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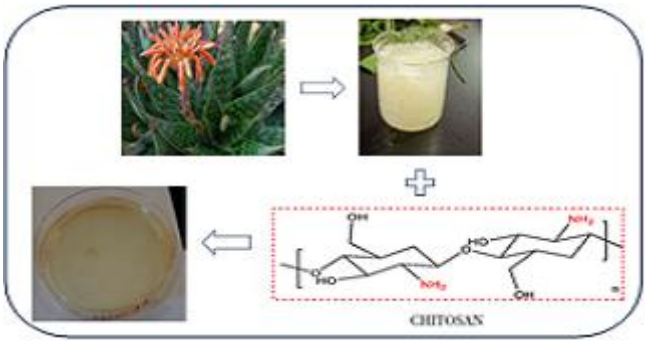
Preparation and Characterization of Aloe Maculata/Chitosan Composite Gel for Wound Healing Application

Hilda Ngomane, Lungiselwa Ndzabandzaba, and Vuyiswa Jane Mkhabela*

Department of Pharmacy, Faculty of Health Sciences, Eswatini Medical Christian University, Lomkiri Portion 69 of Farm 73, Zone 4 Mbabane, Eswatini
*Correspondence: vuyiswa.mkhabela@emcu.ac.sz; vjmkhabela@gmail.com

Abstract: Aloe has been traditionally applied for its therapeutic and medical properties over thousands of years. The medicinal properties of aloe include being used as an anti-inflammatory, antimicrobial, antibacterial, analgesics, anti-allergic as well as antioxidant. In Eswatini, Aloe maculata has been traditionally used for treating various ailments, suggesting potential antimicrobial properties. In this study, the species of aloe was screened for phytochemicals and a composite hydrogel was prepared with varying concentrations of aloe (10%, 20% and 30% w/v) and the natural polymer chitosan. The composite gel's solubility, water absorption capacity, and antibacterial activity was studied for a potential application in wound healing. The incorporation of different amounts of Aloe maculata gel solution into chitosan was observed to increase the water absorption capacity, solubility, and antibacterial activity. The composite gel with a 20% concentration of aloe seemed to possess better overall properties beneficial for an application in wound healing.



Keywords: *Aloe maculata, phytochemicals, chitosan, antibacterial, wound healing*

Introduction

With the persistent evolution of global health sector , the care for wounds is still a great concern since no genuine home or a clinical concentration is of existence.[1] According to Queen and Harding (2023:1), the cost of managing wounds in different healthcare systems around the world is high, and estimated to be the following, (in international billions of dollars); United States (126.846), China (26.9452), United Kingdom (10.1114), South Korea (4.7033), Australia (5.140), Egypt (0.5720), Ethiopia (0.1151), South Africa (1.1630), Lesotho, (0.0099), Mozambique (0.0475) and Eswatini (0.0110).[2]

Most Africans remain destitute because their healthcare services are paid out of pocket. In most African countries, the dressing of wound daily demands a financial requirement beyond the capability of the African families.[3] Wounds are an ignored liability and wound management is an overwhelming decision. The treatment options are very diverse; hence, the choice of treatment option is rather a daunting decision than a relief, requiring proper guidance.[4]

Composite gels, also known as hybrid gels or multifunctional gels, are well described as a form of gel material that combines multiple components to achieve enhanced properties and functionalities.[5] These gels are formed by incorporating different types of materials into a gel matrix, resulting in a synergistic combination of their individual properties. Composite gels incorporate a gel matrix (composed of polymeric network), which maintain the structural integrity and defines the overall properties of the material, enhancing the biological functionality. It also contains components such as nanoparticles, polymers, fibers, or biological molecules, into the gel matrix. These components can be either uniformly dispersed or localized within specific regions of the gel.

Traditionally, Swazis (Eswatini citizens) have utilized various indigenous plants for medicinal purposes, including *Aloe maculata*. Studies suggest that *A. maculata* found in Eswatini possesses a diverse phytochemical profile, potentially contributing to its observed traditional uses.[6] However, limited scientific research has been performed for its specific antimicrobial potential within the Swazi context. While data specific to Mbabane is scarce, studies within Eswatini suggest diverse environmental factors might influence plant secondary metabolite profiles.

Chitosan, a widely studied natural polymer, is crucial in environmental and biomedical fields due to its cationic properties.[7] Despite some drawbacks, enhancing its functionality through appropriate modifications is essential for improving its efficiency. Understanding the chemistry behind altering chitosan's surface traits is vital. The polymer is applied in wound healing due to its antibacterial, anti-inflammatory and antifungal property which reduces infection and promotes fast healing. Chitosan promotes cell adhesion, proliferation and differentiation hence used in tissue engineering.[8] These characteristics make it an ideal candidate for scaffolding materials in regenerative medicine.

The objective of this study was to prepare a composite gel composed of chitosan and *Aloe maculata,* anticipated to possess physicochemical characteristics and antibacterial properties that are ideal for an application in wound healing.

Material and Methods

Chemicals and Media: The chemicals and all reagents used in this study were purchased from Merck (Pty) Ltd., Johannesburg, South Africa. The Eswatini Medical Christian University, Medical Laboratory Sciences department provided distilled water for the experiments. The bacterial strains used for the study were *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative).[9]

Plant Sampling and Extraction of Phytochemicals: The *Aloe maculata* plant was identified and authenticated to ensure accurate species identification at the Malkerns Research Station in Eswatini. Fresh leaves of aloe were collected and thoroughly washed with running tap water to remove any soil or dirt. The thick outer layer of the leaves was removed with a scalpel, and the inner leaf pulp (gel) was

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cut into small cubes, then centrifuged at 5,000 RPM for 15 minutes. The supernatant was discarded, and the resulting clear gel was collected and half of it homogenized with 100 ml of 70% ethanol, agitated for 5 minutes, and stored in a refrigerator for further analysis.[9] The *A. maculata* gel extracted from leaves of the plant is shown in Figure 1. Phytochemical screening tests were conducted as shown in Table 1.

Figure 1. *The extraction of gel pulp from fresh leaves of A. maculata*

Preparation of *Aloe Maculata*/Chitosan Composite: An acetic acid solution was prepared and used to dissolve chitosan. When chitosan was completely dissolved, composites gels were prepared with concentrations of 0%, 10%, 20%, and 30% (w/v) *Aloe maculata* extract. The different composite gel mixtures were dried on petri dishes to form discs.[10]

Characterization of Composite Gel: The prepared composite gel was characterized for its water absorption capacity (% fluid absorptivity), solubility (% gel fraction), and antimicrobial activity.

Water absorption capacity (% fluid absorptivity): The composite gels with different aloe extract concentrations were measured by swelling the gels in distilled water at room temperature.[11] Each sample from the aloe loaded composite gels was cut into small cubes of 4 cm^2, weighed and the mass recorded as M1. The samples were then placed in water and immersed for periods of 5, 10, 20, 30, 40, 50, 60 and 90 minutes. After each period the samples were dried weighed again. This weight recorded as M2. The water absorption capacity was calculated from the following equation:

$$W\ (\%) = ((M2 - M1)) / M1) \times 100$$

Solubility (% gel fraction): For the solubility test, four samples were prepared with each composite gel concentration (0%, 10%, 20%, and 30%). Each sample was weighed (M1) and submerged in a beaker containing water. After immersing for 24 hours, each composite gel was removed from the beaker and dried to a constant weight at 60 degrees Celsius in a Dry oven DHG-9030A. The dried samples were then weighed (M2).[10] The percent gel fraction was calculated using the following equation;

$$\text{Solubility (\% gel fraction)} = ((M1 - M2) / M1) \times 100$$

Evaluation of antibacterial activity: To evaluate antimicrobial activity, all instruments used were sterilized before use. Two strains of bacteria namely, *E. coli* (Gram negative) and *S. aureus* (Gram positive) were selected for the antibacterial activity study. The bacterial strains were cultured on Mueller Hinton (MH) agar medium. 2 ml of solution containing bacteria (concentrated according to McFarland standard), was inoculated onto petri dishes containing MH agar. Small sized discs of the prepared A. *maculata*/chitosan composite gels were placed into agar wells made on the agar plates. The agar plates were then incubated for 24 hours at 37 degrees Celsius. After 24 hours, the zones of inhibition on the agar plates were measured.[12]

Table 1. *Phytochemical analysis of Aloe maculata gel extract.*

Name of Phytochemical	Test Procedure	Results
Alkaloids	Wagner's test: 3 ml of the extract was mixed with 2 drops of Wagner's reagent in a test tube.	-
Flavonoids	Alkaline Reagent Test: To 1 ml of the plant extract, 2 ml of sodium hydroxide and 2 drops of diluted hydrochloric acid were added.	++
Terpenoids	2ml chloroform was added to 5ml aloe extract and 3ml concentrated acid was carefully added. Reaction mixture was boiled for 5 minutes.	+
Saponins	Foam Test: 2 ml of the extract was mixed with 2 ml distilled water and shaken vigorously.	+
Tannins	10% Sodium hydroxide test: 4 ml of 10% sodium hydroxide was mixed with 8 drops of extract and shaken well.	+
Anthraquinones	Bornträger's Test: 10 ml of benzene was mixed with the plant sample and soaked for ten minutes, followed by filtration. 10 ml of 10% ammonia was then mixed in.	+
Phenolic compounds	Ferric chloride test: : 3 drops of 5% ferric chloride solution were mixed with the aloe extract.	++
Protein	2% copper sulfate solution, 1 ml of 95% ethanol, and potassium hydroxide were mixed with 2 ml of plant.	+
Glycosides	Bornträger's Test: 5ml of the sample extract was boiled with 45% ethanol, cooled, and filtered. This was then mixed with chloroform and shaken vigorously. After shaking, ammonium solution was added.	+

Results and Discussion

The *Aloe maculata*/chitosan composite gel showed barrier properties and antibacterial activity which are ideal for wound healing. Phytochemical screening of the *Aloe maculata* leaves revealed the presence of flavonoids, terpenoids, saponins, tannins, anthraquinones, phenolic compounds and proteins. Commonly present in medicinal plants, these secondary metabolites are known to have a number of pharmacological characteristics, such as immune-modulating, antibacterial, anti-inflammatory, and antioxidant effects.

Phytochemical Screening of *Aloe Maculata*

The tests employed for phytochemical analysis and the results obtained from the aloe gel are shown in Table 1. Positive results for flavonoids, terpenoids, saponins, tannins, anthraquinones, phenolic compounds and proteins, were obtained. The positive results of these chemicals constituents in the aloe plant extract suggests it may have therapeutic potential that could be further explored. A study done by Sonam and Tiwari (2016) revealed the presence of these phytochemicals.[13,14]

Negative results were obtained for glycosides. The absence of detectable levels of alkaloids in the extract does not necessarily mean they are completely absent. It could be that the concentrations were below the detection limit of the screening methods used. Alternatively, these phytochemicals may not be the major active constituents in this particular aloe plant sample.

Characterization of *Aloe Maculata*/Chitosan Composite Gel

During the preparation of the composite gel, when chitosan was dissolved in acetic acid, it solidified to form a gel like substance as shown in Figure 2. The addition of the aloe gel solution to the chitosan resulted in small white spots on the gel which increased as the concentration of the aloe increased. After drying, it was simple to gather the composite gels because they didn't break, tear, or lose weight. The drying process of the gels was affected by the water content of the aloe which affected the viscosity of the composite gel. The more the *A. maculata* concentration the less viscous the composite gel was.

The physical characteristics of the composite gel were of an essential aspect due to the anticipated application of the gel to be used in a moist environment. A composite gel in wound healing acts as an absorbent of water and carrier of drugs to protect the applied area from moisture and microorganisms.[15]

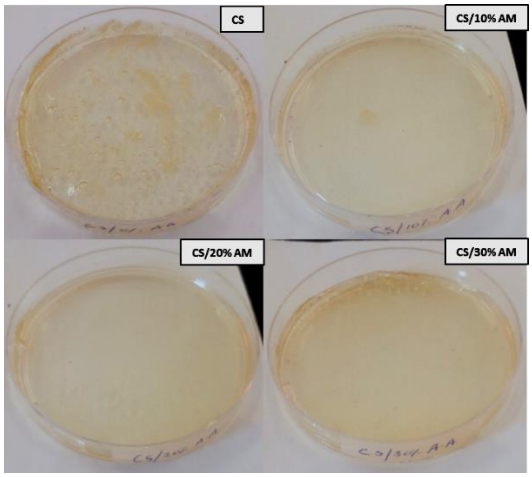

Figure 2: *Composite gels of chitosan with different concentrations of A. maculata.*

The water absorption capacity (% fluid absorptivity) was performed in order to assess the potential of the composite gel to absorb wound exudates, which is an important consideration for gauze and dressing materials in wound care.[16] The results are shown in Figure 3. The sample with chitosan only (0% AM), proved to possess the least ability to absorb water, since at 5 minutes of exposure to water, the water absorption capacity was calculated to be 100 whilst the samples containing *Aloe maculata* (AM) proved to absorb water up to several thousands. This showed a remarkable capacity to absorb water which shows great potential to relieve a wound from exudate, prevent pooling and promote a cleaner wound bed.

On prolonged exposure to water, the CS sample absorptivity increased up to a fewer thousand after 90 minutes. This observation did not surpass the sample containing 10% AM which significantly increased up to 13800 capacity of absorbing water, when it only began at 4650. This evidently shows that, with 10% AM added to chitosan has a potential in inhibiting infections in wounds as large amounts of fluid would be expelled from them. These results suggest the potential use of AM in the composite gels for the management of exudative wounds.

Studies conducted by Trang et al. and Devi M et al,[10] showed different results on the water absorption capacity of their composite gels prepared from *Aloe vera* and chitosan. Their samples with no aloe vera resulted in higher water absorption capacity compared to those samples with the aloe in it. The results obtained in this study are different from other researchers results because of the different method used when preparing the samples and that they used Aloe vera as their incorporated ingredient. When preparing the samples Trang et. al. included glycerol and Devi et. al. included poly-ethylene oxide. Trang chose that method, because he was doing a preservation study of fresh fruits which required the addition of glycerol due to its humectant properties and Devi M was doing a comparative study of different plants in wound healing.

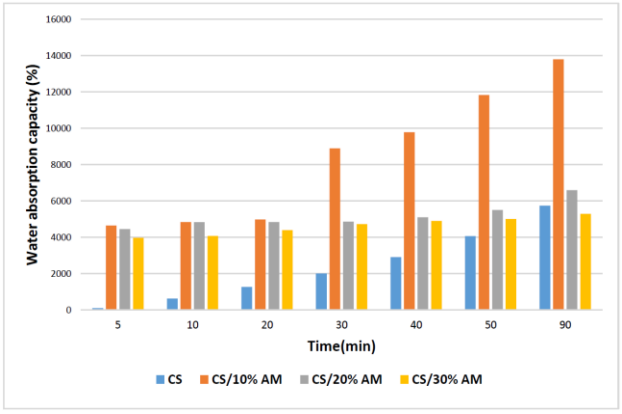

Figure 3: *Water absorption capacity of the different composite gels*

The solubility of the samples was carried out through the immersion of the different composite gels in 100 ml of distilled water for 24 hours. The samples were weighed before and after drying the samples to a constant weight. According to the collected data on Table 2, the mass of the samples was seen to decrease from mass 1 to mass 2. The decrease in mass indicated that the samples were soluble in the water. Generally, the quality of the composite gel increases with its solubility.[10] Generally, the higher the solubility of the composite gel the higher the quality of the composite gel. This is a good characteristic for application in medical situations, as good water solubility tends to release the loaded drug quickly.[17] The % solubility of the composite gels is presented on Figure 4 below.

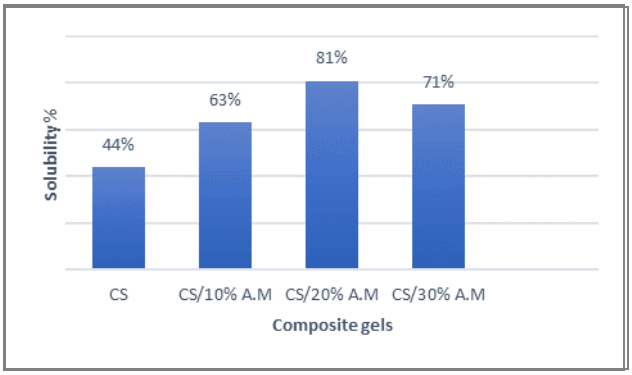

Figure 4: *Percent solubility of the different composite gels.*

Table 2: *The mass of composite gel samples before and after 24 hours of immersion in water.*

Name of composite	Mass 1 (g)	Mass 2 (g)
Chitosan	0.710±0.68	0.400±0.88
CS/10% AM	0.290±0.97	0.106±0.90
CS/20% AM	0.440±0.77	0.083±0.89
CS/30% AM	0.350±1.01	0.096±0.99

Antibacterial activity of the prepared composite gels was investigated by conducting Microbial Susceptibility Testing

(MST) on *E. coli* and *S. aureus*.[18] The zone of inhibition of each sample on MH agar plates with the two different bacterial strains was measured. The results presented in Table 3 showed that antibacterial activity increased when AM was added to chitosan compared to the sample without AM. This is apparent on the inhibition zones of *S. aureus* as CS proved to inhibit 2mm diameter zone and on the CS/10 % AM sample the inhibition zone increased to 6mm and kept on increasing up to 11 mm as the AM concentration was increased. Results for *E. coli* inhibition zones show that for the chitosan only sample, an 8 mm diameter zone resulted and as the aloe concentrations were increased so did the inhibition zones. A standard antibiotic, G-penicillin was used as a positive control.

Table 3. *The zone of inhibition in diameter of the different composite gels on E. coli and S. aureus.*

Name of sample	*E.coli* (mm)	*S. aureus* (mm)
A. maculata	11±0.38	9±0.42
Chitosan	8±0.45	2±0.50
CS/10% AM	4±0.28	6±0.31
CS/20% AM	14±0.11	10±0.22
CS/30% AM	16±1.01	11±0.97
Control	17±0.01	18±0.03

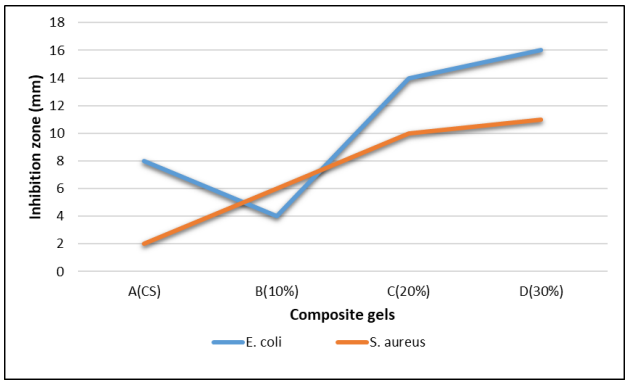

Figure 5: *Microbial susceptibility test of the different composite gels on E. coli and S. aureus after 24 hours.*

Generally, the antibacterial activity of the composite gels was higher as the *A. maculata* content was increased and a higher susceptibility was observed for E. coli as compared to S. aureus. Figure 5 shows a summary of the microbial susceptibility tests on the two bacterial strains. Similar observations were observed in studies by Trang et al., Iqbal et al., and Monzon-Ortega et al. who studied the antibacterial activity of chitosan/aloe vera biofilms. .[10,15,19] In this study, *E. coli* was found to be more susceptible to *A. maculata* compared to *S. aureus* which reached up to 16 mm diameter. The antibacterial activity of aloe species is likely attributed to the phytochemicals identified in the screening, such as flavonoids, terpenoids, tannins, and anthraquinones, which are known to have antibacterial properties. Gram-negative bacteria have lipids in their cell walls, while Gram-positive bacteria have a lot of peptides.[20] The negatively charged microbial cell membranes can interact with the positively charged chitosan molecules, rupturing their integrity and releasing intracellular components of the bacteria.[21] This method significantly inhibits the development and proliferation of bacteria and demonstrates substantial bactericidal and bacteriostatic capabilities. This could be the cause of the composite gel's superior inhibitory action against *E. coli* as opposed to *S. aureus*.

Conclusion

Several phytochemicals with pharmacological and antibacterial qualities were found in the whole leaf extract of *A. maculata*. The composite gels from chitosan supplemented with *A. maculata* with varying contents showed the ability to be effective in biological processes. The study emphasized the construction of a composite gel that has a potential to significantly speed the wound healing process and inhibit bacterial growth. The incorporation of chitosan polymer was to ensure the adhesive potential and enhance the mechanical properties of the composite which are required for the intended application in wound healing. Further studies are required to determine the beneficial effects of the composite gel on the healing of wounds. This will be achieved through fibroblast cell culture studies and the use of animal models. Also further characterization to determine mechanical strength and surface morphology of the composite gel would provide a conclusion of the synergy between the aloe and chitosan in the gel matrix.

Author Contribution Declaration

All authors conceived the research plan and experimental strategy. L. Ndzabandzaba performed the phytochemical screening of the aloe extract, analysed and prepared part of the manuscript. H. Ngomane prepared and characterized the composite gel, and prepared draft manuscript. V. J. Mkhabela confirmed the manuscript preparation and assisted in daily laboratory supervision, designed the research, analyzed the results, and wrote manuscript. The authors have no financial conflicts of interest to disclose.

Data Availability Declaration

The data generated and analyzed is included in this article.

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