EVALUATION OF ANTIBACTERIAL PROPERTY OF METHANOL EXTRACT OF ALOE-VERA GEL AGAINST MDR *S. AUREUS* PHENOTYPE INDUCED ACNE SKIN SITE: FUTURISTIC AI ASSISTED VISION

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Abstract:
Staphylococcus aureus was used to investigate the antibacterial properties of the methanol extract of Aloe-vera gel and the medicines erythromycin and clindamycin (2µg). Once the leaf gel was obtained, the leaf was extracted using methanol. Zones of inhibition were used to gauge the antimicrobial efficacy. The growth of S. aureus (14mm) was decreased by the methanol extract of aloe vera gel, according to an antimicrobial susceptibility test. The study's findings support the widely used methanol extract of aloe vera gel to treat acne caused by S. aureus.
Keywords: Aloe-vera, *S. aureus*, Methanol

Introduction:

Originating in Africa, Aloe vera Linne, also known as Aloe barbadensis Miller, is a succulent belonging to the 400-species Aloe family. The plant can withstand extended droughts thanks to the water source found in its thick leaves (Foster, 1999).

According to some reports, it is a natural cleaner, strong at penetrating tissues, effective at reducing joint and muscle pain, bactericidal, a potent antibiotic, virucidal when exposed to prolonged contact, fungicidal, anti-inflammatory, and helps to increase circulation in the area, break down and digest dead tissue, and moisturize tissues. Aloe vera appears to aid in the opening of the skin's pores so that the skin may absorb moisture and plant nutrients up to four times faster than water. Furthermore, a number of aloe vera compounds have been shown to improve the body's immune system's performance. Additionally, aloe can activate macrophages (Davis, H.R. 1997).

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The antibacterial qualities of aloe liquid have been shown to be effective against both Gram-positive and Gram-negative bacteria. *Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Propionibacterium acne, Helicobacter pylori, Streptococcus pyogenes, Klebsiella pneumoniae,* and *Staphylococcus aureus* have all been demonstrated to be effectively killed, significantly inhibited, or eradicated by the antimicrobial qualities of aloe vera gel. In 2000, J. Lawless and J. Allan

A. vera juice is widely available and is believed to help with a number of gastrointestinal irritants (Foster, 1999). In Germany, concentrated formulations of dried aloe leaves are used as a laxative prior to rectal surgery and as a hemorrhoid therapy. Perhaps the most well-known herbal remedy in the US today is aloe gel, which is used to treat sunburn, thermal burn, and wound healing (Foster, 1999). Additionally, research suggests that aloe gel could help strengthen the immune system (Davis, 1997).

There is an urgent need for a solution to the major health risk of antibiotic-resistant human illnesses (Anandaradje et al., 2020). The resistance issue has

arisen as a result of the inappropriate usage of commercial antimicrobial drugs to treat infectious diseases (Salayov et al., 2021). These resistance mechanisms develop in bacteria due to a variety of enzymatic and genetic changes (Singh et al., 2018; Deljou and Goudarzi, 2016). This situation has forced scientists to look for new antimicrobial therapeutic drugs from a variety of bio-sources, such as medicinal plants and microorganisms, which have been found to be the most efficient sources (Arbab et al., 2021).

Materials And Methods:

Sample Collection:

Samples were taken from a 25-year-old male patient with acne who lived in the Chandrapur District of Maharashtra. The sample was taken from the wound using a sterile swab stick and aspirate (Pus). The swab sticks were collected and then brought to the microbiology lab for examination.

Isolation:

After streaking the sample-containing swab onto the agar medium to inoculate it, it was incubated at 35 to 37 °C for 24 hours. The resultant pure isolates were cultured for 24 hours at 35 to 37 °C after being injected in nutrient broth medium.

Characterization and Identification:

Specimens are cultivated on Mannitol Salt Agar (MSA) to select for Staphylococci. Standard Gram-staining procedure was conducted, further catalase test, coagulase test was conducted for better conclusion. Additional characterisation could involve the Vi-teck system-2 for absolute confirmation

Antibiotic Sensitivity Testing:

For this, the disc diffusion method was applied. For this experiment, a 0.2 mL aliquot of the test organism was transferred from the nutritional broth medium into a sterile petri dish. The antibiotic discs were aseptically inserted onto the surface of the contaminated plates using sterile forceps. Antibiotics, clindamycin (2 µg) and erythromycin discs (60 µg) discs were used.

Methanol Extraction Method:

After washing mature, healthy, and recently harvested Aloe vera leaves longitudinally with clean water, the colorless, parenchymatous tissue (aloe gel) was carefully scraped out with a sterile knife to remove any remaining green fibers. After combining the 790g of collected gel with 100ml of hot water and letting it sit for a full day, the extract was filtered through Whatman filter paper 1 and allowed to evaporate. According to Joshua et al. (2010), 19.0g of the aqueous extract was prepared and kept in the refrigerator at 40C until needed.

Determination of Minimum Inhibitory Concentration (MIC):

According to Kowalska-Krochmal & Dudek-Wicher's approach (Kowalska-Krochmal B and Dudek-Wicher R. 2021), the minimum inhibitory concentration (MIC) of plant extracts was ascertained using the broth dilution method.

Antimicrobial susceptibility testing

Sterile petri plates were filled with sterile agar (at 45°C) and the test organisms were injected onto them. The plates were allowed to gel for an hour. Wells (10 mm in diameter) were punched into the surface of the agar plate using a flamed cork borer. About 1 milliliter of the gel and leaf extracts, respectively, was added to each well. These were incubated at 37°C for 24 hours. High-sensitivity agar plates were used for S. aureus. They were incubated at 25°C for five days. Zones of inhibition were assumed to indicate the presence of antimicrobial action. The average diameter of the evaluated inhibition zones was used to compute the antimicrobial activity based on the observed inhibition zones.

AI Scope:

The data of antimicrobial resistance and concentration of resistance can be feed into software and the possible treatment remedy can be judged. Void data of antimicrobial resistance and research data on herbal drugs across world can be beneficial in using target drug herbal therapy.

Result and Discussion:

Suspected specimens when cultivated on Mannitol Salt Agar (MSA) to select for Staphylococci, it shows yellow halo colonies because of mannitol fermentation. On Gram-staining, Gram-positive cocci shaped bacteria were observed, isolate showed catalase positive confirmed test. In coagulase test, which finds the clumping factor and bound coagulase, is essential for confirmation because it is a trait that distinguishes S. aureus and or growth on blood agar to observe beta-hemolysis. Vi-teck system-2 confirms it as S. aureus.

Table 1: Antibiotic Susceptibility Test.

Antibiotic	Clindamycin (2 µg)			Erythromycin (60 µg)		
Zone of inhibition	T1	T2	T3	T1	T2	T3
	24 mm	24mm	24mm	21mm	21.5mm	21mm
Conclusion	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant

Key: T1, T2, T3 represents repetition of experiment three times.

Table 2: Minimum Inhibitory Concentration (MIC) of Methanol Extract of Aloe-vera Gel Extract.

Test organism	Concentration in mg/ml					
	1.0	0.50	0.25	0.125	0.0625	MIC
S. aureus	+	+	+	+	+	0.125

Key: + = Inhibition, - = No Inhibition

On addition of 1ml of methanol extract of aloe-vera gel for antimicrobial activity it showed 22 mm of zone of inhibition which shows it is effective than Erythromycin and Clindamycin activity.

Table: Antibiotic Susceptibility Test of Methanol extract of Aloe-vera gel extract.

Extract	Methanol Extract		
Zone of inhibition	T1	T2	T3
	14 mm	14mm	14mm
Conclusion	Sensitive	Sensitive	Sensitive

Key: T1, T2, T3 represents repetition of experiment three times.

When AgNPs and chloramphenicol were combined, it was shown that only *K. pneumoniae* was effectively inhibited by the combination. Nevertheless, in individual tests, chloramphenicol had moderate antibacterial efficacy against every bacterium except P. aeruginosa, where it was found to be resistant. Its resistance was verified by testing a gentamicin disc exclusively on *P. aeruginosa*, which showed that it was susceptible to it. Other studies have shown that *P. aeruginosa* has an exceptionally poor sensitivity to antibiotics (Lorusso et al., 2022).

According to Cheesbrough (1984), *S. aureus* is a natural component of the microbial flora found in the epidermis, upper respiratory tract, and digestive tract. Because the gel contains substances including anthraquinones and homones (Davis, 1997) that have antibacterial, antifungal, and antiviral properties, it is also claimed to aid in wound healing. The gel is probably more active than the leaf because the majority of the components are present in the gel rather than the leaf.

Horse skin wounds typically present with discomfort, erythema, edema, heat, and purulent discharge. Other symptoms, such as serous exudates, delayed healing, discolouration, bad odor, and friable granulation tissue at the wound's base, may be seen in later stages. When these wounds are disrupted, pain increases (Wells et al., 1988).

Aloe vera ethanol and chloroform extracts were tested for their antibacterial properties; the ethanol extract of A. vera exhibited the greatest inhibition against *S. aureus*. According to Jothi Karumari et al. (2014), two examples include *B. subtilis* and *S. pneumonia*. In a comparable study, ethanol extract had the greatest impact on *Shigella, Escherichia coli, Klebisella pneumoniae,* and *S. aureus* when compared to pure aloe extract. Methanol extract showed the strongest antibacterial activity against S. aureus 24 mm, followed by B. cereus, according to Rudrangshu et al. (2015).

According to reports, the substance can impede the microbes' enzymatic activity (Weir, T.L. et al., 2004) and lengthen their lag phase (Coopoosamy, R.M. ans Magwa, M.L., 2006). Duke's Phytochemical Databases also show the presence of ascorbic acid in A. vera gel (Duke, J.A., 1992). Ascorbic acid's antibacterial properties are consistent with research by Fite et al. (Fite, A. et al., 2003) and Vilter (Speranza G. et al., 1986). By disrupting their cell membranes, enzymatic activity, or genetic processes, ascorbic acid may suppress microbes (Frazier, W.C. and Westhoff, D.C., 1995).

Conclusion:

The methanol extract of A. vera exhibited strong antibiotic efficacy with erythromycin and clindamycin activity, according to the current investigation. In comparison to antibiotics, this data suggests that the analyzed plant extracts may include bioactive phytochemical substances that have therapeutic potential for treating S. aureus infections. This study's conclusion offers evidence in favor of standardizing the use of these plants as viable candidates for the creation of innovative antibacterial formulations with higher efficacy that can be applied to improve the productivity and health of animals. Therefore, more research on the antibacterial properties of extraction with different

solvents needs to be done. Analyses of such plant extracts' cytotoxicity, antioxidant properties, and affordability should be looked at, as well as their biological activity, especially in vivo.

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