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Study on Characterization of Various Biofilms Prepared by Starch Isolated from *Maranta Arundinacea* L.

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ABSTRACT

In the present study, the rhizome of *Maranta arundinacea* L., Arrowroot, was selected for a rich source of starch for the preparation of biofilm. Firstly, some physicochemical properties of the selected sample were determined by AOAC method. Furthermore, the elemental analysis of the selected sample was carried out by Energy Dispersive X-ray Fluorescence (EDXRF) spectroscopy. Moreover, antimicrobial activities of various solvent extracts were examined by Agar well diffusion method on six tested organisms. And then, the qualitative determination of starch tests such as Iodine test and Tannic acid test were done. In addition, starch from Arrowroot powder was isolated and confirmed by FT IR spectrum. Finally, starch biofilms were prepared by using isolated starch and various ratios of plasticizers PVA, PEG, and Sorbitol. The characterizations of seven kinds of prepared biofilms were measured.

KEYWORDS: *Maranta arundinacea L., Biofilms, EDXRF, AOAC method, FT IR, PVA, PEG, Sorbitol*

1. INTRODUCTION

The Rhizome of *Maranta arundinacea* L., Arrowroot is also a potential source of starch and it is also a staple food for human. *Maranta* is an herbaceous, perennial plant which has cylindrical rhizomes with high starch content. It also belongs to the Marantaceae family. The *Maranta* plant is being cultivated in the Caribbean islands, Southeast Asia, South America, Philippines and India, H. Arachchige, et al [1]. Chemically, starch consists of two important polysaccharides: amylopectin and amylose.

Amylose is an essentially a linear chain ofglucose units and linked by α-1, 4-glycosidic bonds, Amylopectin consists of linear chains ofglucose units that linked by α-1, 4-glycosidic bonds and is highly branched at the α-1, 6 positions. The starch may also contain proteins, lipids, water and a very small amount of phosphorus, magnesium and calcium, Morrison, [2].

Nowadays, environmental pollution has come seriously from consumed polymers such as packaging materials and off-set plastic bags and cups. Thus, the suitability of biomaterials (biopolymers) for film production has been intensively studied Aminabbavi, et al, [3]. Starch is one of the most attractive raw materials for the production of bioplastics because it is quite many branches of the industry because of its respective physicochemical properties. It is also found ingrains, fruits, roots or tubers of manygreen plants and acts as their main storage material. Properties of starch depend on several parameters including the botanical origin, M.N. Madineini, et al, [4].

Plasticizer, film-forming polymers are additives that increase the mechanical properties of biofilms. So, the starch film is needed to add a plasticizing agent to enhance film brittle-less, plasticity and mechanical properties. Plasticizers aregenerally small molecular weight molecules such as polyols like sorbitol, polyethyleneglycol (PEG) and polyvinyl alcohol (PVA) etc. These polyols increase not only flexibility but also water vapor andgas permeability, Bravin, et al, [5]. Bioplastic film coating with encapsulated antimicrobial substances can retard thegrowth of micro-organisms on the surface of food product, S. Ko., et al, [6].

In recent decades, thegrowing environmental awareness has encouraged the development of biodegradable materials from renewable resources to replace conventional non-biodegradable materials in many applications. Among them, polysaccharides such as starches offer several advantages for the replacement of synthetic polymers in plastics industries due to their low cost, non-toxicity, biodegradability and availability, Fajardo et al., [7], Simkovic, [8]. The use of renewable resources that can produce biodegradable materials to reduce waste disposal problems continues to be explored as consumer concern rise on limited natural resources and environmental issues, Tavassoli-Kafrani et al., [9]. The consumer demand has shifted to eco-friendly biodegradable materials, especially from renewable agriculture by-products, food processing industry wastes and low-cost natural resources, Alves et al, [10].

In this study, pure bio-starch films were prepared from the Arrowroot starch and prepared the other six kinds of films. The mechanical properties of various types of prepared biofilms were examined.

1.1. Botanical description

Botanical name - *Maranta arundienacea* L.
Family name - Marantaceae
Myanmar name - Ar-ta-lut
English name - Arrowroot
Part used - Rhizome
Medicinal uses - diarrhea, vomiting, pain or fever, stomach and intestinal disorder, leucorrhoea, ulcer rheumatism, and skin diseases.

Figure 1: Plant, flower and rhizome of Arrowroot

2. MATERIALS AND METHODS

2.1. Sample collection

The rhizomes of Arrowroot were purchased from the local market, Mandalay region. The collected samples were washed with water several times to remove the impurities adhered to the surface and removed the paper-like scales. Then, the samples were cut into small pieces and dried in air at room temperature andgrain the powder.

2.2. Determination of some Physico-chemical characterizations of the rhizome of Arrowroot

Nutritional values of the rhizome of Arrowroot were measured by AOAC (Association of Official Analytical Chemists, 2000)[11] method.

2.3. Determination of elemental analysis in rhizome of Arrowroot

The elemental contents of dried powder of rhizome of Arrowroot were determined by using EDXRF method.

2.4. Antimicrobial activities of the rhizome of Arrowroot

Antimicrobial activities of the rhizome of Arrowroot by using three solvents extracts such as n-hexane, ethyl acetate and ethanol were investigated against *Bacillus subtilis, Staphylococcus aureus, Pseudomenas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli* species by employing Agar well diffusion method.

2.5. Qualitative analysis of starch in the rhizome of Arrowroot

2.5.1. Iodine test

2g of sample was weighed and added with 25mL of distilled water. The solution was heated for 15 minutes and then cooled and filtered. 2mL of filtrate was reacted with 2drops of iodine solution. The blue color was disappeared during heating. Then, it was cooled, the blue color was reappeared. Finally, it was mixed with 5 drops of sodium hydroxide solution. The blue color was disappeared. The starch may be present.

2.5.2 Tannic acid test

2g of the sample was boiled with 25mL of distilled water for 10 minutes. It was cooled and filtered. The filtrate was mixed with 3 drops of tannic acid solution. The white precipitate was obtained. Then, it was heated again for 10 minutes. The white precipitate was disappeared. So, starch may be present.

2.6. Extraction of starch from fresh Arrowroot

Arrowroot was first washed and cleaned of the paper-like scales. After removing these scales, the roots were washed again and cut into small pieces. 100g of fresh sample and 200mL of distilled water were blended toget homogeneous slurry. The milky liquid was filtered with a coarse into a beaker and allowed to stand for 1 hour. The residual containing starch was washed with 200mL of distilled water and blended again. Then the mixture was filtered and the filtrate was allowed to stand for 1 hour. The liquid above the settle was decanted. Finally, the obtained starch was dried at room temperature. The yield percent was calculated.

$$\text{Yield percent} = \frac{\text{Weight of Starch (g)}}{\text{Weight of Arrowroot (g)}} \times 100$$

2.6.1. FT IR spectrophotometric analysis of extracted starch

The functionalgroups of extracted starch were determined by using FT IR spectrophotometer.

2.7. Preparation of pure starch film

2g of starch powder and 50mL of distilled water were put into 250mL beaker. And then, the mixture was stirred and heated at 50°C for 2 hours on the magnetic stirrer to become the homogeneous solution. After degassing, 10mL of the mixture solution was poured into each Petri dish and it was maintained at room temperature for 5 days. Finally, the pure starch film was obtained.

2.8. Preparation of starch-PVA (1:1) film

1g of starch powder and 1g of PVA were added into 250mL beaker. Then, 50mL of distilled water was added into a beaker. The mixture solution was stirred and heated at 50°C for 2hours on the magnetic stirrer to become the homogenous solution. After degassing, 10mL of mixture solution was poured into each petri dish and it was kept at room temperature for 5 days. Starch-PVA (1:1) film was obtained.

2.9. Preparation of starch-PVA (1:2) film

1g of starch powder and 2g of polyvinyl alcohol were added into 50mL of distilled water in a 250mL beaker. Then, the mixture was stirred and heated at 50°C for 2 hours on the magnetic stirrer to obtain a homogeneous solution. After degassing, 10mL of mixture solution was poured into each Petri dish and it was maintained at room temperature for 5 days. And then, starch-PVA (1:2) film was obtained.

2.10. Preparation of starch-sorbitol (1:1) film

1g of starch powder and 1g of D-sorbitol were put into a 150mL beaker and 50mL of distilled water was added. And then, the mixture solution was stirred and heated at 50°C for 2 hours toget a homogeneous solution. After degassing, 10mL of solution was poured into each petri dish and it was kept at room temperature for 5 days. Starch-Sorbitol (1:1) film was obtained.

2.11. Preparation of starch-sorbitol (1:2) film

1g of starch powder and 2g of D-sorbitol were added into a 150mL beaker and filled with 50mL of distilled water. Then, it was stirred and heated at 50°C for 2hours toget a homogeneous solution. After degassing, 10mL of solution was poured into each Petri dish and it was maintained at room temperature for 5 days. Starch-sorbitol (1:2) film was obtained.

2.12. Preparation of starch-PEG (1:1) film

1g of starch powder and 1g of PEG were added into a 50mL of distilled water in a beaker. Then, the mixture was stirred and heated at 50°C for 2 hours on amagnetic stirrer to obtain a homogeneous solution. After degassing, 10mL of solution was poured into each petri dish and it was maintained at room temperature for 5 days. The starch-PEG (1:1) film was obtained.

2.13. Preparation of starch-PEG (1:2) film

1g of starch powder and 2g of polyethyleneglycol were put into a 150mL beaker. Then, 50mL of distilled water was added into a beaker. It was stirred and heated at 50°C for 2 hours on the magnetic stirrer toget a homogeneous solution. After degassing, 10mL of mixture solution was poured into each petri dish and it was maintained at room temperature for 5 days. Finally, starch-PEG (1:2) film was obtained.

2.14. Determination of mechanical properties of prepared films

Mechanical properties of prepared films such as thickness, tensile strength, elongation at breaks, tear strength were measured.

3. RESULTS AND DISCUSSION

In this section, the results obtained from the experimental works such as some physicochemical properties, mineral contents, antimicrobial activity, isolation of starch, preparation of starch films and characterization of starch-based films were discussed.

3.1. Some physicochemical properties of the rhizome of Arrowroot

Physicochemical properties of rhizome of Arrowroot dried powder such as pH, moisture content and ash content were determined. The results were shown in Table 1. All of the resulting data were obtained by triplicate measurements. Mean value was described for each.

Table1: Physicochemical properties of rhizome of Arrowroot

No.	Parameters	Apparatus used	Results
1.	Moisture content	Temperature controlled oven	8.3%
2.	Ash content	Temperature controlled Muffle furnace	2.7%
3.	pH	pH meter	8.5

According to Table 1, the rhizome of Arrowroot contains moisture 8.3%, ash 2.7% and pH 8.5 respectively.

3.2. EDXRF Analysis of Rhizome of Arrowroot

The elemental contents of the rhizome of *Maranta arundinacea* L. were determined by EDXRF method. The results were described in Table 2.

Table2 Elemental Contents in the rhizome of Arrowroot

No.	Symbol	Element	Composition (%)
1.	K	Potassium	0.980
2.	S	Sulphur	0.090
3.	P	Phosphorus	0.076
4.	Ca	Calcium	0.015
5.	Fe	Iron	0.03
6.	Cu	Copper	0.002
7.	Mn	Manganese	0.001
8.	Rb	Rubelium	0.001
9.	Br	Bromine	0.001
10.	Zn	Zinc	0.001

Table 2 shows that potassium was the highest amount in the sample. Decreasing order of mineral content is K > S > P > Ca > Fe > Cu > Mn > Rb > Br > Zn. So, the rhizome of Arrowroot contains essential minerals for human health.

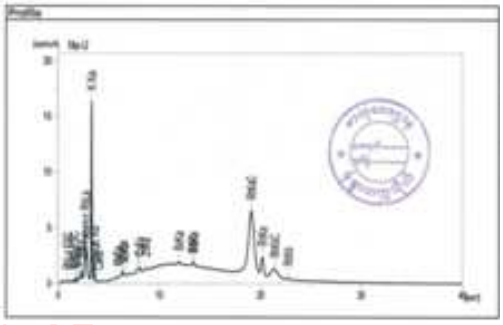

Figure 2: EDXRF spectrum of the rhizome of Arrowroot

3.3. Antimicrobial activities of the rhizome of Arrowroot

The study of antimicrobial activities of the rhizome of Arrowroot was performed by agar well diffusion method on six microorganisms. These results were shown in Table (3) and Figure 3.

Table3: Antimicrobial activities of the rhizome of Arrowroot

Sample	Solvents	Inhibition Zone					
		I	II	III	IV	V	VI
Rhizome of Arrowroot	EtOH	16 mm (++)	16 mm (++)	14 mm (+)	15 mm (++)	14 mm (+)	15 mm (++)
	EtOAc	11 mm (+)	13 mm (+)	13 mm (+)	15 mm (++)	12 mm (+)	14 mm (+)
	n-hexane	12 mm (+)	11 mm (+)	(–)	13 mm (+)	12 mm (+)	11 mm (+)

Zone diameter Organisms

well diameter = 10mm
0mm = (-)
10 mm ~ 14 mm = (+)
15 mm ~19 mm = (++)
20 mm above = (+++)

I = *Bacillus subtilis*
II = *Staphylococcus aureus*
III = *Pseudomonas aeruginosa*
IV =*Bacillus Pumilus*
V = *Candida albicans*
VI = *E-coli*

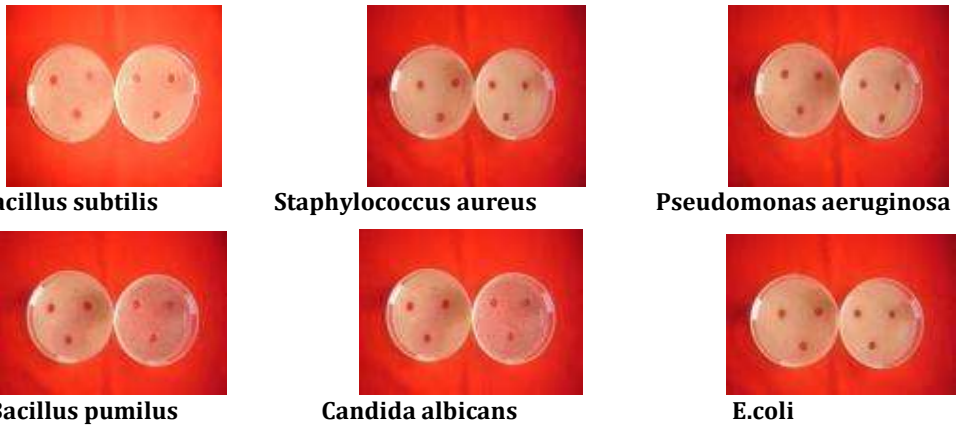

Figure 3: Animicrobial activities of various solvent extracts of the rhizome of Arrowroot

According to the results, ethanol extract of the sample shows medium activities on *Bacillus subtilis, Staphylococcus aureus, Bacillus pumilus*, *E-coli* and low activities on the remaining two organisms. Ethyl acetate extract responds on medium activities on *Bacillus pumilus* and low activities on the other five tested organisms and n-hexane extract has low activities on all tested organisms.

3.4. Qualitative analysis of starch in the rhizome of Arrowroot

The qualitative analysis of starch in the rhizome of *Maranta arundinacea* L. was determined by the following two tests. The observation was described in Table 4

Table4: Special test for starch

No.	Test	Extract	Reagent	Observation	Results
1.	Iodine Test	D/W	I_2 solution, NaOH	Blue color appears Blue color disappear	+
2.	Tannic Acid Test	D/W	Excess Tannic Acid solution	White ppt	+

Figure 4: (a) Iodine test (b) Tannic acid test

From these results, it was observed that the rhizome of Arrowroot sample consists of starch.

3.5. Extraction of starch from fresh Arrowroot

The yield percent of starch from the rhizome of Arrowroot was found to be 27.25 ± 0.001%.

3.6. FT IR spectrophotometric analysis of extracted starch from Arrowroot

The functionalgroups of extracted starch were determined by using FT IR spectrophotometer.

Figure 5: FT IR spectrum of isolated starch

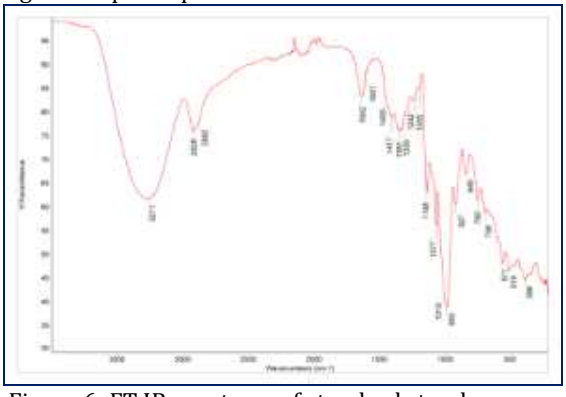

Figure 6: FT IR spectrum of standard starch

3.7. Preparation of various types of biofilms

The various types of films such as Pure starch films, starch-PVA films (1:1/1:2), starch-sorbitol films (1:1/1:2), starch-PEG films (1:1/1;2) were prepared by Casting method. The different types of prepared films were shown in figure-7.


Pure Starch film


Starch-PVA (1:1) film


Starch-PVA (1:2) film


Starch-Sorbitiol (1:1) film


Starch-Sorbitol(1:2) film


Starch-PEG (1:1) film


Starch-PEG (1:2) film

Figure 7: The various types of prepared biofilms based on Arrowroot starch

3.8. Mechanical properties of different types of Biofilms

According to investigations of mechanical properties of prepared biofilms, the pure starch film is thicker than others (0.05 mm thickness). The best tensile strength (25.3 MPa), elongation at break (275%) and the best tear strength (72.5kN/m) were observed at starch- sorbitol (1:1) biofilm. The starch-PVA (1:2) film was found that the second largest of elongation at break (145%), tear strength (60.0kN/m) and thickness (0.09mm).

Table5: Mechanical properties of different types of films

No	Sample	Thickness (mm)	Tensile strength (MPa)	Elongation at Break (%)	Tear strength (kN/m)
1.	Pure Starch	0.05	16.3	67	22.5
2.	Starch-PVA (1:1)	0.10	3.8	45	53.0
3.	Starch-PVA (1:2)	0.09	8.4	145	60.0
4.	Starch- PEG (1:1)	0.11	12.3	45	60.0
5.	Starch-PEG (1:2)	0.10	12.8	93	45.5
6.	Starch-sorbitol (1:1)	0.10	25.3	275	72.5
7.	Starch-sorbitol (1:2)	0.12	6.5	85	15.7

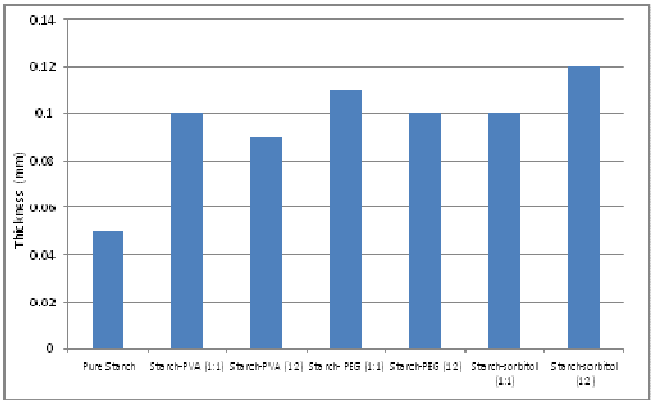

Figure 8(a) Thickness of prepared biofilms

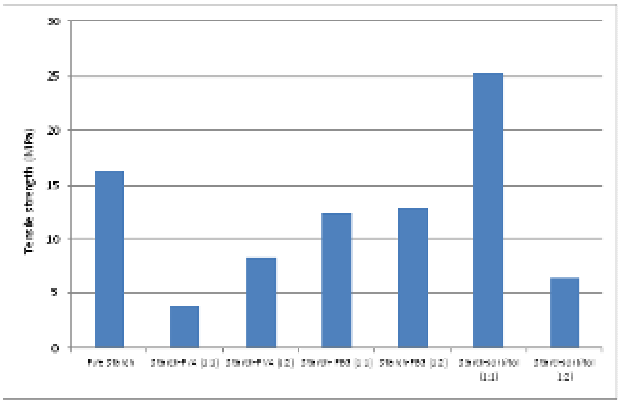

Figure 8 (b) Tensile strength of prepared biofilms

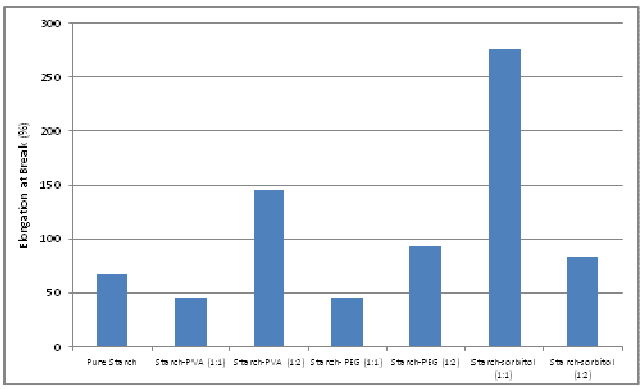

Figure 8 (c) Elongation at break of prepared biofilms

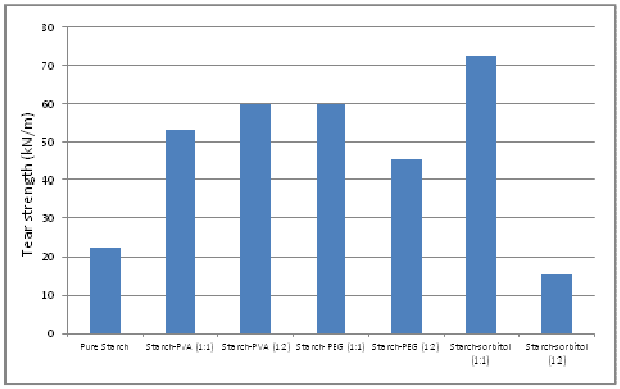

Figure 8 (d) Tear strength of prepared biofilms

4. CONCLUSIONS

In this research work, one of the richest sources of starch, *Maranta arundinacea* L. (Arrowroot) was selected for the preparation of biofilms and other investigations. Seven types of bio-films were prepared and their characteristics were studied. Among them, starch sorbitol 1:1 film is the best mechanical properties than others. Biofilm can harbor human infections agents in the environment, but they also can promote remediation of contaminatedgroundwater and soil. Biofilms can be potential tools to clean up environmental pollution and returning an environment from an altered state back to its natural one with the help of microorganisms. Though collecting oil and running it through a biofilm filter of some sort is not a common method to clean up oil spills today, it may be an interesting option to explore in the future.

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