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Evaluation of the Effect of Incorporation of Micro- and Nanoparticles of Aloe Vera Extract on the Flexural Strength, Surface Properties, and Microbiological Assay of an Experimental Resin Composite

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Abstract

Purpose: The goal of this study was to assess the effect of incorporation of micro- and nanoparticles of Aloe Vera extract on the flexural strength, surface properties, and microbiological assay of an experimental resin composite. **Materials and methods:** An experimental resin composite was prepared into which the silanized micro and nano Aloe Vera filler particles were incorporated with different concentrations (10, 15, and 20 wt%). Moreover, a commercial resin composite was tested as control. Specimens of the experimental and control composites were prepared in stainless-steel molds for flexural strength, surface properties, and microbiological assay testing. **Results:** Regarding flexural strength and roughness test results, the control group recorded the highest mean value, followed by the micro Aloe Vera-filled composite, while the nano Aloe Vera-filled composite groups recorded the lowest mean values. Vickers hardness test results revealed that the control group recorded the highest mean value, followed by nano Aloe Vera-filled composite, however, micro Aloe Vera-filled composite groups had the lowest mean values. The nano Aloe Vera-filled composite had the highest antimicrobial activity in the microbiological evaluation, followed by the micro Aloe Vera-filled composite, while the control group showed the lowest mean value. **Conclusion:** The incorporation of micro- and nanoparticles of Aloe Vera extract improved the surface roughness and microbiological properties of the experimental resin composite, however, the flexural strength and surface hardness were negatively affected.

Keywords: Aloe vera, Atomic force microscope, Flexural strength, Microbiological assay, Resin composite

1. Introduction

Nowadays, Aloe Vera (AV) is one of the plants that has many applications. Because of their medicinal properties, plant species of the Aloe (Liliaceae) genus have been extensively studied. Aloe Vera is one of the species of the Aloe (Liliaceae) genus that has numerous biologically active substances that have anti-inflammatory, antibacterial, hypoglycemic, immunomodulating, wound healing, and regenerative properties [1].

Furthermore, acemannan, a polysaccharide extracted from AV, is a biomolecule with tissue regeneration potential, playing a key role in cell proliferation, extracellular matrix synthesis, and mineralization [2,3].

AV has been used in the dental field as an additive in dentifrices and mouthwashes. It can be used in the treatment of periodontal pockets as a subgingival medication and in oral lesions such as herpetic viral lesions and aphthous ulcers. Moreover, it can be used as a medication for root canals, a

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media for storing avulsed teeth, and for preventing halitosis. The addition of AV to different products was proven by researchers to greatly increase their effectiveness [4,5].

Natural fillers, interacting poorly with the matrix, are water-resistant and less durable. Weaker adhesive bonds between the hydrophilic fillers and the hydrophobic matrix negatively affect composite properties and limit their uses. To compensate for this lack of compatibility, several approaches have been introduced, including the use of coupling agents and/or other surface modification techniques [6,7].

Currently, nanoparticles are used as fillers for reinforcing polymer composites. As the fillers in the nanoscale are mostly free from defects, they can overcome the limitations of the conventional microscale particles [8].

Nanocomposite restoratives are widely used nowadays due to their excellent mechanical properties and good handling characteristics. The smaller particle size allows for greater filler packing (up to 79 %), which reduces the polymerization shrinkage. In addition to their improved mechanical properties, the resulting nanosized filler particles have better surface finish, translucency, and less biodegradation [9,10].

The powder of Aloe Vera can be considered potential resin composite filler because it typically fills in the polymer voids and enhances wear and thermal properties. Aloe Vera has gained popularity due to its eco-friendliness and biodegradability. It has no pollutant effect on the environment. Aloe Vera filler revealed unique characteristics such as stress shielding effect and good electrical and magnetic properties. Thus, conducting a research study in this domain could be of importance to the scientific and industrial communities [11].

Some studies reported the effectiveness of Aloe Vera as a reinforcing filler for resin composites

[12–14]. A study [15] demonstrated enhanced mechanical, thermal, and morphological properties of nanocomposites made of polyvinyl alcohol (PVA) and cellulose nanofibers derived from AV rind. The authors concluded that 2 wt% of cellulose nanofibers was significantly effective in improving the tensile strength and the modulus of PVA. They also reported that adding AV rind fiber increased the thermal stability of the nanocomposite, which could result in a higher degradation temperature.

Therefore, this study was conducted to evaluate the effect of incorporation of micro- and nanoparticles of Aloe Vera extract on the flexural strength, surface properties, and microbiological assay of an experimental resin composite.

2. Materials and methods

Materials: Materials and chemicals used in the current study are listed in [Table 1].

2.1. Sample size calculation

According to the sample size calculation by G power, with reference to the study by Sailesha A et al. (2018), a total sample size of 196 was calculated to be sufficient to detect a large effect size (0.4), with a power of 90 %, and a significance level of 0.05 [16].

This *in vitro* study was approved by the Research Ethics Committee, Faculty of Dental Medicine for Girls, Al-Azhar University REC-MA-22-01.

2.2. Methods

2.2.1. Specimens' grouping

A total of 196 specimens were prepared in this study. Specimens were divided into 7 groups ($n = 28$) according to the type of Aloe Vera powder particles

Table 1. The materials and chemicals used in the current study.

Chemical name	Presentation	Manufacturer	Batch number
Hybrid resin composite/alpha-dent	Paste	Dental Technologies, Inc.	Lot Y219cz
Bisphenol-A glycerolate dimethacrylate (BIS-GMA)	Viscous liquid	Ningbo Inno Pharmchem (China)	1565-94-2
Triethylene glycol dimethacrylate (TEGDMA)	Liquid	Ningbo Inno Pharmchem (China)	109-16-0
3-aminopropyltrimethoxysilane	Liquid	Ningbo Inno Pharmchem (China)	919-30-2
Ethyl-4-(dimethylamino)benzoate ($C_{13}H_{15}NO_2$)	Powder	Ningbo Inno Pharmchem (China)	10287-53-3
Camphorquinone	Powder	Ningbo Inno Pharmchem (China)	465-29-2
Aloe Vera	Powder	Nano Gate Co. (Egypt)	
Nano Aloe Vera	Powder	Nano Gate Co. (Egypt)	
Silica	Powder	Fisher UK	S-0365-60
Acetic acid glacial	Liquid	Fisher UK	A-0360-PB17
Ethanol70	Liquid	Biochem, Egypt	
Sodium hydroxide (NaOH)	Liquid	Elnada Co. for chemical industries	
Buffer solution Duracal (pH 4.01)	Liquid	Fisher UK	J-2880-1

added to the experimental composite (micro and nano Aloe Vera powder particles) and the concentration of Aloe Vera powder added (10 %, 15 %, and 20 wt%). In addition, an unmodified commercial composite was used to act as control. Each group was tested for flexural strength, microhardness, surface roughness, and microbiological evaluation where $n = 7$ for each experimental condition.

2.2.1.1. Preparation of micro aloe vera powder. AV leaves were washed and disinfected to remove any dirt and surface bacteria. The AV extract was then filtered to produce a fiberless extract. The extract was then sterilized using (UV) ultraviolet light. The AV extract was then freeze-dried to yield micro AV powder [17].

2.2.1.2. Preparation of nano aloe vera powder. Nano AV was prepared by the addition of 1 ml glacial acetic acid to 99 ml of distilled water. After that, 0.2 g of chitosan powder was added to them and left on a magnetic stirrer overnight. A weight of 0.3 g of Aloe Vera powder and 0.1 g of tripolyphosphate (TPP) were separately dissolved in distilled water and added to the chitosan/acetic acid solution while on the stirrer in a dropwise manner successively. Finally, the solution was stored in the refrigerator at -30°C for 2 days before freeze-drying. After that, the freeze-dried crystals were grinded by a quartz mortar and pestle to obtain nano Aloe Vera powder [4].

2.3. Characterization techniques

2.3.1. Fourier transform-infrared analysis (FTIR)

The FTIR analysis was used to determine the functional groups of the chitosan nanoparticles (CsNPs), AV and AV CsNPs, as well as to predict the mechanism of attachment between the CsNPs and AV.

2.3.2. Zeta potential

The zeta potential provides information about the potential difference between the dispersion medium and the stationary layer of the fluid attached to the dispersed particles, which aids in determining the stability of the nanoformulations.

2.3.3. Transmission electron microscope (TEM)

To determine the size and morphology of nanoparticles, TEM was performed by a JEOL JEM-2100 high-resolution transmission electron microscope at an accelerating voltage of 200 kV.

2.4. Preparation of the experimental resin composite

2.4.1. Preparation of the resin matrix

The monomer was formulated by combining 69.3 % bisphenol-A glycerolate dimethacrylate (Bis-GMA) and 29.7 % triethylene glycol dimethacrylate monomers (TEGDMA). For the photo-initiator system, 0.5 wt% camphorquinone and 0.5 wt% ethyl 4-dimethyl-aminobenzoate (EDAB) were weighed and gradually mixed with the prepared matrix, which was then stirred for 1 h using magnetic stirrer [18].

2.4.2. Salinization and incorporation of the filler particles

Silane-coupling agent was prepared by proportioning 70 % ethanol in a glass beaker, then gradually adding few drops of acetic acid to lower the pH to 3.5. Finally, 3 wt% 3-aminopropyltrimethoxysilane was added to the solution. For 1 h, a magnetic stirrer was used to stir the mixture. Micro AV, nano AV, and silica fillers were silanized for 2 h before being centrifuged for 30 min (model 800, China). Finally, the precipitate was dried in a hot oven at 110°C for 1 h [18]. For both micro and nano Aloe Vera, 10, 15, and 20 wt% fillers were weighed by an electric balance to be incorporated into the experimental resin matrix. Silica fillers were then added until a packable consistency was obtained. Scanning electron microscopy (SEM) (Quanta 250 FEG SEM, FEI Company, Netherlands) was used to evaluate the filler particle distribution within the resin matrix (Fig. 1b).

2.5. Testing procedures

2.5.1. Flexural strength (FS) testing

Specimens were constructed using a stainless-steel mold ($25 \times 2 \times 2$ mm) according to ISO standard no 4049:2000 for polymer-based filling, restorative, and luting materials [19].

Flexural strength was tested in a universal testing machine (Instron, [19] Model 2519–106, Instron Corp, Norwood, Mass, USA) at a cross-head speed adjusted to 0.1 mm/min. Flexural strength was calculated using the following equation [20]:

$$FS = 3FL/2bd^2$$

where FS represents the flexural strength (MPa) and F represents the maximum load applied to the specimen (Newton), L is the distance between the supports (20 mm), and b and d are the width and height of the specimen in mm, respectively. The deflection of

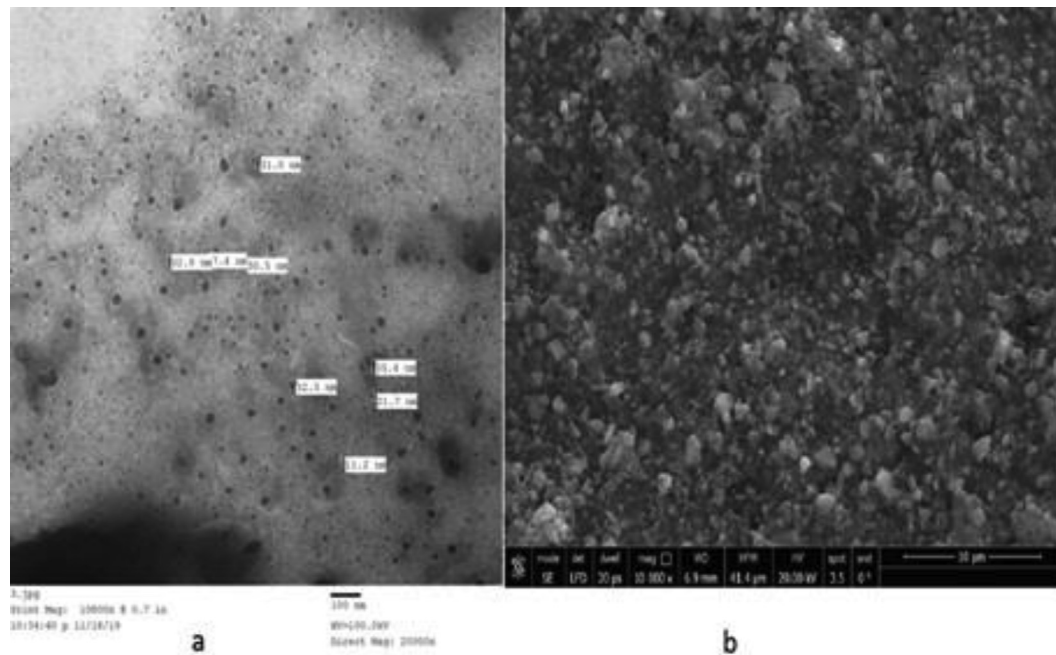


Fig. 1(a). Transmission electron microscope micrograph of nano Aloe vera powder. (b) Scanning electron microscopy images of resin composite modified with 20 wt % nano Aloe Vera.

the specimen was calculated under compressive loading, until the fracture of the specimen [21].

2.5.2. Measurement of vickers microhardness (VHN)

For the microhardness test, disc-shaped specimens (10 mm in diameter x 3 mm in thickness) were prepared according to the ADA specification no 12 for resin-based restorative materials [22]. Vickers microhardness tester with a Vickers diamond indenter (Micromet 2001, Buehler, Illinois, USA) and 20X objective lens was used for testing. For each measurement, three indentations were made randomly on the top surface of each specimen for 15-s dwell time. The length of the diagonals of the indentations was measured by a built-in scaled micrometer and the length values were averaged and then converted into Vickers hardness number (VHN) [23].

2.5.3. Measurement of surface roughness

Surface roughness was performed on the nano-scale using atomic force microscope (AFM) (Thermo microscopes Autoprobe CP research, scanner100 m, USA). The surface of the specimen was imaged in contact mode in air at room temperature. The surface topography was represented in x, y, and z directions. Images were captured using a cantilever. The vertical deflections of the cantilever were used to deduce the topography of the surface. The deflection was measured using a laser spot reflected from the top surface of the cantilever into an array of

photodetector. During scanning, the force between the tip and the surface was kept constant in the 2.5-N range. The scanned areas had dimensions of $25 \times 25 \mu\text{m}$. Computer software was used to record the topographical data of the surfaces [24].

2.5.4. Microbiological evaluation

The 'Agar diffusion test' was used to assess the antibacterial activity of the tested groups. A standardized *Streptococcus mutans* strain was cultured overnight at 37°C on Trypticase Soya agar (TSA) slants to create the inoculum. The prepared blood agar infusion was placed in blood agar petri plates and pre-incubated at 37°C . Using a sterile swab, the *Streptococcus mutans* 24 h inoculum was evenly applied to the surfaces of the blood agar plates. Using sterile tweezers, the specimens were placed on an agar plate containing *Streptococcus mutans*. The diameter of the inhibition zones was measured in millimeters (mm) following a 24 h incubation period under aerobic conditions at 37°C and pH 5 [4].

2.6. Statistical analysis

Data management and statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) version 18. Numerical data were represented as means and standard deviation values. Data were explored for normality using

Kolmogorov–Smirnov and Shapiro–Wilk tests and then analyzed using one-way analysis of variance (ANOVA) test followed by Tukey's post hoc test for pairwise multiple comparisons.

All *p* values are two-sided. *P* values less than or equal to 0.05 were considered significant.

3. Results

3.1. Characterization results of prepared powder and prepared discs

3.1.1. Transmission electron microscope

The micrographs of TEM of nano AV powder revealed uniform AVCsNPs in the range of 37–50 nm at magnification 20000X (Fig. 1a).

3.1.2. Scanning electron microscope

SEM images at 20000X magnification revealed homogeneously distributed nano AV filler particles of 20 wt% concentration within the resin composite, with very low degree of agglomeration (Fig. 1b).

3.1.3. The FTIR analysis of nano aloe vera

The Fourier transform-infrared spectroscopy (FTIR) was used to determine the chemical functional groups of the CsNPs, AV, and AVCsNPs.

FTIR revealed that the peaks for both Aloe Vera and chitosan were observed with no absent or shifted bands (Fig. 2).

3.1.4. Zeta potential

Zeta potential results revealed a dropdown of charge from +37 mV of chitosan to +5.4 mV for

Table 2. Zeta potential of CsNPs, Aloe Vera, and AVCsNPs.

Material	Zeta pot/Mv
CsNPs	+37
Aloe Vera	−15.7
Aloe Vera CsNPs	+5.4

AVCsNPs indicating the combination between the nanoparticles (Table 2).

3.2. Results of testing the resin composite material

3.2.1. Flexural strength (MPa)

The highest mean flexural strength value (MPa) was shown by the control group (126.52 ± 2.04), followed by micro AV 20 wt% (108.91 ± 1.33), micro AV 15 wt% (104.45 ± 1.12), 10 wt% (100.07 ± 1.19),

Table 3. Mean and standard deviation values for the effect of type and concentration of Aloe Vera filler particles on flexural strength (MPa) of the different tested groups.

Group	Mean	SD
Control group	126.52 ^a	2.04
Micro Aloe Vera 10 wt%	100.07 ^d	1.19
Micro Aloe Vera 15 wt%	104.45 ^c	1.12
Micro Aloe Vera 20 wt%	108.91 ^b	1.33
Nano Aloe Vera 10 wt%	83.53 ^e	1.51
Nano Aloe Vera 15 wt%	81.20 ^f	1.10
Nano Aloe Vera 20 wt%	75.39 ^g	0.82
<i>P</i> value	0.000 ^a	

Significance level *P* less than or equal to 0.05. Small letters for intra group comparison under the same group and the means with different symbols are statistically significant difference at $P \leq 0.05$ (It illustrated the different between tested subgroups).

^a Means with different superscript letters are significantly different.

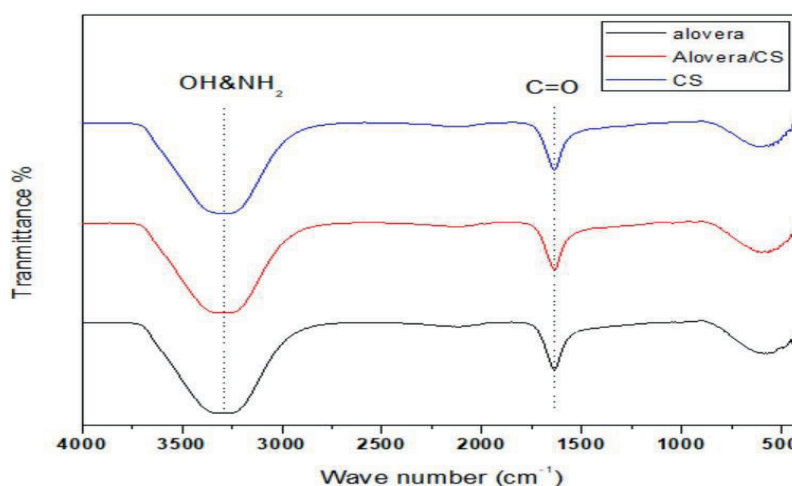


Fig. 2. The Fourier transform-infrared spectroscopy spectra of Aloe Vera, micro Aloe Vera/CS, and CS.

nano AV 10 wt% (83.53 ± 1.51), and then nano AV 15 wt% (81.20 ± 1.10), with the lowest value recorded by nano AV 20 wt% (75.39 ± 0.82). Tukey's post hoc test revealed a statistically significant difference between all tested groups ($P = 0.000$) as shown in Table 3.

3.3. Vickers microhardness results

The highest mean Vickers microhardness value (VHN) was shown by the control group (63.53 ± 0.62), followed by nano AV 20 wt% (56.04 ± 0.58), nano AV 15 wt% (54.40 ± 0.52), nano AV 10 wt% (52.18 ± 0.48), and then micro AV 20 wt% (51.29 ± 0.50), with the lowest value recorded by micro AV 15 wt% (48.44 ± 0.48) and micro AV 10 wt% (47.79 ± 0.48). Tukey's post hoc test revealed a statistically significant difference between all tested groups ($P = 0.000$), except for micro AV 15 wt% and micro AV wt 10 % that showed insignificant difference from each other (Table 4).

3.4. Roughness results

The highest roughness mean value (nm) was shown by the control group (40.80 ± 0.1), followed by micro AV 15 wt% (16.60 ± 0.2), micro AV 20 wt% (14.55 ± 0.15) and 10 wt % (9.40 ± 0.27), nano AV 10 wt% (1.81 ± 0.02), and then by nano AV 15 wt% (1.58 ± 0.05), with the lowest value recorded by nano AV 20 wt% (1.22 ± 0.04). Tukey's post hoc test revealed a statistically significant difference between all tested groups ($P = 0.000$), except for nano Aloe Vera 10 wt% and 15 wt% that showed insignificant difference between each other (Table 5).

Table 4. Mean and standard deviation values for the effect of type and concentration of Aloe Vera filler particles on VHN of the different tested groups.

Group	Mean	SD
Control group	63.53 ^a	0.62
Micro Aloe Vera 10 wt%	47.79 ^f	0.48
Micro Aloe Vera 15 wt%	48.44 ^f	0.48
Micro Aloe Vera 20 wt%	51.29 ^e	0.50
Nano Aloe Vera 10 wt%	52.18 ^d	0.48
Nano Aloe Vera 15 wt%	54.40 ^c	0.52
Nano Aloe Vera 20 wt%	56.04 ^b	0.58
P value	0.000 ^a	

Significance level P less than or equal to 0.05. Small letters for intra group comparison under the same group and the means with different symbols are statistically significant difference at $P \leq 0.05$ (It illustrated the different between tested subgroups).

^a Means with different superscript letters are significantly different.

Table 5. Mean and standard deviation values for the effect of type and concentration of Aloe Vera filler particles on surface roughness (nm) of the different tested groups.

Groups	Mean	SD
Control group	40.80 ^a	0.10
Micro Aloe Vera 10 wt%	9.40 ^d	0.27
Micro Aloe Vera 15 wt%	16.60 ^b	0.20
Micro Aloe Vera 20 wt%	14.55 ^c	0.15
Nano Aloe Vera 10 wt%	1.81 ^e	0.02
Nano Aloe Vera 15 wt%	1.58 ^e	0.05
Nano Aloe Vera 20 wt%	1.22 ^f	0.04
P value	0.000 ^a	

Significance level P less than or equal to 0.05. Small letters for intra group comparison under the same group and the means with different symbols are statistically significant difference at $P \leq 0.05$ (It illustrated the different between tested subgroups).

^a Means with different superscript letters are significantly different.

3.5. Antibacterial evaluation

The highest antibacterial value was shown by nano AV 20 wt% (5.49 ± 0.27), followed by nano AV 15 wt% (4.60 ± 0.12) and 10 wt% (4.25 ± 0.22), micro AV 20 wt% (3.88 ± 0.15), and then by micro AV 15 wt % (3.76 ± 0.10) and 10 wt% (3.75 ± 0.07), with the lowest value that was recorded by the control group (0.049 ± 0.01). Tukey's post hoc test revealed a statistically significant difference between all tested groups ($P = 0.000$), except for the micro Aloe Vera groups that showed insignificant difference between each other (Table 6).

4. Discussion

Fibers derived from Aloe Vera, opuntia, pineapple, corn-husk, areca nut, and Palmyra sprouts have recently gained popularity due to their ability to produce high-performance biocomposites for a

Table 6. Mean and standard deviation values for the effect of type and concentration of Aloe Vera filler particles on antibacterial effect of the different tested groups.

Group	Mean	SD
Control group	0.049 ^e	0.01
Micro Aloe Vera 10 wt%	3.75 ^d	0.07
Micro Aloe Vera 15 wt%	3.76 ^d	0.10
Micro Aloe Vera 20 wt%	3.88 ^d	0.15
Nano Aloe Vera 10 wt%	4.25 ^c	0.22
Nano Aloe Vera 15 wt%	4.60 ^b	0.12
Nano Aloe Vera 20 wt%	5.49 ^a	0.27
P value	0.000 ^a	

Significance level P less than or equal to 0.05. Small letters for intra group comparison under the same group and the means with different symbols are statistically significant difference at $P \leq 0.05$ (It illustrated the different between tested subgroups).

^a Means with different superscript letters are significantly different.

variety of applications [25]. Composite properties can be altered by varying the reinforcing fillers and matrix phases. Natural fillers have numerous benefits over synthetic fillers, including their abundance, availability, low density, as well as low cost. As a result, there is an increase in the commercial use of natural filler-based composites in various fields [26].

The micro AV used in this study was prepared using freeze-drying process at -30°C for two days. All the beneficial properties of the Aloe powder were preserved in this process and none of the vitamins or minerals were destroyed. During the freeze-drying process, Aloe Vera gel was simply separated from the water, resulting in a concentrated 200 : 1 extract, which means that 1 gm of powder equals 200 gm of raw Aloe Vera leaf gel [27] (Fig. 1a).

Nano Aloe Vera particles encapsulated by chitosan nanospheres were prepared by ion gelation method. This is a simple method that does not rely on the use of harmful organic solvents and retains the bioactivity of macromolecules during preparation [4]. Characterization of the prepared freeze-dried powder was performed by means of Fourier transform-infrared spectroscopy (FTIR), TEM, and zeta potential.

FTIR is routinely used for identification of organic compounds owing to its simplicity, ease of maintenance, rapid data collection, and ease of data interpretation and reproducibility. The presence of peaks (Fig. 1b) for both Aloe Vera and chitosan with no absent or shifted bands indicated encapsulation of Aloe Vera by chitosan rather than occurrence of a chemical reaction between them.

The uniform spherical particles of chitosan around Aloe Vera, as revealed by the difference in electron density (Fig. 1b), may further confirm the formation of chitosan nanospheres that encapsulated the Aloe Vera particles. The particle size of the prepared powder (27–50 nm), lied within the range normally reported for ion gelation method [28].

Due to their small size, nanoparticles are energetically unstable. Therefore, as the particles undergo Brownian motion, colloids tend to balance between the attractive van der Waals' forces and the electrical repulsion due to surface charges. If the zeta potential falls below a certain level, the colloids tend to agglomerate/aggregate due to the attractive forces. The potential charges on the surface of the particles reflect their stability. The electric potential at the boundary of the double layer is known as the zeta potential, which was determined and shown in Table 3. Zeta potential results showed a dropdown of charge from +37 mV of chitosan to +5.4 mV for

Aloe Vera CsNPs, which indicated that loading and combination took place between them [28].

Following powder preparation and characterization, the prepared powder of both micro- and nanoparticles of AV was added in different concentrations 10, 15, and 20 wt% to the experimental resin composite. These weight percentages were selected to exceed the minimum inhibitory concentration (MIC) of AV (12.5 g/ml) for *Streptococcus* mutants as it was recommended to use a higher concentration of AV to ensure its effectiveness [4,29].

For resin matrix preparation, Bis-GMA was used as a major monomer in the resin structure. It is one of the most traditionally used monomers that yields adequate mechanical properties and low polymerization shrinkage. However, it has high viscosity owing to its high molecular weight and increased strength of hydrogen bonding. Therefore, low-molecular-weight TEGDMA was added as a diluent to lower the viscosity. TEGDMA has been frequently used in dental composites as a functional monomer. It produces high number of double bonds on a flexible backbone and so, improves the conversion process [18].

In the current study, the photo-initiator system was based on camphorquinone and a tertiary amine, ethyl diethyl amine benzoate. The amine serves as a proton donor that accelerates free radical production, while camphorquinone has a wide spectrum of absorption wavelengths from 360 to 510 nm (blue visible light), which is compatible with the light-emitting diodes (LED) [18].

Flexural strength results of nano Aloe Vera resin composites were increased at low filler loading 10 wt % (83.525 MPa), while at higher filler loading, it showed a significant decrease for 15 and 20 wt% groups (81.203, 75.386 MPa, respectively, (Table 3). This was explained by the fact that at lower concentration, dispersion of the fillers increased the surface area of these particles, producing a large polymer/nanofiller interfacial area, facilitating the transmission of forces, and increasing the strengthening effect [28]. On the other hand, the tendency of nanoparticles to agglomerate at high concentrations decreases their surface area, resulting in poor interfacial interaction and prevents efficient stress transfer between the components of the systems and hence decreases the strength that explains the reduction in the properties of groups with higher concentrations of nano Aloe Vera [30].

Another explanation for the decrease in the flexural strength at high filler loading of nano AV is that the reinforcement expected due to the higher concentration of particles present in the resin

composite was counteracted by the increase in the mechanical failure between resin matrix and inorganic fillers [31].

On the other hand, micro Aloe Vera composite showed a decrease in the flexural strength at a concentration of 10 wt% (100.073 MPa) with an increase in flexural strength at higher filler loading (15 and 20 wt % 104.450, 108.914 MPa, respectively) (Table 3). This might be explained by the decrease in interconnecting spacing and voids, indicating a better adhesion between micro Aloe Vera and resin matrix particles representing an increase in the flexural strength at this high filler loading [32].

This result may be due to the uniform distribution of microparticles of AV, which allowed them to seal spaces between the linear chains of polymer matrix, resulting in enhanced flexural strength [33].

Because of the ease of specimen preparation, simplicity of the test method, and availability of the equipment, hardness has been widely used as a method of investigating factors that influence the degree of conversion of resins and for characterization of the mechanical quality of a polymer. Moreover, Vickers hardness test can be used with a variety of dental materials ranging from soft to hard, it is also a nondestructive testing method, so the test specimen can be reused [34].

The results of this study revealed that the micro AV-filled composite had the lowest hardness values (Table 4). Such finding could be explained on the basis that large filler size resulted in an increased light scattering of the curing light, thus decreasing the degree of conversion and hence the hardness value [35]. However, nano AV-filled composite was found to have significantly higher (VHN) values than micro AV-filled composite (Table 4) due to the higher filler content and densely packed nanofillers into the experimental resin composite [36].

According to this study, increasing the filler content in both micro AV- and nano AV-filled composites increased the hardness value (Table 4). This could be attributed to the fact that filler content influences the hardness values of dental materials. Fillers are employed to strengthen and reinforce composites. Stresses generated because of the action of occlusal forces are transmitted by the organic phase to the reinforcing fillers where the inorganic fillers are harder than resin matrixes. With high filler content, additional strengthening effects might occur between the organic and inorganic phases. As the percentage of filler increases, the initial interparticle spacing decreases. This space further decreases with increasing conversion of the organic matrix, which could cause interactions between the filler surface and the densifying matrix. This process

might result in the additional reinforcement of the composite network [37,38].

Atomic force microscope is one of the most common methods for measuring surface roughness. It was used in this study because it was fast, simple, and reliable for comparative assessment of surface roughness property [39]. Nano AV-filled composite had the least surface roughness (Ra) at different concentrations (Table 5), which could be explained by the fact that nanofiller particle size adhering to the resin matrix, decreased the interconnecting distance between neighboring particles resulting in a smooth surface. As the smaller filler size reduced interparticle spacing causing less strain localization around the filler, so it lowers the fatigue failure [39]. On the other side, the surface roughness of the micro AV-filled composite was higher than nano AV-filled composite. This was due to the fact that larger filler particles have higher stress concentration, so they start to pull away from the surface, increasing the roughness of these composites [39].

A potential reason for the higher roughness of the control group than micro AV-filled composite was that the micro AV-filled composite has a closer distance between neighboring particles than the commercial composite, even though the filler-to-volume fractions were the same across the groups [39]. In accordance with the results of this study, a study was conducted to investigate the effect of incorporating AVCsNPs into the compomer on the surface roughness. The study concluded that addition of 15 wt% of freeze-dried AV enclosed in chitosan nanospheres improved surface roughness [4].

The goal of this research was to formulate a composite with an antibacterial property by incorporating micro Aloe Vera and freeze-dried AVCsNPs. This was performed by the 'Agar diffusion test.' Agar diffusion test is a simple technique accepted and approved by the clinical and laboratory standard institute for bacterial and yeast testing [40].

For the antibacterial test results, Tukey's post hoc test showed no significant difference between micro AV 20 wt %, 15 wt %, and 10 wt % (Table 6). This could be explained by the fact that micro Aloe Vera contains compounds like anthraquinones and saponin that had direct antibacterial activities as they could inhibit protein synthesis from bacterial cells. This results in disruption of membrane permeability, so bacterial growth was inhibited. Other components of AV gel, such as acemannan, can also exert indirect bactericidal activity by stimulating leukocyte phagocytosis. Saponin has antiseptic properties because it dissolves lipoproteins in the bacterial cell membrane. This caused bacterial lysis and death by causing damage to the cell membrane [29].

Nano Aloe Vera at 20, 15, and 10 wt% (Table 6) showed higher values than micro Aloe Vera. This could be attributed to the hydrolysis of the chitosan coating, which occurs under acidic conditions and is pH-sensitive, releasing AV into the media containing *Streptococcus mutans*. The direct interaction between *Streptococcus mutans* and nano AV caused bacterial lysis and death by causing damage to the cell membrane. As a result, AV inhibition zone would be expanded [29,41]. In accordance with the results of this study, a study was carried out to determine the impact of adding AVCsNPs to a composite material on its antibacterial properties. They concluded that the addition of 15 wt% of freeze-dried AV encased in chitosan nanospheres to the composite material had an antibacterial effect on *Streptococcus mutans* [42].

4.1. Conclusion

Within the limitations of this study, it could be concluded that the incorporation of micro and nano AV extract improved the surface roughness and microbiological properties, however, the flexural strength and surface hardness of the experimental resin composite were negatively affected.

4.2. Recommendations

- (1) It is recommended to use higher percentages of AV and nano AV fillers and study their effect on the mechanical properties.
- (2) Further investigations should be performed to understand the mechanism of release of Aloe Vera from chitosan nanosphere.

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Conflicts of interest

The authors have no conflict of interest.

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