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Mechanical Properties of Moulded Specimens of Hydroxyapatite with Varying Particle Size Incorporated with Different Propolis Compositions

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ABSTRACT

Incorporating hydroxyapatite (HA) with propolis would be beneficial for dental applications, providing an alternative dental biomaterial. The propolis is a resinous substance produced by bees with antibacterial, antifungal and anti-inflammatory that increase its potential as a biomaterial. This study explores the mechanical properties of different particle sizes of HA; micro-HA (μ-HA) and nano-HA (*n*-HA) by incorporating different compositions of propolis from 2.5 wt.% to 10 wt.%. The materials were sieved for 15 min and mixed on the mixing glass using polybasic carboxylic acid as a binder to form a homogenize composite HA and propolis. The composites were moulded into a 5 mm × 5 mm moulded. The water absorption increased with the addition of propolis indicating that more porous and potentially less durable in an aqueous environment The mechanical properties show improvement of *n*-HA compared to μ-HA due to better particle interaction and bonding. However, the incorporation of propolis from 2.5 wt.% to 10 wt.% decreased the compressive strength from 4.3 MPa to 0.8 MPa, vickers hardness from 500 HV to 420 HV and shore hardness from 90.6 mN to 76.6 mN due to the lower density of propolis which resulted in high porosity of moulded specimen. Accordingly, the mechanical properties of HA_2.5Propolis and HA_5.0Propolis are comparable to Mineral Trioxide Aggregate (MTA) thereby ensuring adequate mechanical stability and leveraging the antimicrobial properties of propolis.

Keywords: Hydroxyapatite; propolis; water absorption; mechanical properties; particle size

INTRODUCTION

Hydroxyapatite (HA), a calcium phosphate salt with a hexagonal structure and a stoichiometric Ca/P ratio of 1.67, is a primary component of tooth enamel and dentin, and constitutes a significant part of human bone [1]. HA's structural similarity to bone apatite

makes it ideal for dental applications, as it closely mimics the inorganic matrix found in teeth and bones. Propolis, a resinous substance collected by bees, is composed of plant resins, wax, essential oils, pollen, and other minor constituents such as sugars, amino acids, and vitamins [2].

Propolis serves several physiological and biological purposes in addition to offering physical protection. Propolis has been demonstrated to be protective against microbiological pathogens in bee colonies [3,4,5]. Propolis's antibacterial and anti-inflammatory properties make it a valuable addition to HA composite. The efficacy of three intracanal medicaments and propolis against E. faecalis reported that propolis prevented the growth and proliferation of E. faecalis and had suitable antibacterial efficacy [6]. Compared propolis and calcium hydroxide as intracanal medicament and showed that propolis was an effective intracanal medicament and quickly eliminated E. faecalis [7].

Incorporating hydroxyapatite with propolis would be beneficial for dental applications, providing an alternative to Mineral Trioxide Aggregate (MTA). MTA has proven to be a promising material for root filling [8], despite its lengthy setting time and requirement for humidity. A previous study evaluated the antimicrobial activity of two pastes containing propolis and calcium hydroxide against polymicrobial cultures taken from the root canals of 16 necrotic primary molars [9]. They used the agar well-diffusion technique to determine the antimicrobial activity of the pastes from 11% ethanolic extract of propolis and calcium hydroxide and 11% extract of propolis without ethanol and calcium hydroxide. The results showed that the anti-microbial activity of the latter paste was higher than the former paste. They concluded that a combination of propolis and calcium hydroxide is effective to control dental infections. In order to generate a dental remineralization gel, HA was manufactured from oyster shells and was combined with propolis resulted in strong antibacterial activity against lactobacillus acidophilus, streptococcus mutans, and streptococcus sanguinis [7]. It also showed high cell viability (92.80%) in MC3T3-E1 cells, suggesting that it may be useful in promoting caries prevention and dental enamel remineralization.

To address bonding challenges between propolis and hydroxyapatite, a polybasic carboxylic acid binder is used. Binders play a crucial role in composite materials by enhancing adhesion and cohesion between constituents [10]. The choice of binder is essential for creating a strong and functional composite structure. There are also composites incorporated with propolis from the last research such as Glass ionomer cements (GICs)-propolis [11] and CPP-ACP-propolis [12]. However, composites show different effects on mechanical properties in addition to propolis. Glass ionomer cement (GICs)-propolis incorporated with Ketac Fil Plus and ChemFlex binder showed that propolis reduced the diametral tensile strength and compressive strength [11]. However, GICs with ethanolic extract of propolis with ethanol as a binder increased the microhardness of the moulded specimen and did not affect the performance of the GICs in terms of microleakage [13].

To ensure the lifetime and durability of dental restorations, composites used in dentistry need to have a high mechanical strength to withstand the large pressures applied during chewing and biting. Under the continuous pressure of regular oral activities, materials with higher mechanical strength resist fractures, wear, and deformation [14]. Furthermore, its characteristics improve marginal integrity and guard against secondary caries, increasing the overall efficacy and durability of dental therapies. Therefore, for successful and long-lasting dental restorations, choosing composites with good mechanical strength is essential.

Upon conducting a thorough literature study, it is evident that there exists a research lacuna concerning the impact of different HA particle sizes in a PLA matrix. Instead, most investigations used HA in micro- or nano-sized particles [15,16]. In composites, particle size plays a crucial role as a reinforcing ingredient that greatly affects the material's overall quality. The surface area, dimensions, projected area, volume, or cross-sectional area are important factors in particle size analysis. Particle sizes vary and show different functions. For example, smaller particles are more dispersible because they have a higher exposed surface area that interacts with the polymer matrix.

This study investigates various formulations of different particle size HA composites with propolis contents ranging from 2.5 wt.% to 10 wt.% using polybasic carboxylic acid as binder. The porosity and mechanical properties, including micro-hardness, compressive strength and shore hardness are assessed to understand the impact of propolis concentration on the composite's performance. The goal is to optimize the mechanical properties of HA and propolis composites for improved performance in dental applications.

MATERIALS AND METHODS

Materials

HA powder was synthesized from clamshell using the precipitation method. Propolis ethanolic extract from *Heterotrigona itama* purchased from Raub, Pahang. Polybasic carboxylic acid was purchased from Glass Ionomer luting and lining cement, GC Corporation, Japan and Mineral Trioxide Aggregate (MTA) the 'gold standard' in endodontology was purchased (MTAflow white, Ultradent Products Inc, US).

Ball Milling

Milling of HA was accomplished by means of a Fritsch Planetary Ball Mill Machine to transform synthesized micro HA (μ-HA) into nano HA (*n*-HA). The grinding media (balls) are made of zirconia and the ball mill jars are from alumina. The ball mill ran for 6 h at 360 rpm to maintain a ball-to-powder ratio (BPR) throughout the process of 5:1. A particle size analyser was used to determine the reduction of the size.

Composite HA_Propolis

The composite was prepared by adding propolis powder to HA powder at different compositions 2.5 wt.% to 10 wt.% as shown in Table 1 into a sieve for 15 min to homogenize these two powders. Afterward, the sieved powders were mixed on the mixing glass using polybasic carboxylic acid as the binder to form a composite hydroxyapatite with propolis (HA_Propolis). The composites were moulded into a 5 mm × 5 mm mould. The steps were repeated replacing μ-HA with *n*-HA powder.

Table 1. Formulation of HAp composite

Composite	HA (g)	Propolis (wt.%)	Propolis (g)
HA (control)	2.0	0.0	0.00
HA_2.5Propolis	1.95	2.5	0.05
HA_5.0Propolis	1.90	5.0	0.10
HA_7.5Propolis	1.85	7.5	0.15
HA_10Propolis	1.80	10.0	0.20

Physical Properties

The visual appearance of the composite was photographed using a commercial handphone camera (Iphone 11, Apple). All composites were let to set for at least 24 h before further testing. This is to ensure the composite was fully dried. Scanning electron microscopy (SEM) JSM-7600F, JOEL was used to evaluate the surface morphological structure of the composite. The sample was coated with platinum using a sputter coater (Polaron SC 7620). A low vacuum was used to introduce the prepared sample into the microscopy chamber.

Water Absorption

For water absorption and solubility analysis, the composite samples were dried at room temperature for 72 hours. The sample was weighed (W_0). The samples were then immersed in a beaker containing 50 mL of distilled water for 24 hours, drained, and weighed again (W_1). The percentage of water absorption was measured using eq. (1). The method was based on ASTM D570-63.

$$water\ absoption\ (\%) = \left(\frac{W_1 - W_0}{W_0}\right) x\ 100. \quad (1)$$

Mechanical Properties

The mechanical properties of the moulded specimen (compressive strength, microhardness and shore hardness) of the HA propolis composite were measured. For the compressive strength, the moulded specimens were measured in accordance with the ASTM C1424-10 using a universal testing machine (H50KT, Instron 3382, Titinus Olsen). The sample was placed in a vertical position, and a force load (F) was applied with a crosshead speed of 1 mm/min. The compressive strength (*Cs*) was computed using eq. (2).

$$Cs = \frac{F\ (N)}{\pi d^2} \quad (2)$$

The microhardness (HV) of moulded specimen was performed by Vickers micro-hardness tester (Akashi AAV-500 series). The samples were grinded using 600 grit sandpaper and polished using 1 µm Polycrystalline diamond solution. The applied load was 9.81 N with an indentation time of 10 s. The shore hardness test was measured using a shore durometer.

RESULTS AND DISCUSSION

Particle Size Analysis

Figure 1 shows the SEM image of HA powder and particle size distribution for µ-HA and *n*-HA. The HA powder is irregular in shape and agglomerate provides binder to fill the interstitial space during mixing. Table 2 shows the parameters of the particle size distribution. The particle size distribution shows that µ-HA and *n*-HA shows bimodal system. The parameters of the particle size distribution were analyze from the three distribution points identified as D_{10}, D_{50} and D_{90}. Under ideal conditions, the value for S_w using eq. (3)

$$S_w = \frac{2.56}{log_{10}\left(\frac{D_{90}}{D_{10}}\right)}, \quad (3)$$

should be either less than 2 or greater than 7 indicating that a very broad (S_w= 2) or a very narrow distribution(S_w= 7). From Table 2, the value of S_w for µ-HA and *n*-HA are 1.43 and 1.14, respectively, corresponding to the relatively broad distribution. A small value of S_w corresponds to a broad particle size distribution that more powder could be packed in volume. The reason is that using particles of various sizes fills more space because small particles tend to occupy the space left between larger particles making possible high loads of the powder [17].

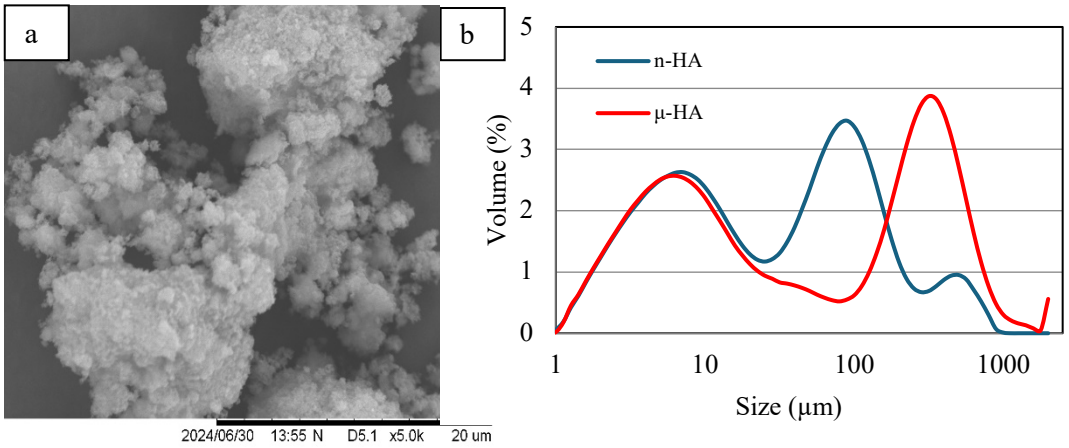

Figure 1. (a) SEM image of HA powder and (b) Particle size distribution for µ-HA and *n*-HA

Table 2. Parameters of the Particle Size Distribution

HA powder	Particle size (um)			Specific surface Area (m^2/g)	Particle Width Distribution (S_w)
	D_{10}	D_{50}	D_{90}		
µ-HA	2.928	38.834	500.010	0.676	1.147
n-HA	2.950	28.724	181.082	0.701	1.432

Physical Appearance

Figure 2 shows the physical appearance of moulded specimens. The control HA sample appeared white. Adding 10% propolis to HA as shown in Figure 2(e) created a dark brown colour compared to only 2.5%–7.5% propolis. Due to oxidation, it affects the film colour, resulting in a brownish composite.

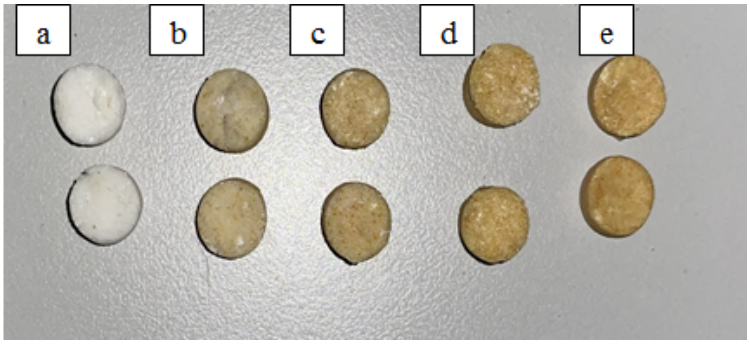

Figure 2. The physical appearance (a) HA (control), (b) HA_2.5Propolis, (c) HA_5.0Propolis, (d) HA_7.5Propolis and (e) HA_10Propolis

Water Absorption Test

Propolis repel water molecules due to the hydrophobic properties thus propolis can help to improve the durability of the composite by absorbing less water [18]. Water absorption tests conducted on the composites provide insight into the porosity and potential durability of the materials. Figure 3 shows the water absorption of HA with different propolis content. It shows that increase propolis increased the water absorption. This result is due to the irregular structure of HA create voids or gaps within the composite as shown in SEM image (Figure 4). These voids contribute to an increase in porosity. A similar trend was documented by previous studies [11], where addition of an ethanolic Brazilian green propolis to a GICs, increased water absorption. Propolis also seems to make the GICs more soluble in water and drastically lowers their compressive strength.

As shown in Figure 3, the HA_10Propolis results in low water absorption of 0.01 and 0.51 for µ-HA and *n*-HA, respectively. Adding 10 wt.% propolis to HA composites caused the water absorption value to decrease. This anomaly can be attributed to the hydrophobic nature of propolis, which repels water [19]. As the amount of propolis increases, its hydrophobic behavior becomes more pronounced leading to less water being absorbed by the composite. The material may become more porous and maybe less durable in an aqueous environment resulting in decreased water absorption.

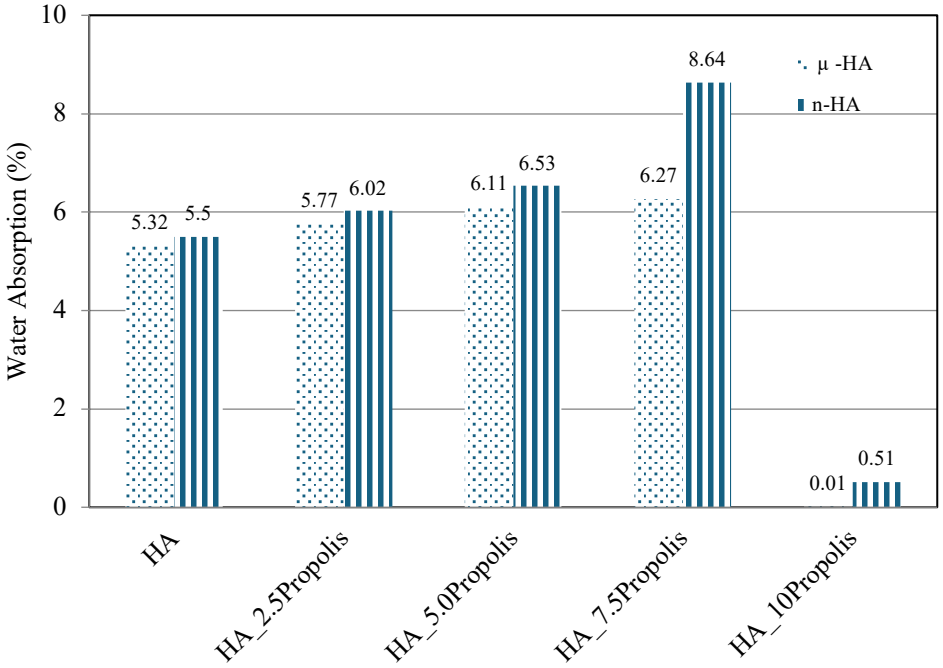

Figure 3. Water absorption test for composite HA with different propolis content

Propolis tends to be less dense than HA, with density of $0.953 \pm 0.001\ g/cm^3$ and $3.16 g/cm^3$ respectively, hence adding propolis to HA composites tends to reduce the total density leading to decreased in mechanical strength. As shown in Figure 3, HA_Propolis moulded specimen had higher water absorption, indicating larger porosity. Because of its increased porosity, the material may become less dense and less resilient in an aquatic environment. Higher propolis content and increased porosity are correlated, which implies that the composite becomes more prone to water penetration, thereby compromising its longevity and functionality as a dental material [20,21].

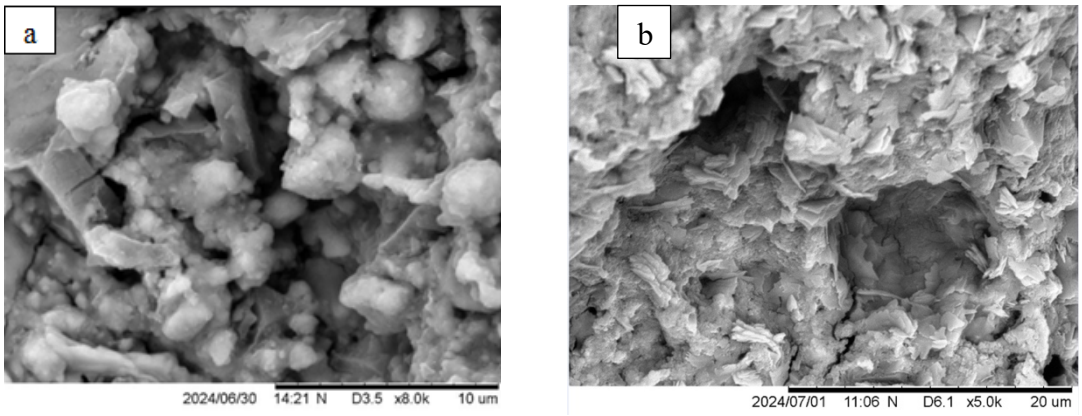

Figure 4. The SEM Image of HA composite (a) HA (control) and (b) HA_10Propolis

Compressive Strength and Microhardness Test

The compressive strength with different propolis concentrations for μ-HA and *n*-HA are shown in Figure 5. It shows that an increase of propolis content incorporated with HA resulted in the decrease of compressive strength from 4.0 MPa to 0.7 MPa. In general, lower compressive strength is correlated with high porosity. Reduced compressive strength results from increased porosity since there are more voids in the material and less solid material to support the applied loads. Higher propolis incorporation levels cause the HA composites to become more porous, which increases water absorption and decreases mechanical strength. This result was supported by previous studies [22], where propolis extract at 1% was sufficient to lower the compressive strength of GICs by about 7.5%. The effect of different particle size of HA can be observed where *n*-HA composed higher compressive strength compared to μ-HA due to *n*-HA particles that have a significantly higher surface area-to-volume ratio compared to micron-sized particles. This increased surface area allows for better particle-particle interaction and bonding, leading to a denser and more cohesive material. Compared to MTA (Mineral Trioxide Aggregate) composite, which has a compressive strength of approximate 1.2 MPa as shown in Figure 5, it is evident that HA composites with propolis concentrations between 2.5 and 5 wt.% considered acceptable. Without appreciably affecting the composite's compressive strength, density, or hardness, this range would probably offer some antibacterial advantages [23].

Figure 6 shows the microhardness test for different HA composites with propolis. It shows that, an increase propolis to HA composite is associated with a decrease in the hardness of the moulded specimens, falling from 433HV for HA_2.5Propolis to 374HV for HA_10Propolis for μ-HA. A significant difference was found between *μ*-HA *n*-HA where *n*-HA composites exhibit higher microhardness values of 475 HV while *μ*-HA shows 433HV for HA_2.5Propolis moulded specimen. MTA has a significantly lower hardness (348 to 350 HV) than the HA-propolis composite (~420 HV), comparing the microhardness of μ-HA and n-HA moulded specimens. This distinction shows that propolis inclusion does not lower the hardness of HA-propolis composites relative to MTA. However, the addition of propolis resulted in a drop in hardness (from 500 HV to 420 HV), indicating a trade-off between preserving mechanical strength and improving antibacterial characteristics [24]. In comparison to MTA, HA-propolis composites have a higher hardness, which implies that they may offer superior wear resistance in dental applications. However, the noticeable decrease in hardness that occurs with an increase in propolis content suggests that the amount of propolis added needs to be carefully optimized to balance its advantageous qualities with the required mechanical

performance. Based on the observed trends, maintaining a lower concentration of propolis (around 2.5 wt.% to 5 wt.%) might provide a more balanced outcome, ensure adequate hardness while enhance antimicrobial properties.

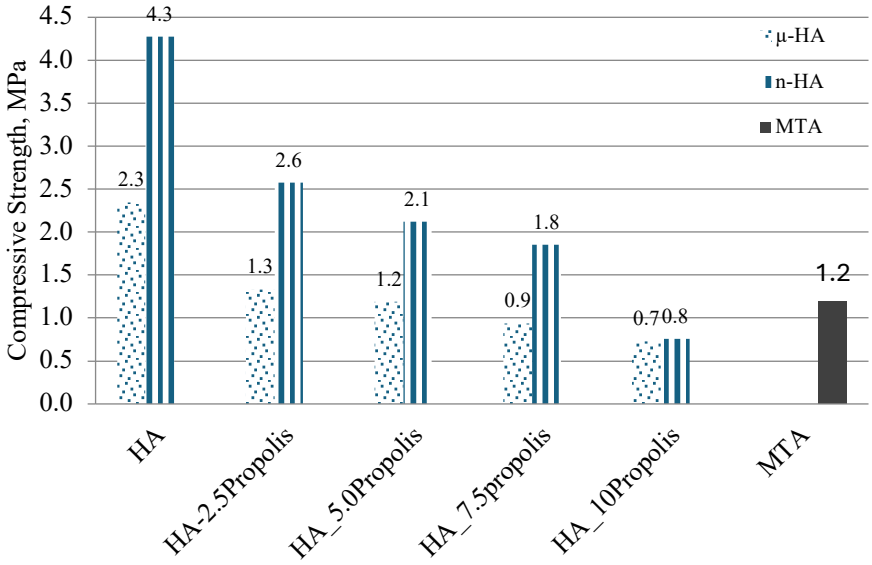

Figure 5. Compressive Strength of the composite with different propolis content

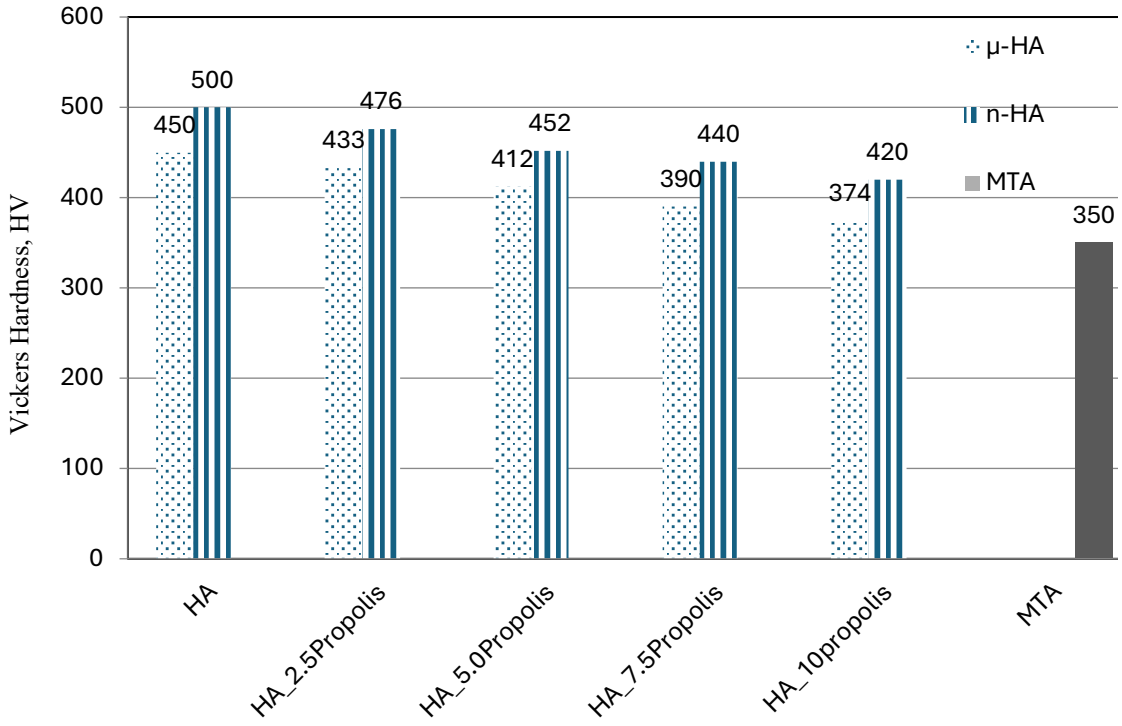

Figure 6. Microhardness of the composite with different propolis content

Shore Hardness Test

The results of the shore hardness test for the composite HA powder with varying propolis show significant patterns regarding the characteristics of the material as illustrated in Figure 7. High shore hardness values of 91 mN for μ-HA and slightly higher at 95 mN for *n*-HA are attributed to the inherent mechanical strength and stiffness of pure hydroxyapatite (HA). This implies that HA's smaller particle size helps create a composite

material that is tougher [25]. Because *n*-HA has a higher surface area to volume ratio, the composite matrix is probably better able to interact and connect, which increases the hardness of the material [26]. Figure 7 shows that the shore hardness of both µ-HA and *n*-HA composites decreases steadily with increasing propolis concentration. Propolis is softer than other substances, which explains why the mechanical properties decrease with increasing propolis content. Because propolis has a less stiff structure than other resinous substances [27], it lowers the composite material's overall hardness. Propolis has a noticeable effect that decreases the hardness significantly when more of it is added. This trade-off indicates that to balance the composite's mechanical and antibacterial qualities, the propolis concentration needs to be carefully optimized.

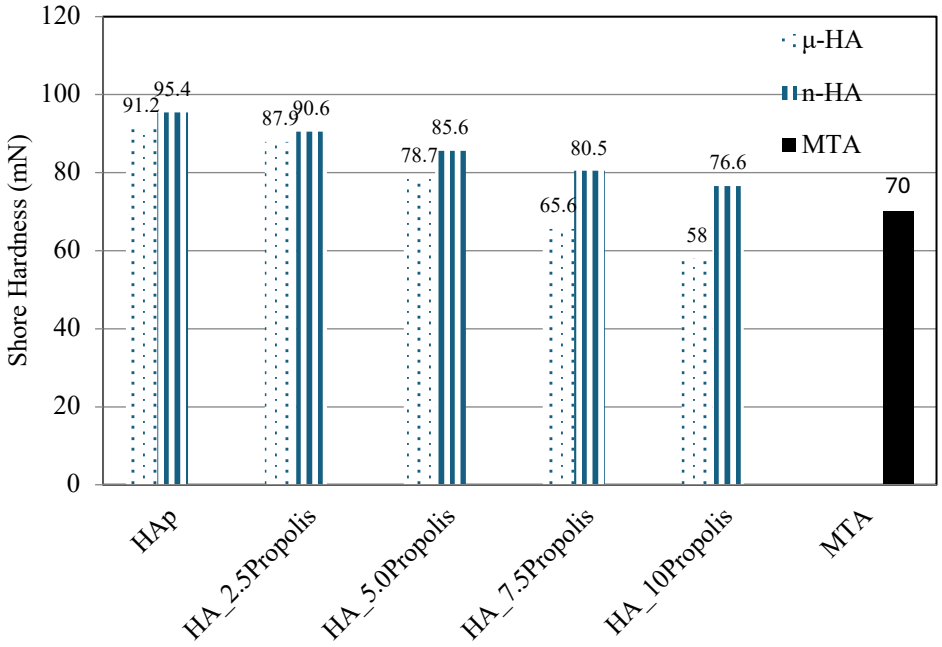

Figure 7. Shore hardness strength of the composite with different propolis content

The MTA composites exhibited shore hardness values of 70 mN, which is consistent with their robust mechanical performance, as indicated by their known mechanical properties, including durability and compressive strength [28]. The mechanical results indicate that a propolis concentration of 2.5% to 5% by weight is appropriate. Within this range, the HA composites sustain shore hardness values that are comparable to MTA, thereby ensuring adequate mechanical stability and leveraging the antimicrobial properties of propolis. This optimization guarantees that the composites continue to be viable as dental materials by remaining within the hardness range of MTA.

CONCLUSIONS

The study showed that incorporating propolis into HA composites using polybasic carboxylic acid as a binder generally decreased the compressive strength, microhardness and shore hardness of the moulded specimen. However, nano-sized HA composites demonstrated higher mechanical strength compared to micro-sized HA composites due to better particle-particle interaction and bonding. HA_Propolis composites incorporating 2.5–5.0 wt.% propolis represent a promising alternative biomaterial for dental applications, balancing mechanical stability with bioactivity with slightly better strength with HA_2.5Propolis.

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