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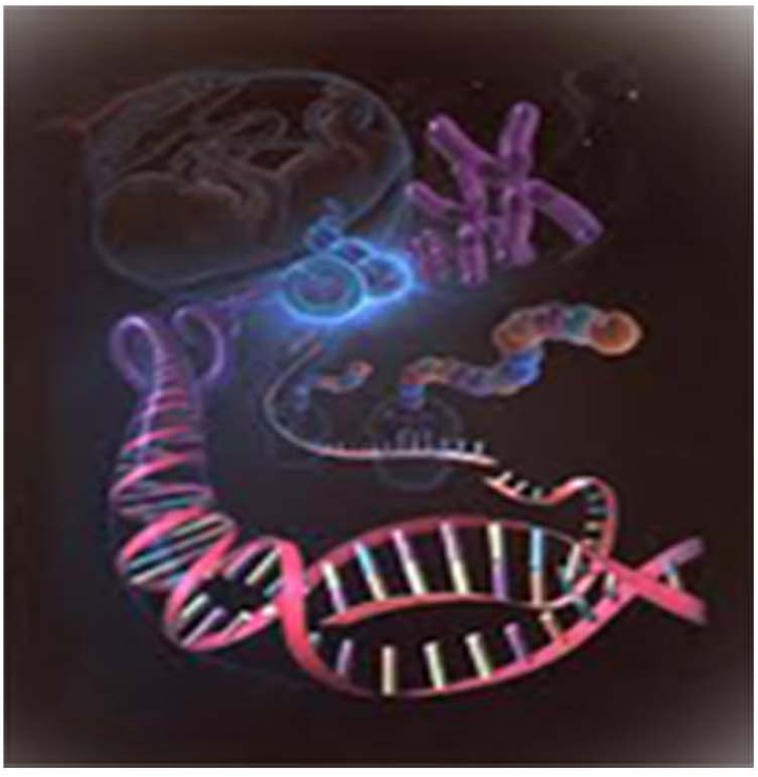

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Research Paper

ANTIMICROBIAL SENSITIVITY OF *ALOE-VERA* EXTRACTS ON *STAPHYLOCOCCUS* AND *PSEUDOMONAS* SPECIES ISOLATED FROM WOUND *SEPSIS*

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The antimicrobial sensitivity of *Aloe-vera* extracts on clinical isolated *Staphylococcus* and *Pseudomonas* species were carried out using the Agar gel and Disc diffusion methods. The solvent of extraction were water (cold and hot) and ethanol. Based on this analysis the effects of *Aloe-vera* extracts were dose dependent. Both water and ethanol extracts were evaluated using varying concentrations of 10 ml/mg, 5 ml/mg, 2.5 ml/mg, 1.25 ml/mg and 0.313 ml/mg. The effects of hot water extracts were high on *Staphylococcus* spp. at 10 ml/mg (5 mm) and on *Pseudomonas* spp. (2 mm) at 10 ml/mg. *Pseudomonas* spp. in cold water had the highest effect at lowest concentration of 0.313 ml/mg (4.5 mm). The effect of ethanol extract was high at lowest concentration of 0.313 ml/mg (4.5 mm) and the minimum inhibitory concentration of water and ethanol extracts was (0.313 ml).

Keywords: Antimicrobial sensitivity, *Aloe-vera, Pseudomonas* spp., *Staphylococcus* spp., Agar gel and Disc diffusion methods

INTRODUCTION

Aloe-vera has been well known for centuries for its healing properties. Both oral intake and topical dressings have been documented to facilitate healing of any kind of skin wound and burns (Yate, 2002). The raw plant is the best, but commercial preparations can also be used. Other topical uses include for healing/curing acne, sunburn, frostbite (it appears to prevent decreased blood flow), shingles, screening out x-ray radiation, preventing scaring, warts, wrinkles from aging and eczema (Lyon, 2008). Internally *Aloe vera* is showing real promise in the fight against AIDS, and the virus has become undetectable in some patients who used it on a regular basis, due to its immune system stimulating properties. It is also used in the prevention of opportunistic infections in case

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of HIV and AIDS (Ernest, 2000). It appears to be of help in curing cancer patients (including lung-cancer) by activating the white blood cells and promoting growth of non-cancerous cells (Marshall, 2000).

The national cancer Institute has recommended further testing of *Aloe Vera.* Due to its apparent use in fighting of cancer (Newton, 2000). Taken orally, *Aloe vera* also appears to work on heart burn, Arthritis, rheumatic pains and cancer. Studies have shown its positive effects on lowering of the blood sugar in diabetics (Yates, 2002). Other situations in which it appears to work when taken internally include congestion, intestinal worms, indigestion, stomach ulcers, colitis, liver infections, urinary tract infections, prostate problems and as a general detoxifier (Gong and Wang, 2002).

Commercially *Aloe vera* can be found in pills, sprays, ointments, lotions, and creams and also available in other numerous products. Unfortunately, the *Aloe vera* industry is virtually unregulated and some products that advertise *aloe* content actually have little or none (Ombrello, 2008). As there has been a lot of antibiotic resistance in the treatment of wounds, the aim of this study was to investigate the antimicrobial sensitivity of *Aloe vera* extract on the two isolated bacterial *Staphylococcus and Pseudomonas.*

MATERIALS AND METHODS

Conical flask, test tube, glass bottle, petri-dish, pipette, cork borer, mortal, glass stirrer, slide, beaker, measuring cylinder, cotton wool and aluminium foil.

Hard Wares

Incubator, autoclave, weighing balance, Bunsen burner, rack stand, water bath and wire loop.

Media and Reagents

Kovac's regent, peptone water, distilled water, acetic acid, alcohol, ammonium solution, safranin, crystal violet, Lugol's iodine, MacConkey agar, nutrient agar and cled.

Sterilization

Autoclavable materials such as agar were aseptically sterilized in an autoclave at 121^{o}C for 15 min. Properly washed pertri-dishes, beakers, wash bottles, test-tubes, pipettes, conical flasks, spatula, inoculating needles and forceps were sterilized using hot air oven at a temperature of 180 ^{0}C for 2 h. The wire loops were sterilized by heating in the blue flame of the Bunsen-burner until red-hot and allowed to cool. 70% alcohol was used to swab the working bench area to prevent contamination. This process was carried out aseptically.

Collection of The Plant

Fresh *Aloe vera* was collected from experimental garden at Uwani Enugu, Nigeria.

Preparation Of The Plant

The collected *Aloe vera* was mashed with the aid of a blender, labelled and kept in a conical flask until extraction.

Extraction

The extraction was done using soaking method. The blended *Aloe vera* samples were extracted using cold water, hot water and ethanol. The sample was soaked overnight for 24 h. After 24 h, the sample was filtered with muslin cloth and the filtrate was collected in a round bottom flask.

Preparation of Culture Media

The media for culturing were aseptically prepared according to the manufacturer's instructions and autoclaved at 121^{o}C for 15 min.

Identification of Test Organisms

Test organisms were collected from the microbiology department of the National Orthopaedic Hospital, Enugu. The isolated microorganisms were subjected to gram staining and Biochemical tests for proper identification.

Gram Staining

A smear was made on the slide. The heat-fixed smear was laid across a staining rack and placed over a sink. Dry smear was flooded with crystal violet and allowed to stand for 1 min, the smear was washed off with water. Lugols iodine was used to flood the smear to remove excess stain. After a minute, it was washed with water and 95% acetone was applied and allowed to stand for 30 min. Thereafter, the smear was washed with water and flooded with safaranin for 30 s. It was washed off after 30 s and allowed to air dry. After drying the smear was viewed using oil immersion microscope. Here two colors were observed: gram positive bacteria showed blue black and gram negative bacteria which showed pink or red.

Indole Test

The tryptophan broth was inoculated with the test sample at 37°C for 28 h then 0.5 ml of the Kovac's reagent was added, gently agitated and examined for 1 min. The upper layer of the liquid of the test tube that turns red indicates a positive result.

Motility Test

This test was done using the hanging drop method. A drop of the test organisms in a saline suspension was placed on a cover slip. The cover slip was inverted and placed on a cavity slide, this was viewed under the microscope; a sharp darting movement in different direction across the field viewed of the microscope indicated a positive motility result.

Sugar Test

1.0 g each of different types of sugar like glucose, lactose and sucrose were dissolved separately. To each 10 ml of peptone water was added in test tube. This was followed by the addition of 2 drops of 0.1% phenol red. Durham tubes were also placed on to the test tubes inverted in order to detect the production of gas and acid. The tubes were autoclaved at 110°C for 15 min. And after cooling, the tubes were inoculated with bacterial culture using sterilized wire loop under aseptical condition. Un-inoculated tubes were used as the control tubes were then incubated for 48 h at 37°C. A color change to yellow showed acid production and was recorded as positive fermentation.

Catalase

A small amount of the culture was picked from agar slope, using a clean sterile platinum wire loop. This was inserted in drops of H_2O on a clean microscope slide. The production of gas bubbles was indicated a positive.

Coagulase Testing

A loopful of colony was placed on a slide. About few drops of normal saline was also placed and used to emulsify the colony. Formation of smooth milky suspension of agglutination which was visible to the eye showed positive reaction while no agglutination reaction showed negative reaction. This can also be done using human plasma if the normal saline could not form chimp.

Reconstitution of Extract

The blended extracts were reconstituted by homogenizing using 100 ml of ethanol and water (hot and cold) respectively. The mixture was filtered using the sterile Whatman's No 1 filter paper to remove impurities and other contaminants. The stock solution was further

dissolved at different concentrations and then stored in sterile conical flasks, for further analysis.

Antimicrobial Screening of the *Aloe vera* Extracts

Two methods were employed for the antimicrobial testing which are the

- Agar gel diffusion method.
- Disc diffusion method.

AGAR GEL DIFFUSION METHOD

The anti-microbial screening of ethanol *Aloe vera* extract was done as described by Lino and Deogracious (2006). Nutrient agar, was poured into sterile Petri-dishes and allowed to solidify. 1 ml of the cultured organism was dropped on the solidified agar and the organism spread all over the surface of the agar using spreader.

Wells of approximately 5 mm in diameter were made on the surface of the agar medium using a sterile cork borer.

The plates were turned upside down and the wells labelled with marker. Each well was used as control for the culture plates that were incubated aerobically at 37°C for 24 h. Sensitivity of the organisms to the extract was recorded.

DISC DIFFUSION METHOD

The locally prepared sterile discs were soaked in the water and ethanol extracts for some hours and nutrient agar medium was poured in sterile petri-dishes that were allowed to solidified. 1 ml of the test organism was placed on the solidified agar and spread all over the surface of the agar. The soaked discs were picked using forceps. The plate was incubated at 37°C for 24 h. Sensitivity of the organisms was recorded.

RESULTS AND DISCUSSION

The result of antibacterial effect of *Aloe vera* on the test organisms *Staphylococcus* spp. and *Pseudomonas.* spp. were obvious through the zone of inhibition made by the extract. The concentration and the solvents used in extraction affected the activity of the extract as illustrated in Tables 2, 3, 4 and 5. The hot water extract had MIC of 0.63 ml/mg and 1.2 ml/mg on *Staphylococcus* spp. and *Pseudomonas* spp. respectively as shown in Table 3. The cold water extract had MIC of 10 ml/mg and 5 ml/mg on *Staphylococcus* spp. and *Pseudomonas* spp., respectively as illustrated in Table 4 above. While, the ethanol extract had MIC values of 0.313 ml/mg, respectively for both *Staphylococcus* spp. and *Pseudomonas* spp. From results obtained above the organisms used are gram positive and gram negative bacterial and both ferment sugar to produced either acid or gas or both.

Table 1: Morphological Characteristics of Test Organisms Isolated From Wound Sepsis

Morphological characteristics of colony Isolated	Grams Reaction	Presumptives Identification
Small round milky raised colonies of 0.5 µm-1.0µm in diameter on nutrient agar medium.	Gram positive *cocci*	*Staphylococcus Spp.*
Yellow: colonies, Rod-smell shape motile organism Cled and reddish on MacConkey agar.	Gram negative (–ve) rod shaped organism with flagella	*Pseudomonas Spp.*

Table 2: Biochemical Tests for Identification of The Test Organisms

Biochemical Test		Sugar fermentation		Isolated organism
-	+ Cataglase	A	A Lactose	*Staphylococcus* Spp.
-	+ Coagulase	G	A Sucrose	*Pseudomonas* Spp.
+	- indole	A	AG Glucose	
+	- motility	-	AG Manitol	

Key: (+) = Positive reaction; (AG) = Acid and gas produced; (-) = Negative reaction; (A) = Acid produced.

Table 3: The Antimicrobial Effects of Hot Water Extract of *Aloe vera* on *Staphyloccous* Spp. and *Pseudomonas* Spp.

Yeast Organism	Concentrations of the Extract						MIC (ml/mg)
	10ml/mg	5ml/mg	2.5ml/mg	1.25ml/mg	0.63ml/mg	0.313ml/mg	
Staphylococcus Spp.	5mm	3mm	2mm	1.5mm	1mm	0.0mm	0.63ml/mg
Pseudomonas Spp.	2mm	1mm	0.5mm	0.2mm	0.0mm	0.0mm	1.2ml/mg

Key: MIC is the smallest concentration that inhibits the growth of test organism.

Table 4: The Antimicrobial Effects of Cold Water *Aloe vera* Extract on *Staphyloccocus* Spp. and *Pseudomonas* Spp.

Yeast Organism	Concentrations of the Extract						MIC (ml/mg)
	10ml/mg	5ml/mg	2.5ml/mg	1.25ml/mg	0.63ml/mg	0.313ml/mg	
Staphylococcus Spp.	2.5mm	3.5mm	3.7mm	4.0mm	4.2mm	4.2mm	10ml/mg
Pseudomonas Spp.	0.0mm	0.3mm	1.0mm	2.5mm	3mm	4.5mm	5ml/mg

Key: MIC is the smallest concentration that inhibits the growth of test organism.

Table 5: The Effects of Ethanol Extract of *Aloe vera* Extract on *Staphylococcus* Spp. and *Pseudomonas* Spp.

Yeast Organism	Concentrations of the Extract						MIC (ml/mg)
	10ml/mg	5ml/mg	2.5ml/mg	1.25ml/mg	0.63ml/mg	0.313ml/mg	
Staphylococcus Spp.	3.00mm	3.5mm	3.9mm	4.0mm	4.2mm	4.5mm	0.313ml/mg
Pseudomonas Spp.	0.4mm	0.7mm	1.0mm	1.3mm	1.8mm	2.2mm	0.313ml/mg

Key: MIC is the smallest concentration that inhibits the growth of test organism.

Based on the analysis of antibacterial effect of *Aloe-vera* extract with water (hot and cold) and ethanol, *Aloe vera* should be used in the treatment of wound infected by *Staphylococcus* spp. and *Pseudomonas* spp.

CONCLUSION

In conclusion, *Aloe vera* could be a potent wound healing agent for wounds that are infected by *Staphylococcus* spp. and *Pseudomonas* spp.

RECOMMENDATIONS

We hereby recommend that cold water extract of *Aloe vera* should be used in the treatment of infections caused by *Staphylococcus* spp. and Pseudomonas spp. instead of ethanol extract, since both of them have almost the same effect.

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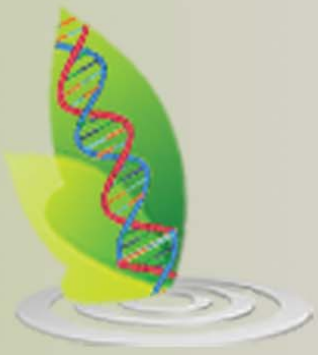

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