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Study of characterization of natural biomaterials *Nypa fruticans* extract for scaffold fabrication using 3D bio-printing method

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Abstract

Nypa fruticans (Arecaceae) is a tropical mangrove plant with potential as a natural biomaterial due to its galactomannan content, a water-soluble hetero-poly-saccharide suitable as a base material for bio-ink. This study aims to evaluate the potential of *Nypa fruticans* extract as a sustainable natural biomaterial for scaffold fabrication using 3D bio-printing technology. To optimize its mechanical and structural properties, glycerol and sorbitol were added as plasticizing agents. The research methods consist of (a) maceration method extraction of *Nypa fruticans* fruit, subsequent characterization by Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography-Mass Spectrometry (GC-MS) analysis, (b) formulation of biomaterials with glycerol and sorbitol at varying concentrations (5%, 10%, and 15%), and (c) scaffold fabrication using 3D bio-printing. The scaffolds were then assessed for printed line width, density, and porosity. FTIR analysis confirmed the presence of hydroxyl (O-H) functional groups at 3600–3300 cm⁻¹, while GC-MS detected 5-hydroxymethylfurfural, indicating polysaccharide derivatives consistent with galactomannan content. The addition of sorbitol significantly influenced the physical properties and printability of the formulated bio-ink, with the optimal composition obtained at 85% *Nypa fruticans* extract and 15% sorbitol. This formulation successfully produced stable CAD-based scaffold architectures with cubic and rhombic pore configurations, achieving precise deposition widths (± 1.18 mm) relative to the 0.5-mm nozzle diameter. An increased sorbitol concentration of 15% correlated with higher scaffold density (up to 0.971 g/cm³) and reduced porosity (down to 0.436). These findings demonstrate the potential of *Nypa fruticans* extract as an efficient and sustainable natural bio-ink for tissue engineering applications.

Keywords:

Nypa fruticans extract, 3D bio-printing, scaffold, tissue engineering, density, porosity.

1 Introduction

In the field of tissue engineering, the development of new materials is crucial for addressing the limitations of natural resources and environmental concerns. The development of eco-friendly biomaterials has become a primary focus in biotechnology and tissue engineering. The use of thermoplastic materials has been increasingly popular, with around 6,300 metric tons of plastic waste, including medical waste, recorded in 2015 [1,2]. Plastic waste has become a significant global problem; these commodity materials are difficult to degrade, take a long time to break down, and release toxins into the air [3].

The use of plastics continues to increase due to the demands of a practical and consumptive lifestyle. Given the conventional plastics currently in use, they pose a threat to the stability of the environmental ecosystem. Plastic waste also has a limited lifecycle, during which it can only be recycled into other products of certain quality, after which it still ends up as waste [4]. Consequently, environmentally friendly alternative materials are being advocated to replace these commodity plastics.

Rapid Prototyping (RP) technology has become an essential part of product development across various industries, including the biomedical field [5], [6]. This technology allows for the rapid and efficient creation of prototypes using a wide range of materials, including biomedical materials [7]. The ability of biomaterials to be rapidly and precisely reshaped makes them an ideal choice for rapid prototyping processes. The bionic extruder mechanism in 3D bio-printing is the most important component in pushing the bionics out of the nozzle. This system requires high accuracy and precision to form the product [8]. Yu Shrike Zhang et al. (2021) explained that the extruder mechanism in 3D bio-printing is a technology well-suited for various biomedical applications due to its ability to produce structurally sophisticated and robust tissue constructs for tissue fabrication [9]. Biomaterials are engineered devices designed to interact with biological systems, restoring body functions or supporting tissue regeneration [10-12].

The selection of *Nypa fruticans* fruit as a raw material for biomaterial production is driven by its significant potential in terms of availability. The *Nypa* plant is widely distributed across Indonesia, with an estimated area of 700,000 hectares, covering regions such as Sumatra, Kalimantan, Java, Sulawesi, Maluku, and Papua New Guinea [13]. *Nypa fruticans* fruit has distinct and notable physical characteristics, appearing round to oval in shape with varying sizes, typically ranging from 15 to 25 cm, as shown in (Fig. 1(a), Fig. 1(b), and Fig. 1(c)). The outer skin is dark brown to nearly black, with a complex and scaly texture [14]. In addition to its abundant availability, this plant contains galactomannan compounds, as illustrated in (Fig. 1(e)), which are polysaccharides composed of mannose and galactose chains. Galactomannan is widely applied as an excellent thickening and stabilizing agent [15]. According to research conducted by Mahfiroh (2017), *Nypa* fruit has high galactomannan content, reaching up to 11.59% [16].

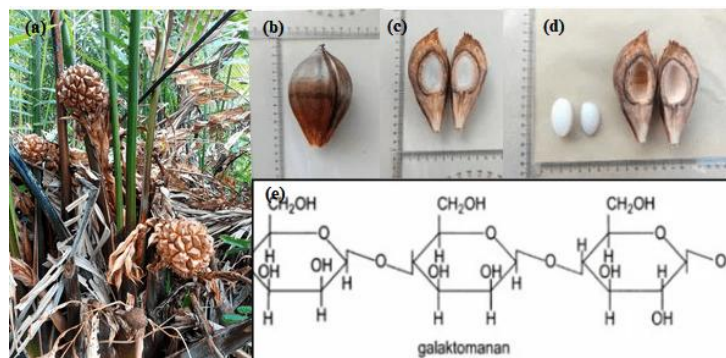


Fig. 1. *Nypa fruticans* plant. (a) *Nypa* plant with fruits, (b) single *Nypa* fruit, (c) cross-section of a *Nypa* fruit, (d) showing the inside of the *Nypa* fruit with seeds removed, (e) galactomannan compound from *Nypa*.

Previous studies have investigated various natural biomaterials for 3D bio-printing applications, such as gelatin, to improve viscosity and printability [17], and chitosan as a multifunctional biomaterial for bone regeneration [18]. However, research specifically focusing on the potential of *Nypa fruticans* extract as a bio-ink precursor for scaffold fabrication remains limited. Therefore, this study aims to evaluate the feasibility of *Nypa fruticans* extract as a novel plant-based biomaterial for 3D bio-printing applications. It emphasizes the physicochemical characterization of the extract through Fourier Transform Infrared (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS)

analyses to identify active galactomannan components. It assesses its printing performance as an initial indicator of suitability for tissue engineering applications.

The contribution of this research is to provide a comprehensive characterization of *Nypa fruticans* extract using FTIR and GC-MS to identify its functional groups and chemical compounds relevant to biomaterial formulation. Furthermore, this study introduces an injection consistency test to evaluate the printability and flow stability of Nypa-based biomaterial during extrusion-based 3D bio-printing. These findings offer a new insight into the potential of *Nypa fruticans* as a sustainable and biocompatible material for scaffold fabrication.

2 Materials and methods

2.1 Equipment and materials

This study utilized primary material in the form of Nypa fruit, obtained from the west coast of Samatiga District, West Aceh Regency, Indonesia. The physical characteristics of Nypa fruit are oval, with a size of 8-13 cm, and dark brown in color. The Nypa fruit extraction process was carried out using two different methods, namely the Soxhlet method with ethanol solvent for the needs of GC-MS tests with a ratio of material to solvent of 1:10 (w/v), and the extraction method with distilled water (H₂O) solvent for the needs of biomaterial manufacturing with a ratio of 1:2 (w/v).

Characterization of the compounds in the *Nypa fruticans* extract was performed using Fourier Transform Infrared Spectroscopy (FTIR, Two Lita L1600300). At the same time, identification of chemical components was conducted through GC-MS analysis (Shimadzu GC-MS-QP2010 Ultra). The extract obtained from Soxhlet extraction was then processed using a rotary evaporator (Strike 280) to remove the solvent and obtain a concentrated extract. Scaffold printing was performed using a 3D printer (Creality CR-10 Max), supported by a computer (Lenovo, 4 GB RAM, Windows 10 operating system), which was used for designing the scaffold model based on CAD. In the formulation of the material, *Nypa fruticans* extract was used as the primary component, and two types of polymerization agents were added: glycerol (C₃H₈O₃) and sorbitol (C₆H₁₄O₆), which served to enhance the flexibility and stability of the biopolymer structure, respectively.

2.2 Preparation and characterization test

This study investigated the fabrication technique of biomaterials from galactomannan *Nypa fruticans*-based paste gel for use in rapid prototyping 3D bio-printing machines to create soft tissue scaffolds. The initial stage involved the extraction of Nypa flour using the maceration method with an ethanol solvent at a ratio of 1:10 [19], [20], followed by a 3-day incubation at room temperature [21]. The extract was then subjected to evaporation to obtain the Nypa extract. Characterization of the Nypa extract was performed using FTIR and GC-MS tests to detect the presence of galactomannan compounds.

Subsequently, the preparation of Nypa biomaterials involved several stages, including stripping, cleaning, refining, pressing, and evaporation of Nypa liquid, as presented in Fig. 2(a) and Fig. 2(b). Various biomaterial compositions were mixed with sorbitol and glycerol polymerization agents at different concentrations (5%, 10%, 15%) [22]. The resulting biomaterials were manually cast using a syringe and oven-dried for 2 hours [23]. The physical properties of the biomaterials were observed before testing their scaffold form using 3D bio-printing technology. The next stage involved the fabrication of scaffolds using 3D bio-printing technology, which formed scaffolds with cubic and rhombic pore patterns [24], as shown in Fig. 2(c). Evaluation of the scaffold structure included measurements of scaffold deposition, density, and porosity. The results were analyzed to assess the feasibility of Nypa biomaterials as scaffold implant materials based on the ASTM F2150-19 standard.

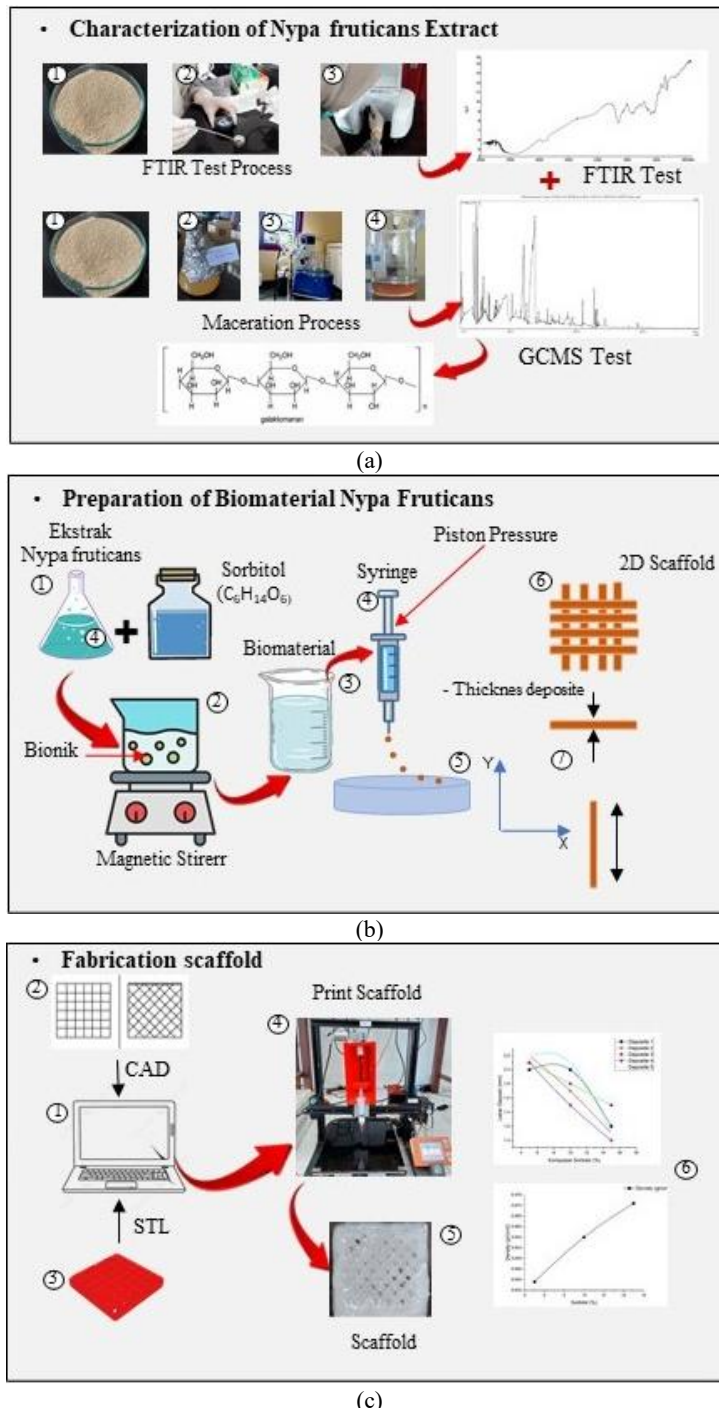


Fig. 2. Biomaterial preparation procedure from Nypa extract for scaffold printing application, (a) characterization of *Nypa fruticans* extract, (b) biomaterial preparation scheme, (c) scaffold fabrication.

Fig. 2 illustrates the research workflow in developing bio-ink derived from natural biomaterials based on *Nypa fruticans* extract for scaffold fabrication using 3D bio-printing technology. The initial stage involves characterizing active compounds through FTIR and GC-MS analyses to identify galactomannan as the primary component. In the next stage, the Biomaterial extracted from *Nypa fruticans* is formulated into bio-ink by adding sorbitol as a polymerizing agent, followed by homogenization using a magnetic stirrer. The resulting bio-ink is then utilized for 2D scaffold printing via a manual extrusion system, which uses a syringe to precisely control the thickness and printing pattern based on the operator's expertise. The final stage of scaffold fabrication begins with a CAD design that is converted into STL format and uploaded to a 3D printer for printing.

2.3 Scaffold fabrication

In this study, scaffold fabrication based on extrusion applies rapid 3D bio-printing technology. The material used is a bionic material produced from Nypa extract, combined with sorbitol as an additive to

enhance physical properties and improve material stability during the printing process, while also achieving good mechanical properties. This rapid 3D bio-printing technology is designed to utilize a piston-based bionic injection mechanism, which is specifically engineered to inject biomaterials in a controlled manner. The setup parameters for the process are shown in Fig. 3. This mechanism is supported by

a 12V DC dynamo torque, which is capable of producing variable rotation speeds between 6 to 30 rpm, providing precise control over material flow during the extrusion process. A 10 ml syringe with a 0.5 mm nozzle diameter is used. Fig. 3 shows the scaffold fabrication process using rapid prototype 3D bio-printing based on an injection piston.

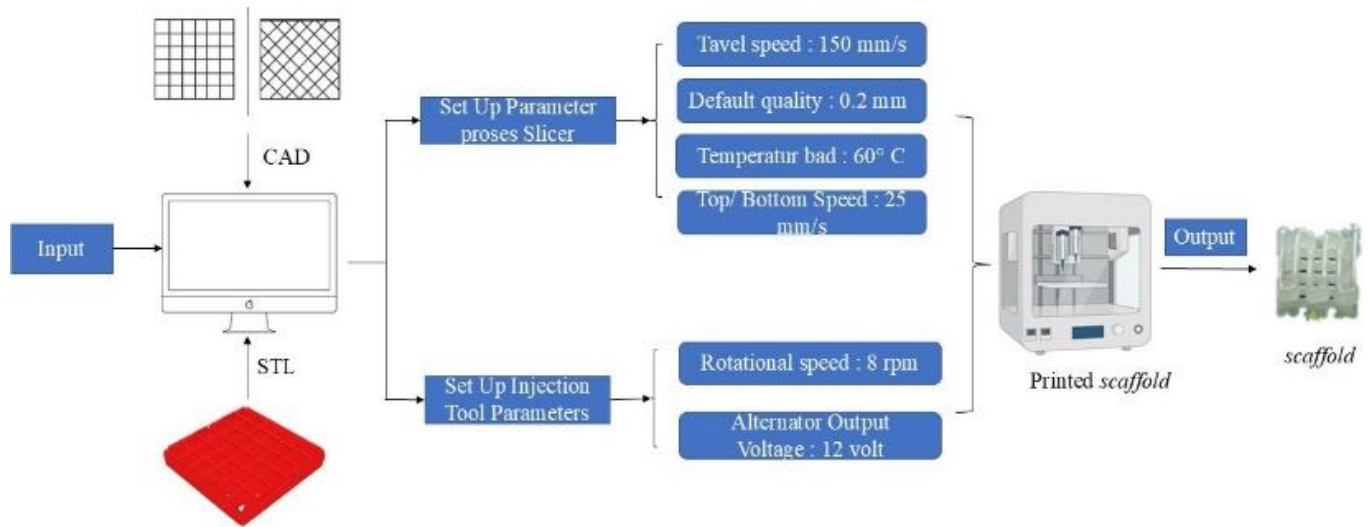


Fig. 3. Setup parameters for scaffold fabrication process using 3D bio-printing.

The bionic extruder process utilizes a nozzle, as shown in Fig. 4, which serves as a container for extruding *Nypa* biomaterial that is then formed into a scaffold through a 3D bio-printing technique. The *Nypa* extract is first processed by reducing the water content through heating at a temperature of 40-60°C using a magnetic stirrer for 7 minutes, then sorbitol is added and stirred for 3 minutes to produce a homogeneous mixture, resulting in a total preparation time of 10 minutes.

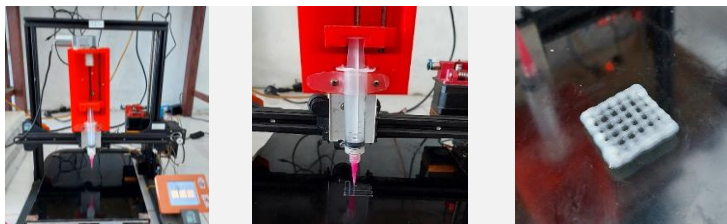


Fig. 4. Scaffold fabrication using 3D bio-printing. (a) piston-based 3D bio-printing, (b) printed scaffold, (c) final printed scaffold.

After preparation, the material is inserted into the syringe for printing using a piston-based injection system driven by a 12V DC ZGY370 motor with a low speed of 6-30 rpm. The motor rotation is transmitted to the screw shaft, which drives the nut on the syringe pusher, producing a linear motion that pushes the piston and injects the bio-ink through the nozzle. The syringe pusher frame structure, with a stainless steel shaft support, maintains the stability and alignment of the piston movement. The injection volume is controlled by adjusting the motor speed using a DC power supply (0-30V, 0-5A), allowing the system to produce stable and precise injection pressure during the scaffold fabrication process. The piston-based injection mechanism features a simple design that is easy to operate, with relatively low fabrication costs compared to other injection mechanisms [25]. The printed scaffold is subsequently evaluated in terms of its structural characteristics, including deposit width measurement, density testing, and porosity analysis.

The process of testing the density and porosity of the scaffold involves obtaining the density value by measuring the dry weight of the object (W_k) and its volume (V) using Eq. (2). The volume of the object is then calculated using Eq. (1). Where V_a is the initial water volume (cm^3), and V_b is the final water volume (cm^3). The porosity test is conducted by measuring the difference between the wet weight (W_b) and dry weight (W_k) per volume of the scaffold (V), which corresponds to Eq. (3).

$$V = V_b - V_a \quad (1)$$

Thus, the density of the object can be obtained using Eq. (2). Where W_k is the dry weight of the object (g), V is the volume of the object (cm^3).

$$D = \frac{W_k}{V} \quad (2)$$

Furthermore, the porosity value can be calculated using Eq. (3). Where P is the porosity, V is the volume of the object (cm^3), W_k is the dry weight of the object (g), and W_b is the wet weight of the object (g).

$$P = \frac{W_b - W_k}{V} 100\% \quad (3)$$

3 Results and discussion

3.1 Characterization test of *Nypa* extraction

3.1.1 The result of the FTIR test

The Fourier Transform Infrared (FTIR) spectroscopy test method is one of the testing methods used to identify functional groups in a material based on the intensity of infrared light absorbed by the material. The test results of *Nypa fruticans* produce an analysis graph as shown in Fig. 5.

Based on the functional groups in Fig. 5, it is explained that the broad absorption band with a range of 3600-3300 cm^{-1} indicates the presence of the Hydroxyl (O-H) functional group, which has a vigorous intensity. The absorption location, with a range of cm^{-1} , shows the double bond region, characterized by the functional group (C=C), with medium intensity. The region with absorption from 1500 to 400 cm^{-1} is the fingerprint region. Peaks with absorption from 1300 to 1000 cm^{-1} indicate ester (C-O) and C-N functional groups, marked with vigorous intensity. The 2928.67 cm^{-1} peak shows the Amine (C-H) region, characterized by a medium spectrum, at 1637.44 cm^{-1} (Table 1).

FTIR absorption also characterizes *Nypa* flour, showing the presence of ether bonds ($\text{CH}_2\text{-O-CH}_2$) 885.01 cm^{-1} [25]. Therefore, it can be concluded from the FTIR test results that hydroxyl (O-H) groups are identified with strong absorption intensity, which indicates the presence of a compound in galactomannan in *Nypa* flour that belongs to the polysaccharide group. These test results are in line with the research of Sari Purnawit [26].

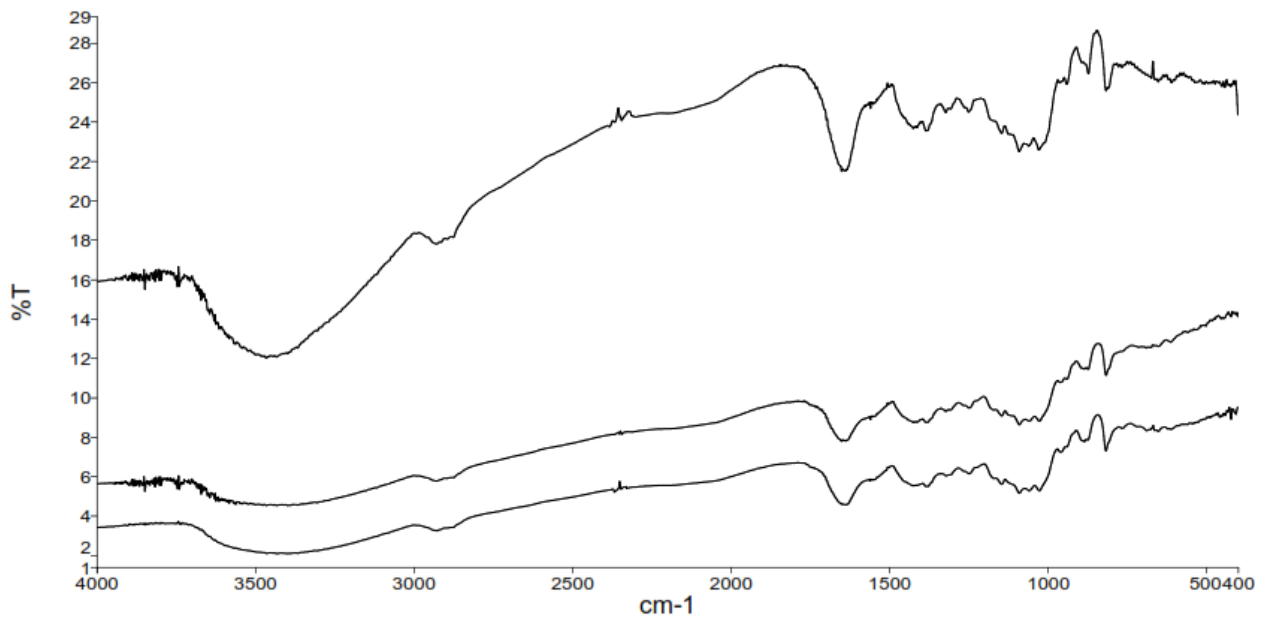


Fig. 5. FTIR spectrum curve of *Nypa fruticans*.

Table 1. Characteristic wavenumbers for functional groups in chemical compounds

Function group	Absorption location (cm ⁻¹)	Absorption intensity
Hidroksil (O-H),	3600 – 3300	Strong
Amina (C-H)	3000 - 2800	Moderate
Double bond area (C=C)	>1600	Moderate
Karbonil (C=O)	1600 – 1450	Moderate
Ester (C-O)	1300 - 1000	Strong
Fingerprint area	1500 - 400	-

3.1.2 The result of the GC-MS test

The GC-MS test method is used to analyze complex mixtures of organic compounds in a sample. The GC-MS test utilizes an extract from *Nypa* flour that was prepared using the maceration method

with an ethanol solvent at a 1:10 ratio. The GC-MS analysis curve of *Nypa* is presented in Fig. 6.

Polysaccharides are macromolecules composed of many monosaccharide units connected through glycosidic bonds [27]. These compounds can be found in various natural materials such as cellulose, starch, and hemicellulose [28]. Based on the GC-MS analysis results of the *Nypa* sample in Table 2, several compounds were identified as indicators of the presence and degradation of polysaccharides or complex carbohydrates. The compound 5-Hydroxymethylfurfural (HMF) had the highest area of 41.83% at a retention time of 13.736 minutes. HMF is a major product of the dehydration of simple sugars such as glucose and fructose, indicating thermal or chemical degradation of polysaccharides in the sample [29].

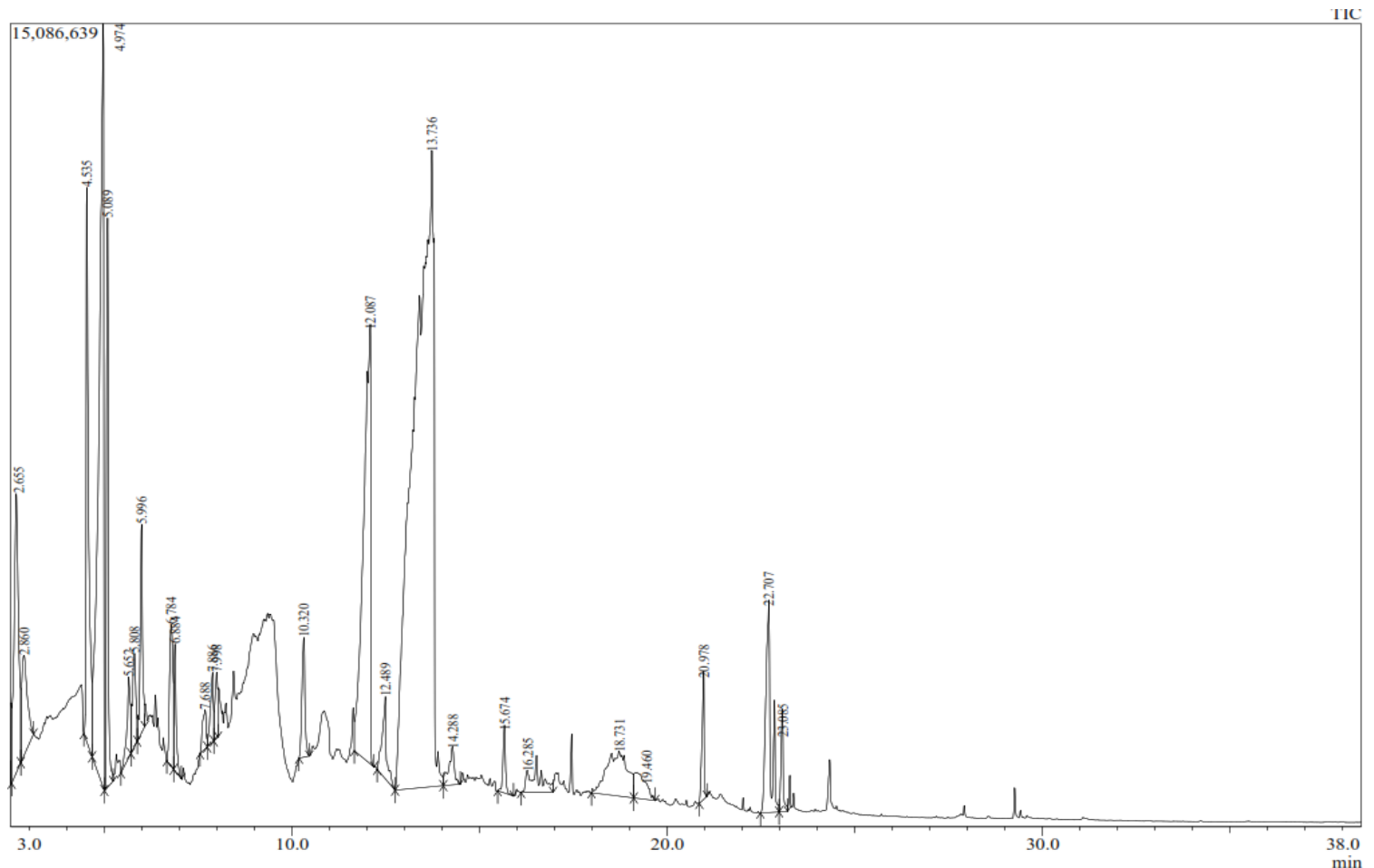


Fig. 6. GC-MS analysis curve of *Nypa* extract.

Table 2. GC-MS analysis results of *Nypa* extract

Peak#	R. Time	Area	Area%	Name
1	2.655	43564106	4.22	Acetic Acid, Anhydride with Formic Acid
2	2.860	20216382	1.96	2-Propanol, 1-methoxy- (CAS) 1-Methoxy-2-propanol
3	4.535	46764272	4.53	Formic acid (CAS) Biorin
4	4.974	120487436	11.68	Acetic acid (CAS) Ethylic acid
5	5.089	35586228	3.45	2-Propanone, 1-hydroxy- (CAS) Acetol
6	5.652	9244053	0.90	2,2'-Bioxirane, (R*,R*)-(.,+.-)-
7	5.808	11523969	1.12	Formaldehyde, methyl(2-propynyl) hydrazone

Additionally, the compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, was detected with an area of 10.36% at a retention time of 12.087 minutes, which is often associated with degradation products of simple sugars or results of the Maillard reaction that occurs during heating. These results overall indicate that the *Nypa* sample contains polysaccharides or related compounds that undergo degradation during sample treatment, with HMF as the dominant component that has the potential to be used as a chemical marker of polysaccharide degradation with chemical chains. Previous research has shown that HMF is a major product of the dehydration of simple sugars such as glucose and fructose, indicating thermal or chemical degradation of polysaccharides in the sample [30].

3.2 Evaluation of composition *Nypa fruticans* biomaterial

This section discusses the process of measuring material deposits that utilize *Nypa* extract as the primary component, along with sorbitol and glycerol as additive materials that serve as polymerization agents. In this process, the physical properties of each biomaterial composition were investigated, including viscosity, deposit width, texture, and the ability of the biomaterial to return to its original shape. The biomaterial synthesis was designed with three variations: 5%, 10%, and 15%. It was found that the percentage of *Nypa* extraction at 85% and sorbitol at 15% presented the appropriate results, as shown in Fig. 7.

Based on manual observation and measurement of biomaterials produced from *Nypa fruticans* extract with the addition of glycerol and sorbitol as softeners, the results of biomaterial composition tests are shown in Table 3. Viscosity, texture, and shape recovery

behavior were evaluated qualitatively by pressing the sample with a spatula and observing its physical response.

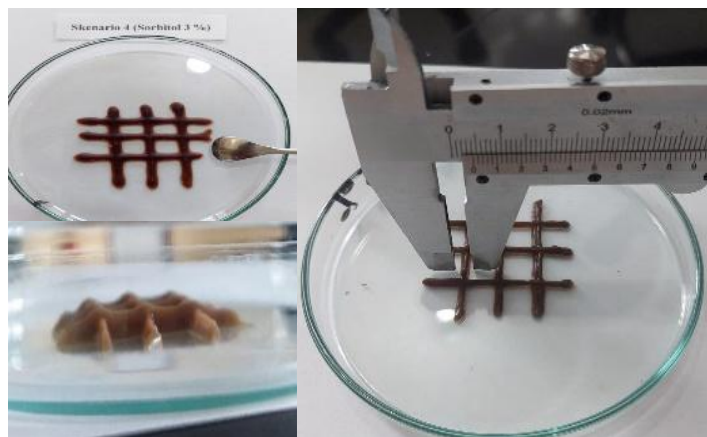


Fig. 7. Results of the synthesis of *Nypa fruticans* biomaterials and measurements of scaffold geometry.

The mixture of *Nypa fruticans* extract with glycerol produced biomaterial samples that were soft when pressed, with some samples slightly adhering to the spatula, indicating higher fluidity and lower viscosity. The pressed areas became irregular or damaged, non-elastic, and did not return to their original shape, showing weak structural integrity. In contrast, the mixture with sorbitol produced denser samples that remained compact under pressure, showed slight elasticity, and did not stick to the spatula, indicating better viscosity and shape recovery behavior.

Table 3. Results of biomaterial composition testing of *Nypa fruticans* reinforced with glycerol and sorbitol

Additive	Figures		
	5%	10%	15%
Glycerol			
Sorbitol			
Glycerol and sorbitol			

The combination of both glycerol and sorbitol did not show significant improvement; the samples appeared soft and semi-fluid, spread easily when pressed, and partially adhered to the spatula, suggesting poor elasticity and porosity formation.

From these observations, it can be concluded that the addition of glycerol tends to produce biomaterials that are softer and less elastic, which are suboptimal for applications requiring mechanical strength and elasticity. Conversely, the addition of sorbitol increased density, viscosity stability, and elasticity, resulting in a denser structure. The best results were obtained with a composition of 85% *Nypa* extract and 15% sorbitol. Therefore, sorbitol is recommended as the preferred softener to improve the mechanical performance of *Nypa fruticans*-based biomaterials.

These findings are consistent with previous studies, which have shown that sorbitol concentrations of up to 15% significantly affect visual appearance, tensile strength, and elongation [31]. Similarly, Mei Liu (2014) reported that sorbitol increases molecular mobility, enhances flexibility, and improves strain while reducing stress [32].

3.3 Evaluation of scaffold fabrication results

The experiment of printing scaffold prototypes with 3D bioprinting refers to the ability of *Nypa* biomaterial paste to form cubic and rhombic pore scaffold structures. As shown in Fig. 8, the scaffold printing follows a pattern with dimensions of 25×25×3 mm, where it can be clearly seen that the *Nypa* biomaterial paste with a sorbitol mixture can form a scaffold structure capable of building a scaffold following the CAD model of cubic and rhombic pores [33].

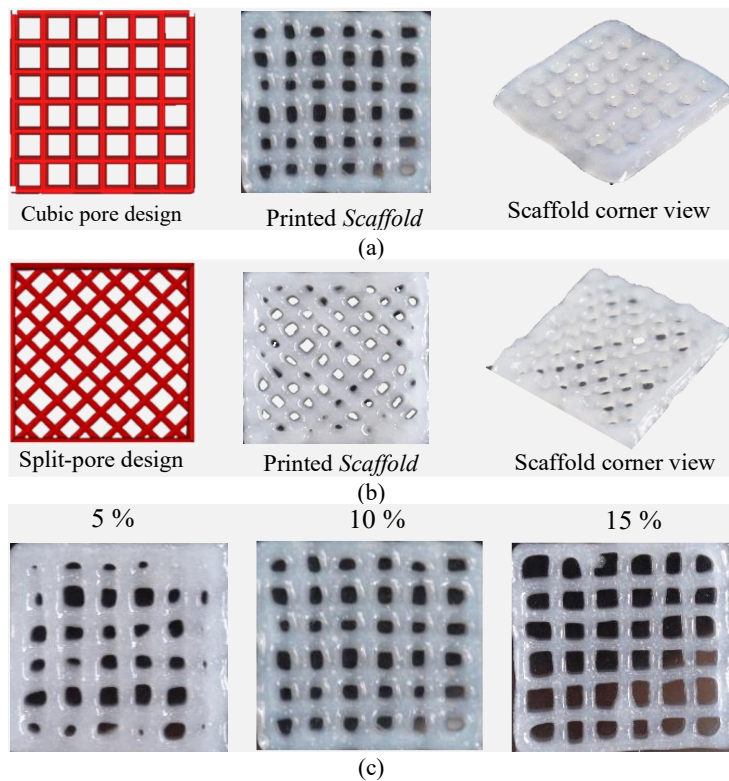


Fig. 8. Scaffold from *Nypa* biomaterial, (a) scaffold with cubic pore configuration, (b) scaffold with rhombus pore configuration, (c) prototype of 3-layer scaffold with 3 variations of sorbitol composition.

Fig. 8 shows the results of scaffold fabrication using an additive manufacturing technique with two different pore configurations and variations of sorbitol content. In part (A), the scaffold design with cubic pore configuration is shown, which is printed into a three-dimensional physical form and exhibits a regular and symmetrical porous structure. Fig. 8(b) shows the design of rhombus-shaped pores, which, after printing, also form a three-dimensional scaffold with a diagonal mesh-like pore pattern. Both configurations demonstrate the success in maintaining the digital design shape in the physical scaffold form.

Fig. 8(c) illustrates a three-layer scaffold prototype, each made with a variation of sorbitol composition of 5%, 10%, and 15%. It is observed that the increase in sorbitol concentration affects the physical appearance of the scaffold, particularly in terms of density and material clarity. At a 5% composition, the scaffold exhibits significant deposit widening and large pores. In contrast, at 10% and 15%, the scaffold displays smaller pores and a more compact and precise surface, indicating an increase in density and possibly mechanical strength. Sorbitol acts as a plasticizer that can influence the flexibility and microporous structure of the scaffold [34]. These results suggest that variations in sorbitol concentration impact the physical properties of the scaffold [35].

3.3.1 Scaffold deposit measurement

The measurement of scaffold geometry includes measuring the width of the deposit produced after the fabrication process. The fabrication process uses a nozzle with a diameter of 0.5 mm. Measurements are taken after oven drying for 2 hours at a temperature of 45°C [36]. Fig. 9 shows the graph of the measurement results of scaffold deposit width.

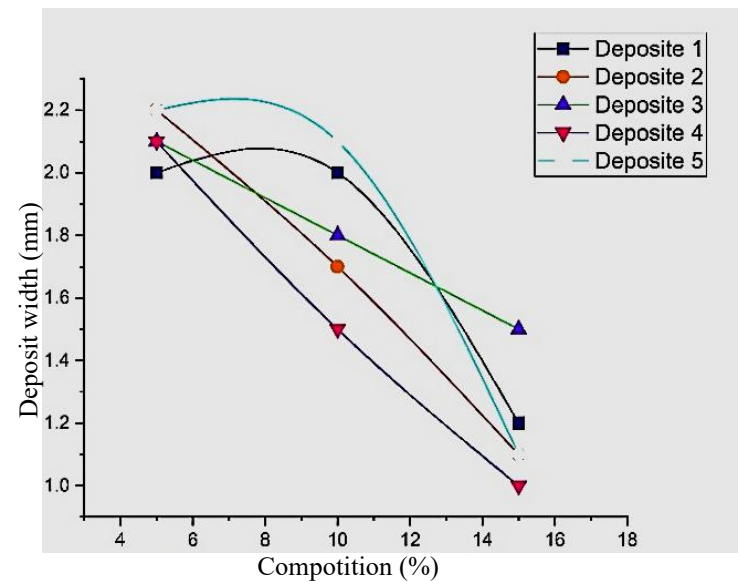


Fig. 9. Graph of scaffold deposit width measurement results for each composition.

Based on the measurement results of scaffold deposit width from five samples mixed with sorbitol in three variations of concentration (5%, 10%, and 15%) in Fig. 9, a clear relationship is observed between the increase in sorbitol content and the decrease in deposit width. At 5% concentration, the average deposit width is recorded at ± 2.12 mm, while at 10% it decreases to ± 1.82 mm, and at 15% sorbitol, the deposit width drastically reduces to ± 1.18 mm. This decrease in deposit width reflects the increase in solution viscosity resulting from the addition of sorbitol, which limits the material's spread when exiting the nozzle. Sorbitol, as a plasticizer agent, can form hydrogen bonds with active compounds in *Nypa* extract, increasing the bio-ink viscosity [37] and hindering its flow during the printing process.

The assessment of scaffold print quality can be seen from the conformity between the deposit width and the diameter of the nozzle used, which is 0.5 mm. In this context, the smaller the deposit width produced, the closer it is to the nozzle size, which means the print precision of the scaffold is higher. Based on the results obtained, the 15% sorbitol composition produces a deposit with the smallest width (around 1.0-1.2 mm), although it does not reach 0.5 mm. However, compared to the 5% and 10% compositions, the value at 15% shows the best tendency in approaching the ideal size. Therefore, it can be concluded that increasing the sorbitol content up to 15% significantly increases the bio-ink viscosity [31] and produces more precise scaffold prints, characterized by smaller and more controlled deposit widths.

This finding aligns with the principles of 3D bio-printing, where dimensional precision is crucial for the success of printed tissue structures [38]. Proper viscosity contributes to maintaining the geometric shape of the print and preventing excessive spreading after extrusion [39]. Therefore, the addition of sorbitol at an optimal concentration becomes an important strategy in formulating bio-ink based on natural materials, such as *Nypa* extract, to achieve high-quality scaffold prints.

3.3.2 Density and porosity test of scaffold

The density and porosity test of the scaffold is crucial in tissue engineering because these two parameters affect the ability of the scaffold to support cell growth and tissue regeneration. Proper density and porosity enable the scaffold to interact optimally with body tissues [40]. The density test is conducted to determine the mass density per unit volume of the scaffold, which plays a role in determining the mechanical strength and structural stability of the scaffold when used as a support for new tissue growth. Meanwhile, the porosity test aims to determine the volume of pores in the scaffold. Ideal scaffold porosity is essential because it enables the diffusion of nutrients, oxygen, and waste removal, while also supporting cell penetration and migration into the scaffold [41].

The samples tested are cubic pore scaffolds made from *Nypa* biomaterial with sorbitol mixtures of 5%, 10%, and 15% variation, with a scaffold geometry of 25×25×3 mm. The measurement of scaffold density and porosity is conducted after the sintering process at a temperature of 50°C for 2 hours [42]. The data on scaffold density and porosity measurement results are presented in Table 4.

Table 4. Density and porosity test results of scaffold

Test	Sorbitol		
	5%	10 %	15%
Density (gr/cm ³)	0.934	0.955	0.971
Porosity (%)	0.773	0.737	0.436

Based on the results of density and porosity testing presented in Table 4, the addition of sorbitol as a plasticizer to scaffolds made from *Nypa* extract has a significant influence on their physical characteristics, particularly density and porosity. The density of the scaffold increases with the increasing concentration of sorbitol, as shown in Fig. 10, from 0.934 g/cm³ at a 5% concentration to 0.955 g/cm³ at a 10% concentration, and reaching 0.971 g/cm³ at a 15% concentration. This increase in density suggests that sorbitol can enhance the density of the scaffold structure by filling the gaps between molecules in the polymer matrix [43]. This finding aligns with reports that the addition of plasticizers, such as sorbitol, strengthens bonds between polymer chains through hydrogen interactions, thereby increasing the density of biopolymer materials [44].

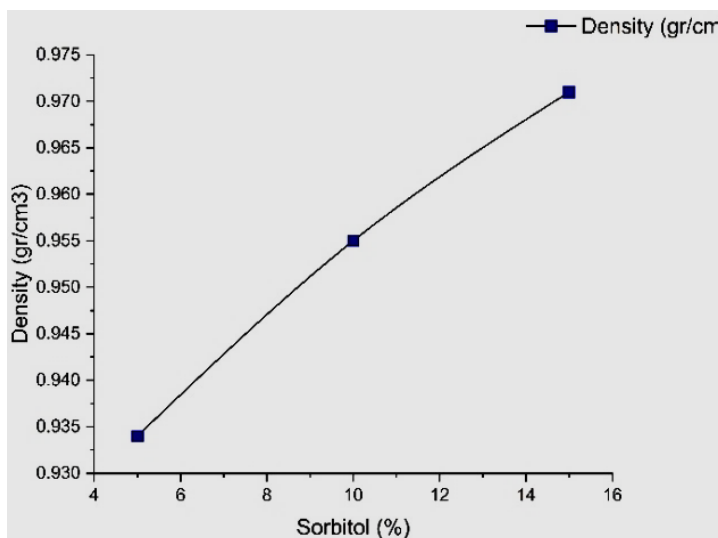


Fig. 10. Effect of sorbitol concentration on scaffold density.

Increasing the sorbitol concentration up to 15% significantly affects the physical properties of the scaffold. The increase in density indicates that sorbitol enhances the compactness of the scaffold structure by filling intermolecular voids within the polymer matrix. This finding is consistent with previous studies, which report that the addition of plasticizers, such as sorbitol, strengthens the intermolecular interactions between polymer chains through hydrogen bonding, thereby increasing the density of biopolymer materials [45-47]. Furthermore, the formation of hydrogen bonds between the hydroxyl (–OH) groups of sorbitol and the polar groups of galactomannan in the *Nypa fruticans* extract restricts the mobility of the polymer chain. This condition enhances the cohesive forces and internal friction within the bio-ink system, which directly contributes to the increased viscosity observed during the printing process [48].

In contrast, the porosity values of the scaffold show a decreasing trend with increasing sorbitol concentration shown in Fig. 11, from 0.773% at 5% concentration, decreasing to 0.737% at 10%, and reaching the lowest value of 0.436% at 15%. This decrease in porosity can be explained by the properties of sorbitol, which is able to attract and bind water during the scaffold formation process, resulting in a decrease in the formation of cavities or pores in the scaffold structure [49]. These results are consistent with studies that explain that the addition of plasticizers can reduce pore formation by filling the space between molecules and minimizing the free space formed during drying or scaffold printing [50].

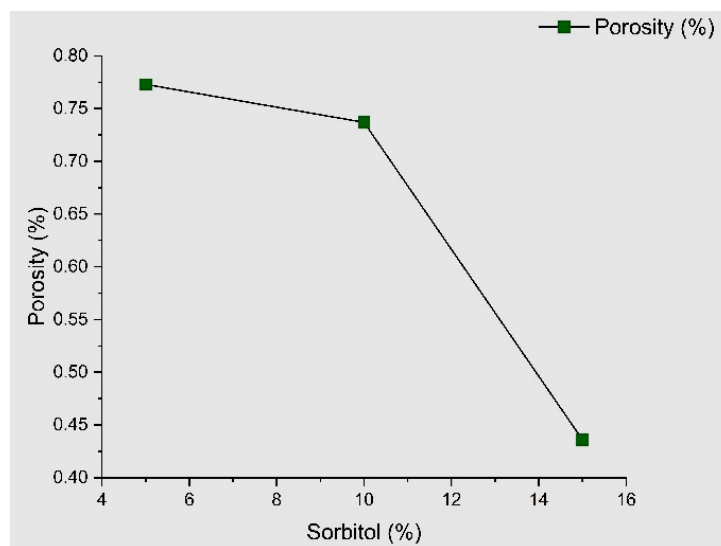


Fig. 11. Effect of sorbitol concentration on scaffold porosity.

Porosity is a crucial parameter in scaffolds because it facilitates the diffusion of nutrients, oxygen, and cell migration within the structure [51]. Therefore, although increasing density can enhance the mechanical strength of the scaffold, the decrease in porosity at higher sorbitol concentrations can limit the scaffold's effectiveness in supporting tissue regeneration [52]. Thus, the use of sorbitol must be optimized to achieve a balance between mechanical strength (enhanced by increased density) and bioactivity (facilitated by adequate porosity), according to the purpose of tissue engineering applications.

4 Conclusions

This study demonstrates that biomaterials derived from *Nypa fruticans* extract exhibit strong potential as natural bio-ink candidates for scaffold fabrication using 3D bio-printing technology. FTIR analysis confirmed the presence of hydroxyl (O–H) functional groups at 3600–3300 cm⁻¹, while GC–MS identified 5-Hydroxymethylfurfural, a polysaccharide-derived compound indicative of galactomannan content within the extract. The incorporation of sorbitol as a plasticizer significantly influenced the physical properties and printability of the formulated bio-ink, with

the optimal composition achieved at 85% *Nypa fruticans* extract and 15% sorbitol. This formulation successfully produced CAD-based scaffold architectures with stable cubic and rhombic pore configurations, yielding precise deposition widths (± 1.18 mm) that closely approach the nozzle diameter (0.5 mm). Increasing 15% sorbitol concentration correlated with higher scaffold density (up to 0.971 g/cm³) and reduced porosity (down to 0.436%). Overall, sorbitol enhancement effectively improved flow consistency, viscosity, and dimensional stability during the printing process, confirming the feasibility of *Nypa fruticans* extract as a promising natural biomaterial for 3D bio-printing applications.

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