). In addition, the identified organisms thrive in moist areas of the skin (Grice and Segre, 2011; Fyhrquist *et al.*, 2016).

For the cultural, morphological and biochemical characterization, all bacterial isolates obtained from individuals with acne were all gram positive organisms. Fifty percent (50%) were observed as coccoid in shape, paired and clustered with slight variation in colour (white, cream and creamy yellow), texture (moist, dry and viscous) and surface (smooth and rough). These organisms were further identified as *Staphylococcus* species. *Staphylococcus* species are catalase positive and ferment glucose, sucrose, and lactose to bring about acid production. In the presence of lactose,

they produce gas (Public Health England, 2014). *Staphylococcus* sp. is a prevailing organism of the skin because it is able to colonize the skin surface and mucous membrane as well as cause superficial infections of the inhabited sites (Cogen *et al.*, 2008; Fyhrquist *et al.*, 2016).

The 16S rRNA gene is used generally to identify and characterize microorganisms from different samples (Matsuo *et al.*, 2021). Many researches showed that 16S rRNA gene was successfully amplified using specific PCR primer amplification of gene of strains of *Staphylococcus* sp. to confirm the presence of 16s rRNA gene in the strains at a molecular weight of 1500 bp (Mohammad, 2018). The BLAST and multiple sequence alignment confirmed that the isolate was *Staphylococcus sciuri* with 100% similarity to the database sequence since the 16S rRNA is a phylogenetic maker to detect the closeness of microbes, the MEGA 7 pro software was

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used in the evaluation of the phylogenetic relationship (Kumar *et al.*, 2016).

It was evident that *Staphylococcus sciuri* strain bn1 is closely related with *Staphylococcus sciuri* strain CB212 using the Tamura-Nei Model. Also the RAST software was used to check the subsystems features with an indication that *Staphylococcus sciuri* strain bn1 is showing a multidrug resistance that necessitate the use of *Sida acuta* plant to show case its effectiveness.

Staphylococcus sp. which is regarded as the most frequent aerobic bacteria isolated from acne patients' aside *Propionibacterium acnes*, had the highest percentage occurrence which agrees with studies of Dhillon and Varshney, (2013) and Yousif and Dabbagh, (2016). Also, more species of *Staphylococcus* were isolated from other skin infections such as wound infection which indicated that *Staphylococcus* sp. is a resident flora of the human skin which agrees with Ekpo and Etim, (2009) and Zeeuwen *et al.*, (2012).

Staphylococcus sp. was susceptible to ciprofloxacin (CFX) antibiotic, the standard used for this study. Bacterial isolates were resistant to the aqueous leaf extract of the plant which is in line with the findings by Ekpo and Etim, (2009), whereby aqueous leaf extracts has no significant effect on the Gram-positive isolates used, which can be attributed to the solubility of the active components of powdered leaves in water. The ethanol leaf extract inhibited the growth of *Staphylococcus* sp. at 75 µg/ml showed better inhibition against the gram positive organisms of the same phylum compared to the aqueous leaf extract, which is in

agreement with Karou *et al.*, (2005) and Oboh *et al.*, (2007). For the root aqueous extract, *Staphylococcus* sp. was inhibited at 25 µg/ml and 100 µg/ml while at 25 µg/ml for the root ethanol extract. This can be attributed to the fact that the root of *Sida acuta* Burm. F. is a rich source of phytochemicals especially alkaloids known to show high inhibitory activity as shown in the pharmacological characterization of *S. acuta* root by Pradhan *et al.*, (2012) as well as the alkaloid activity of *S. acuta* root against *Staphylococcus aureus* by Jindal and Kumar, (2012). Altogether, the leaf ethanol extracts had more inhibition for the bacterial isolates but the aqueous root extract had the maximum inhibition of 45mm at 100 µg/ml for *Staphylococcus* sp. an aerobic organism targeted for acne treatment.

From the statistical analysis, the hypothesis (H_0) that, the bacterial isolates were not resistant to leaf extracts was discarded, because the Z calculated (4.5) is greater than Z critical (1.96) at 5% level of significance. It is therefore deduced that the bacterial isolates were resistant to the leaf extracts but show antibacterial activity. This also was done for root extracts of *S. acuta*. The hypothesis that bacterial isolates are not resistant to root extracts was formulated to guide in achieving the one of the objective of this study. Likewise, the Z-Calculated (4.833) is greater than the Z-Critical (1.96) at 5% level of significance as seen in table 4.7. Hence, the root extract *S. acuta* was potent against the bacterial isolate but had less zones of inhibition as recorded in leaf extracts.

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The study further compared whether there is difference in the antibacterial activity of leaf and root extracts. Since the Z statistics shows that there is resistance to both leaf and root extracts, the mean value obtained for the leaf and root are therefore used as comparison. Since the mean value of 32.5 for root extract is higher than the mean value of 31.5 obtained for leaf extract, it is concluded that the antibacterial activity of the root extracts were effective against the bacterial isolates. Since the mean value of 22.5 for leaf extract is higher than the mean value of 20.5 obtained for root extract, it is deduced that the leaf extracts have high antibacterial activity on the bacterial isolates.

Conclusion

From this study, high zones of inhibition were recorded for the leaf extracts which indicates high antibacterial activity than the root extract at 100 µg/ml. Hence, *Sida acuta* Burm. F. is a potential alternative treatment for acne.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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