SYNTHESIS OF BIODEGRADABLE PLASTIC FROM (*Artocarpus heterophyllus Lam*), (*Zea mays*) AND (*Piper betle*) USING GLYCEROL AS PLASTICIZER

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ABSTRACT

This study aims to produce starch-based bioplastic that is environmentally safe and reduces pollution. This research aims to investigate the effect of betel leaf extract as an antimicrobial agent on the characteristic of bioplastic based on jack fruit seed starch. Bioplastics are the only solution to replace plastics. As a result, these nonbiodegradable plastics remain in the environment for a long time and cause an increase in solid waste production. Starch is one of the major sources in the development of bioplastics. The bioplastic was produced from the starch of jackfruit seed along with glycerol as a plasticizer and analyzed for its physical and mechanical properties. The bioplastic produced was analysed for its antimicrobial activity. The produced bioplastic showed better physio mechanical properties and can be used widely in industries.

Keywords: Bioplastics, Glycerol, Jackfruit, Cornleaf, Betel leaf.

INTRODUCTION

Environmental sustainability has been a real challenge for living beings due to the rising population, urbanization, and standard of living for years, plastic waste has become one of the biggest problems due to its excessive use, its difficulty in decomposition, also its huge of mass cause a lot of negative impact to landfill and water pollution in daily life, plastics are used almost everywhere in the world for various purposes because plastics are inexpensive, readily available, durable and versatile **(1)**. The use of plastic waste continuously increases from time to time, creating problems in the increase in a huge amount of plastic waste. However, the main raw material in producing plastics derived from petroleum is declining and non-renewable. In addition, plastics cannot be destroyed quickly and naturally by destructive microbes in the soil **(2).** Bioplastics are the only solution to replace plastics. As a result, these nonbiodegradable plastics remain in the environment for a long time and cause an increase in solid waste production. Efforts have been made to handle plastic waste issues. Strategies have been applied from the recycling process to the most extreme process (i.e., the burning process). Although the method can reduce the amount of waste, both methods still create new problems. For instance, recycled plastic can cause side effects on contaminating the product, especially when it is used as a food packaging material due to the presence of certain potentially carcinogenic substances. Burning plastic waste can pollute the air because the smoke contains hazardous chemicals such as dioxin. Since plastic cannot be replaced by other materials, some researchers suggested developing bioplastics that are certainly more environmentally friendly **(3).** Polysaccharides, being the most abundant macromolecules in flora and fauna, are one of the most suitable raw materials for bioplastics in the form of starch, which is not only renewable and sustainable but also plentiful and cheap. Starch also has favorable thermoplastic properties and is biodegradable **(4).**

Starch is one of the major sources in the development of bioplastic. Many previous studies have been conducted by using starch as a natural biopolymer. Starch consists of a long chain of two glucose units joined together, namely branched polymerized amylopectin and amylose, which gives its granular structure. Due to its large availability, low cost, renewability, and biodegradability, starches are commonly used in the production of bioplastics. Starch can behave like a thermoplastic in the presence of a plasticizer, with the application of heat and mechanical treatment. As native starch-based films are limited to high water affinity and brittleness, other natural biopolymers are often added as fillers to modify and improve films' properties. Mostly agricultural wastes are used to produce bioplastics **(5).**

The production of starch-based bioplastics is simple, and they are widely used for packaging applications. The tensile properties of starch are suitable for producing packing materials, and glycerol is added to the starch as a plasticizer. The required characteristics of the bioplastics are achieved by fine-tuning the quantities of the additives. For trade applications, starch-based plastics are regularly mixed with eco-friendly polyesters. Bioplastics are also relatively costly compared to plastics. So, the alternative process of using agricultural waste for bioplastic production may decrease the cost of the bioplastic products. Plasticizers are added to these bioplastics to reduce brittleness and stiffness while improving flexibility. Starch-based bioplastics produced without plasticizers boast low elasticity, are very stiff, and have high tensile strength. Glycerol is one of the most used plasticizers. It is non-hazardous and safe for food manufacturing. Glycerol worked as a classical starch plasticizer. It is perhaps the thermoplastic starch's most investigated and utilized plasticizer. This is due to its low price, nontoxicity for human food and biomedical applications, and relatively high boiling point (292°C) **(1).** Starch is composed of two polymers, branched amylopectin, and linear amylose, which normally constitute about 75% and 25% of corn starch, respectively. Betel leaf is known as a traditional medicinal plant that contains polyphenol compounds such as tannins and flavonoids. Tannins and flavonoids are phenolic compounds that have antifungal and antibacterial inhibitory properties. This research will try to make an active packaging material using jack fruit seed starch, corn leaf, and betel leaf extract as a bioplastic manufacturing material. Betel leaf has antimicrobial properties which are expected to prevent microbial growth. The addition of betel leaf extract certainly affects the physical properties of the bioplastics produced. **(6)**

This research aims to investigate the effect of glycerol as a plasticizer on the characteristics of bioplastic based on jackfruit seed starch, and corn leaf starch. Besides the glycerol addition, betel leaf extract is also added to the bioplastic to improve the biological resistance properties of starch against bacteria and fungi. Both betel leaf extract and bioplastic are analyzed in this study. The analysis of betel leaf extract is yield, tannin content, and flavonoid content. The analysis of density, water absorption, biodegradability test, tensile test, moisture content, solubility test, and antimicrobial activity testes are carried out.

MATERIALS AND METHODS

The materials used in this study are Jack fruit seeds ***(Artocarpus heterophyllus Lam)***, corn leaf **(*Zea mays)***, betel leaf ***(Piper betle*)**. Jack fruit seeds was collected from Thiruvilwamala, Thrissur, Kerala, India. The fresh, disease free leaves of corn leaf were harvested from local farmland, Irugur, Coimbatore, Tamil Nadu, India. Betel leaf was collected from local market, Irugur, Coimbatore, Tamil Nadu, India.

Starch Extraction

Strach was extracted to prepare raw materials for making bioplastics. The outer shell of raw jackfruit seeds removed and overnight soaked in water. Then washed with tap water and the brown skin was removed. The seed was then blended using blender to obtain a slurry. The slurry was then filtered by a muslin cloth to obtain crude starch while the supernatant was discarded. Then the starch cake was dried in daylight for 12 hours. **(5)**

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Extraction of corn leaf and betel leaf

The fresh, disease-free leaves of corn leaf were harvested from local farmland, Irugur, Coimbatore, Tamil Nadu, India. The collected leaves were washed with tap water, excess wetness was removed by drying for 30 minutes at room temperature. Then leaves were ground by clean mortar and pestle. About 5g of grounded corn leaves were blended with 100 ml of distilled water and boiled at 100°C for 15 minutes. Then the boiled extract was filtered by the filter paper and stored at room temperature for further study **(7)**. The betel leaf was washed with tap water and dried in oven at 50^0C for 24 hours then grounded by using blender. The obtained slurry was sieved by using 50 mesh. The 5g of betel leaf powdered were added in a 50ml ethanol with ratio 1:10 (w/v) and left for 24 hours. Then the extract was filtered using filter paper. The suspension of filtrate was stored as a filler for fabrication of bioplastic **(6).**

Production of bioplastic

The next process after extracting the starch is bioplastic film production. The proportions of ingredients were selected to produce bioplastics as shown in the Table 1. These ratios were taken for easy comparison of results. Glycerol and distilled water were mixed and heated in a hot plate. Starch mixture was added to the glycerol. Mixing this starch mixture in the hot plate gives gel like substance. The gel like substance was poured into the aluminium foil paper. After that the bioplastic film was dried in oven for 4 hours at 50^0C.The bioplastic film was produced.

Table 1. Bioplastic film fabrication

SAMPLE IN DIFFERENT RATIO	JACK FRUIT SEED STARCH(g)	CORN LEAF EXTRACT(g)	BETEL LEAF EXTRACT(g)	GLYCEROL (ml)	WATER (ml)
Sample in ratio 1 (20g)	15.0	4.9	0.1	12.0	150
Sample in ratio 2 (25g)	20.0	4.8	0.2	15.0	150
Sample in ratio 3 (30g)	25.0	4.7	0.3	18.0	150

Solubility test

The film samples were cut into square sections of 2.0 mm, and the dry film mass was weighed accurately and recorded. The samples remained immersed in 100 ml distilled water and fixed agitation at 180 rpm was carried out for 6 hours at 50^0C. The lasting portions of the film were filtered after 6 h. They were then dried in a hot air oven at 100^0C until an ultimate fixed weight was found. Glycerol has a good water solubility range. The percentage of total soluble matter (% solubility) was calculated.

$$WS\ (\%) = \frac{(W_0 - W_f) \times 100}{W_0}$$

where WS is solubility in water; W_0 is the weight at the beginning of the bioplastics; and W_f the final weight of the bioplastics **(8).**

Thickness Measurement

The thickness of the bioplastics was measured by using the thickness gauge. The thickness was measured by holding the workpiece between stylus and anvil, reading the value directly ***(8*)**.

Test for Moisture content

By measuring the weight loss of films, the moisture content was estimated. The samples were cut into square pieces of 2.0 cm. The samples were weighed accurately. The dry film mass was recorded upon drying in an oven at 100C until a fixed dry weight was acquired. Each films moisture content was measured, **(9).**

$$\text{Moisture Content in (\%)} = \frac{(W_i - W_f) \times 100}{W_i}$$

where W_i is the weight at the beginning and W_f is the final weight.

Biodegradability test

The specimen was cut into pieces of 4.0 cm. Found near the roots of plants which are rich in nitrogenous bacteria, 300g of soil (having slight moisture content) was collected and stored in a pot. All three sample was buried inside the soil at a depth of 2.0 cm, 15 days under the conditions of the room. The weight of the specimen was measured before and after the testing. The biodegradability test was measured by Equation, **(8).**

$$\text{Weight Loss (\%)} = \frac{(W_0 - W) \times 100}{W_0}$$

Where W_0 and W are the weights of samples before and after the test.

Tensile test

A tensiometer was used to test for the strength of the biodegradable film. Three different proportion were tested, and their strength compared with each other. The three specimens were cut into the same specific sizes. Tensile strength testing uses universal tensile strength using both ends of the film sheet clamped to the testing machine. The measurement results in the form of force (F) and sample extension are included in the formula, **(10) (11)**.

$$\text{Tensile strength } (kgf/mm^2) = \frac{Force\ (F)}{\text{Surface area } (A)}$$

Elongation test

The sample is tested for elongation and measured by the formula, **(11)**.

$$Elongation = \frac{\text{Final gage length} - \text{initial gage length}}{\text{Initial gage length}} \times 100\%$$

Modulus young test

The relationship between tensile strength and elongation results in the Modulus Young value in the formula, **(11).**

$$Modulus\ young = \frac{\text{Tensile strength } (MPa)}{Elongation}$$

Antibacterial analysis of bioplastic and betel leaf

Muller–Muller-Hinton agar (MHA) and Nutrient Broth (NB) were used throughout the study for determining the antibacterial study. Bacillus subtilis is the bacterial strain used for the study of antibacterial effect. The media was adjusted to pH and autoclaved at 121^{0}C for 15 minutes. The experimentation was carried out by a laminar air-flow chamber. 25ml of Molten agar medium was taken in a sterile Petri dish and broth cultures of test isolates (0.1 ml) containing 1.0 X 10^{5} CFU/ml of bioplastic film, betel leaf extract along with the bacterial strains were added. The contents were thoroughly mixed and solidified. The wells were punched over the agar plates using the sterile gel puncher at various concentrations. The bioplastic and betel leaf were added in 25µl, 50µl, 75µl, and 100µl concentrations in each species and also to the wells. The sample was transferred into the well using a micropipette. The plates were incubated for 24 hours at 37^{0}C. After incubation, the diameter of the inhibitory zones formed around each disc and was measured (cm) and recorded **(12).**

Antifungal analysis of Bioplastic film and betel leaf

To test the anti-fungal effect, *Rhizoctonia solani* strain is used in this study. The bioplastic film and betel leaf extract solution of two different ratio was prepared by adding 20µl CH3COOH solution and 1 mL dd-H2O to the samples, and then vortex for 3 to 5 minutes. The sample was prepared by adding 20µl CH3COOH solution and 1 mL dd-H2O to bioplastic film and betel leaf, and then vortexing for 3 to 5 minutes. Then we put a round filter paper (diameter 2 cm) with a dissolved bioplastic film and betel leaf extract in the middle of the plate and calculated the diameters of the inhibition zone **(12).**

RESULT AND DISCUSSION

Production of Bioplastic

Bioplastic film ratio 1 | Bioplastic film ratio 2 | Bioplastic film ratio 3

Figure 1. Production of bioplastic film

Solubility test

Water solubility of all samples was observed in Table 2. The sample in ratio 3 showed the least water Solubility. Water solubility of the bioplastic films is shown in Table 2. According to **(8)** the sample has the highest content of rice starch, which may be due to the rice starch, where the solubility is lower. Water solubility was the main property able to distinguish the starch type through the film formation methods, based on starch being related to the amylose content in the grains.

Table 2. Water solubility of bioplastics

SAMPLE	INITIAL WEIGHT W_0 (G)	FINAL WEIGHT W_F (G)	WATER SOLUBILITY (%)
SAMPLE IN RATIO 1 (20g)	0.288	0.231	19.79
SAMPLE IN RATIO 2 (25g)	0.297	0.242	18.51
SAMPLE IN RATIO 3 (30g)	0.318	0.274	13.8

Thickness measurement.

The thickness of the bioplastics is measured using a thickness gauge. Three different ratios of bioplastic film thickness were measured. The thickness of the bioplastics is shown in Table 3. As per the regulations of the Government of India, the thickness of plastic bags should not be less than 50 microns. The results show that the prepared bioplastics have more thickness and, hence, can be used for preparing carry bags. In the current work, the thickness is higher, which may be due to the presence of jackfruit seed starch.

Table 3. Thickness of bioplastics.

sample	Thickness(mm)	Thickness in Micron
SAMPLE IN RATIO 1 (20g)	0.27	270
SAMPLE IN RATIO 2 (25g)	0.25	250
SAMPLE IN RATIO 3 (30g)	0.24	240

Moisture content

Table 4. Moisture content of bioplastics

SAMPLE	MOISTURE CONTENT %
Sample in ratio 1 (20g)	76.92
Sample in ratio 2 (25g)	83.5
Sample in ratio 3 (30g)	92.85

A moisture content test for taken for all three ratios. The sample in ratio 3 (30g) shows high moisture content. The sample in ratio 2 (25g) shows the least moisture content.

Biodegradability test

A biodegradability test was conducted for three ratio bioplastic films for 10 days. The weight loss of the samples indicated the process of biodegradation of the bioplastics by the microorganisms. The biodegradability test in the soil was recorded value shown in Table 5. Biodegradability results are shown in Table 6 and Figure 17. The biodegradability test is shown in Table 5 and Figure 19. The sample in ratio 1 has the least biodegradable value due to the low amount of starch. The sample in ratio 3 (30g) has a high biodegradable value due to the high amount of starch. Visual modifications of the samples were seen after the finish of the testing practice in the pot. The samples got broken into pieces when touched. Therefore, from the weight loss shows, it could be concluded that the bioplastics prepared from jack fruit seed starch, corn leaf, and betel leaves are biodegradable. According to (**8**) Biodegradability of 48.73% was achieved in 15 days for the sample placed in the soil at a depth of 3.0 cm. From the corn and rice starch-based bioplastic film based experiment, it may be concluded that the burial depth did not show a significant role in biodegradability.

Table 5. Biodegradability test in soil recorded value

Days	Bioplastic weight(mg) Sample in ratio 1(20g)	Bioplastic weight(mg) Sample in ratio 2(25g)	Bioplastic weight(mg) Sample in ratio 3(30g)
0	900	900	900
2	870	852	830
4	828	810	802
6	772	754	732
8	745	739	725
10	700	691	683

Table 6. Biodegradability percentage of recorded value

Concentration	Sample in ratio 1 (20g)	Sample in ratio 2 (25g)	Sample in ratio 3 (30g)
Biodegradability (%)	22.22	23.22	24.11

Physical analysis of Bioplastic film

Tensile strength is the amount of maximum strength needed to break the bioplastics film. Tensile modulus is defined as the stress change divided by change in strain within the linear viscoelastic region of the stress/strain curves. Elongation at break is the indication of the amount of the variation of extreme film length while attaining tensile strength until the film breaks, related to the original length. The tensile strength of the bioplastic film, Young's Modulus, and the elongation of the film at the breaking point was found for the samples and shown in Table 7. Glycerol, as a plasticizer, made the film more flexible as the intermolecular bonds between the polymer chains were reduced and the mechanical properties were modified.

Table 7. Physical analysis of bioplastics

Parameters	Sample in ratio 1 (20g)	Sample in ratio 2 (25g)	Sample in ratio 3 (30g)
Thickness (mm)	0.42	0.28	0.21

Tensile strength (Mpa)	5.2	3.6	8.0
Elongation at break (%)	32.02	28.03	25.01
Elasticity (Mpa)	950	928	1200

Tensile test

Table 8. Tensile test results

Samples	Tensile strength (Mpa)
SAMPLE IN RATIO 1 (20g)	5.2
SAMPLE IN RATIO 2 (25g)	3.6
SAMPLE IN RATIO 3 (30g)	8.0

The bioplastic tensile strength measurements results presented in Table 8. and figure 20. show that the bioplastics have the highest tensile strength of 8.0 MPa. The tensile strength of bioplastics increases with the addition of the corn leaf extract (4.7g) and betel leaf extract (0.3g) while the bioplastics with the addition of corn leaf extract (4.8g) and betel leaf extract (0.2g) decrease. This shows that the addition of corn leaf extract and betel leaf extract affects the tensile strength of the bioplastics produced.

Elongation test

Table.9 Elongation test results

Sample	Elongation%
SAMPLE IN RATIO 1 (20g)	10.37
SAMPLE IN RATIO 2 (25g)	7.60
SAMPLE IN RATIO 3 (30g)	13.47

The elongation value of bioplastics with the effect of corn leaf extract and betel leaf extract addition can be seen in Figure showing that the measurement results shown in Table 9 and Figure 21. The elongation/percent of bioplastic elongation with corn leaf and betel leaf variations obtained the highest elongation value at 7.6 in Sample in ratio 2. Corn leaf and betel leaf variation of Sample in ratio 3 has the smallest bioplastic elongation value at 13.47 in Sample in ratio ratio3. The percent elongation produced has decreased in various variations of the different ratios.

Modulus young test

Table 10. Modulus young test

Sample	Young's Modulus (Gpa)

SAMPLE IN RATIO 1 (20g)	0.501
SAMPLE IN RATIO 2 (25g)	0.473
SAMPLE IN RATIO 3 (30)	0.593

The value of the bioplastic modulus Young's test shows the results in Table 10. From these results Sample in ratio 3 shows the highest elongation value whereas S2 has a very low elongation value due to the different ratios.

Antimicrobial test

Antibacterial and Antifungal analysis of Bioplastic film

Table 11. Antibacterial and Antifungal results of Bioplastic film

Organisms Concentration	***Bacillus subtilis***	***Rhizoctonia solani***
20 g	3 mm	4 mm
25 g	5 mm	6 mm
30 g	5.5mm	8 mm
Standard (Chloramphenicol)	5 mm	10 mm

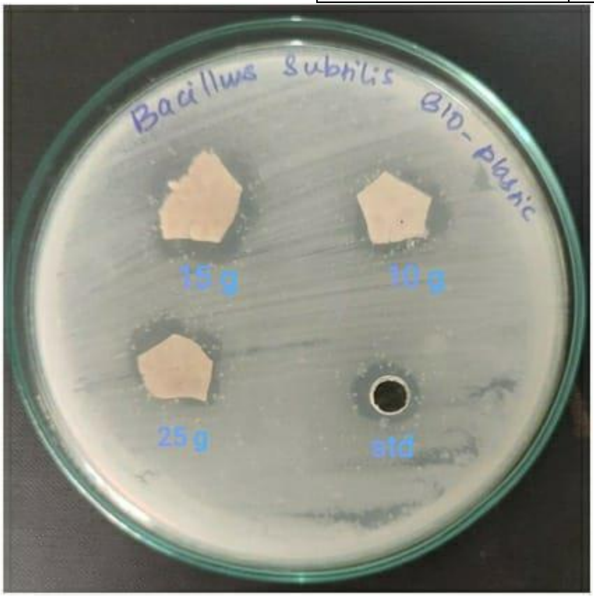

Figure 2. Bacillus subtilis for Bioplastic film

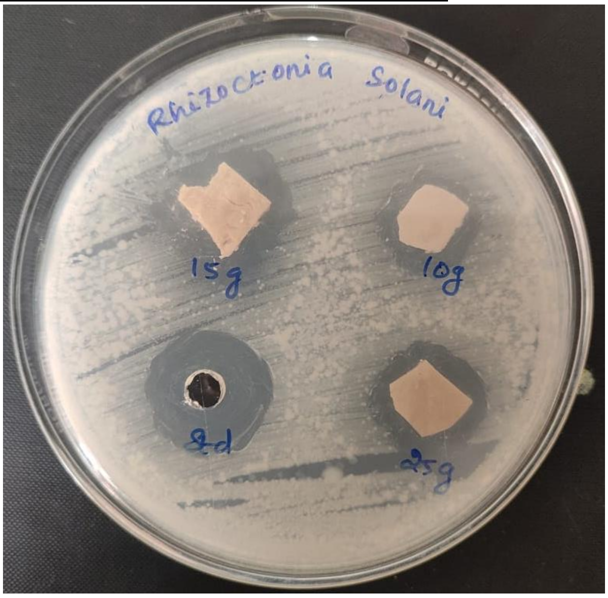

Figure 3. Rhizoctonia solani for Bioplastic film

The antibacterial screening revealed that the bioplastic film possesses antimicrobial properties against bacteria and fungi shows in Figure 2 and Figure 3. The effect of this extract was found to increase in the following order of concentration against test organism, Bacillus Subtilis (gram-positive), a highest zone of inhibition in Bacillus Subtilis at 30g concentration. Chloramphenicol was used as a positive control for antibacterial activity. The antifungal screening was evaluated with *Rhizoctonia solani*, a fungal strain which revealed that the effect of bioplastic film showed a higher zone of inhibition in Table 11. against *Rhizoctonia solani*, at 30g concentration. Fluconazole was used as a positive control for antifungal activity.

According to **(6)** the antifungal activity of bioplastic films. All variation of bioplastics donot have inhibition for *Aspergillus niger* growth. It shows that both betel leaf extract and glycerol have no ability to inhibit the fungi growth. Fungi have a more complex structure than bacteria. Fungi are multicellular organisms and have cell walls. Meanwhile, bacteria have a simpler structure which is unicellular and does not have a cell wall. This is the reason why the betel leaf extract shows inhibitory properties of bacteria but not fungi. The betel leaf extract addition is increased the diameter of inhibitory zone. An antimicrobial agent consists of ether, ester, carbonate or carbamate derivatives of polyols. Antimicrobial agents are derived from organic compounds containing three until six hydroxyl groups. Antimicrobial agents are used to kill microbes on contact such as in disinfection applications as well as to preserve and protect materials against microbial attack.

Antibacterial and Antifungal analysis of Betel leaf extract

Table 12. Antibacterial and Antifungal results of Betel leaf extract

Betel leaf extraction Concentration	***Bacillus subtilis***	***Rhizoctonia solani***
A (50 µl)	6 mm	10 mm
B (100 µl)	4 mm	7 mm
Standard (Chloramphenicol)	6 mm	10 mm

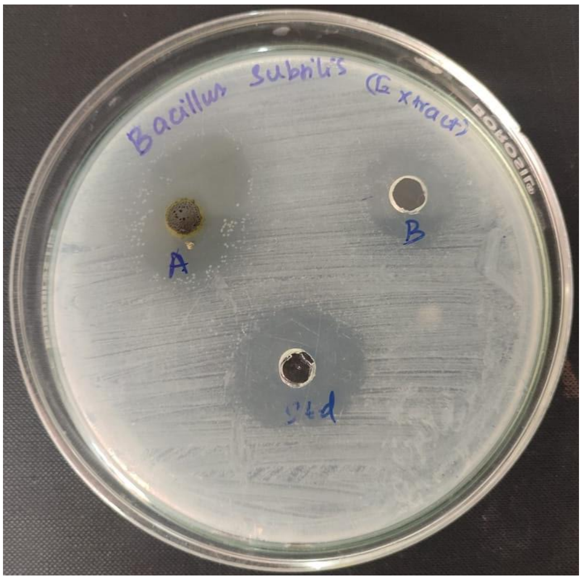

Figure 4. Bacillus subtilis for Betel leaf extract A and B

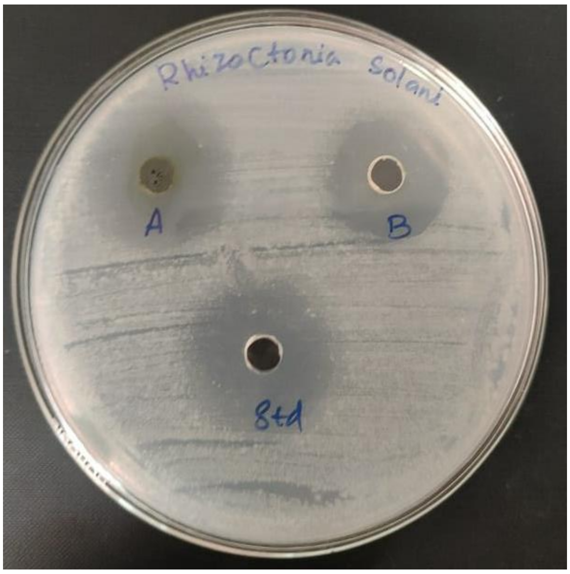

Figure 5. Rhizoctonia solani for Betel leaf extract A and B

The antibacterial screening revealed that the betel leaf extract possesses antimicrobial properties against bacteria and fungi shown in Figure 4 and Figure 5. The effect of this extract was found to increase in the following order of concentration against the test organism, Bacillus Subtilis (gram-positive), the highest zone shown in Table 12. of inhibition in Bacillus Subtilis at 50 µl concentration. Chloramphenicol was used as a positive control for antibacterial activity. The antifungal screening was evaluated with Rhizoctonia solani, a fungal strain which revealed that the effect of betel leaf extract showed a higher zone of inhibition against Rhizoctonia solani, at 100 µl concentration. Fluconazole was used as a positive control for antifungal activity.

According to **(6)** the diameter inhibitory zone of antibacterial activity of betel leaf extract concentrated and betel leaf with 2% dilution is 44 mm and 14,4 mm respectively. This means that betel leaf extract has more inhibition ability better than betel leaf extract with 2% dilution.

The antifungal analysis of the betel leaf extract concentrated shows a large inhibition area which means it has the potential to inhibit the fungal growth. However, betel leaf extract with 2% (2 g/ 100 mL) dilution has no inhibition for the *Aspergillus niger* growth. The diameter inhibitory zone of antifungal activity of betel leaf extract concentrated is 45,8mm. It shows that actually betel leaf extract has the potential to inhibit fungal growth. The betel leaf extract at 2% concentration is too dilute so it is unable to inhibit the growth of *Aspergillus niger*

SUMMARY AND CONCLUSION

This paper presents a process for making an environmentally friendly bioplastic from jackfruit seed starch, corn leaf fiber, and betel leaf extracts with antimicrobial activity. Jackfruit seed has a high starch content (about 70.22% of fresh seed weight) and it was easy to collect in large quantities and extract starch. The bioplastic was formulated with three different ratios of starch and glycerol. The bioplastic was then analyzed for its solubility, thickness measurements, moisture content, biodegradability, tensile strength, elongation analysis, modulus young analysis, and analyzed for its antimicrobial activity. The bioplastic sample in ratio 3 had the least solubility in water while the sample in ratio 1 showed the best solubility in water. The bioplastic sample in ratio 3 had the best thickness of 0.24 mm. Bioplastic with a ratio of 30:18 has the best tensile strength. The sample in the ratio 3 was found to be 13.47% elongated. The bioplastic was found to be degraded in soil within ten days of analysis. The antimicrobial analysis of bioplastic of three different ratios and betel leaf extract exhibited the best inhibition zone of antibacterial and antifungal analysis. In addition, the simple processes of starch extraction and bioplastic fabrication also promise that this proposed method can be applied to industrial production shortly.

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