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COLOCASIA ESCULENTA (L.) LEAF BIO-WAX AS A HYDROPHOBIC SURFACE COATING SUBSTANCE FOR PAPER FOR PREPARING HYDROPHOBIC PAPER BAGS

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ABSTRACT
The surface of Colocasia esculenta (L.) (taro) leaf is covered with a layer of highly hydrophobic layer of bio-wax. The main objective of the project was to isolate the bio wax layer of the leaves using organic solvent extraction method using chloroform and coat it on to the surface of paper to obtain hydrophobic paper which might be later used for making biodegradable hydrophobic paper bags. Also, the isolated bio-wax was subjected to various tests like heat test, hydrophobicity test, anti-microbial test, quantitative analysis to check its viability for industrial uses. The result of quantitative analysis showed that 1 gram of sample leaf contained about 0.116 gram of wax. It was also observed that the paper coated with the bio wax attained hydrophobic property which was similar to the Colocasia leaf. Heat sensitivity test showed that the bio-wax retains hydrophobicity even at high temperature. The bio-wax also possesses anti-bacterial property.

KEY WORDS
Anti-bacterial, Bio-wax, Colocasia esculenta (L.), *Hydrophobic, Heat sensitivity, Quantitative*

INTRODUCTION
Plastic bags, without any doubt, have found usefulness due to the properties like high durability, non-corrosiveness, light weight, electrical and thermal insulation, etc, that plastics possess [1]. But in spite of having such varying range commercially useful properties, plastic bags have become a global concern because of the fact that they possess serious threat to the environment because of their non-biodegradable nature. Even process like incineration which is carried out with the intention of destroying the plastic bags produce large amount of greenhouses gases and other toxic gases like carbon dioxide, carbon monoxide, sulphur dioxide gases, etc [2] which does more harm than good to the environment by causing air pollution. Plastic bags are also the major cause of blockage of drainage systems, one of the reasons behind urban floods and water pollution. Not only plastic bags cause pollution to the environment, but also cause death of the animals when swallowed by terrestrial or marine [3, 4] and the alarming concern is that the production of plastic bags has increased several folds over few decades. Around 5 billion plastic bags are used across the globe [3]. So, attempts are made to create alternatives like biodegradable plastic bags. But many studies show that biodegradable plastic requires a favorable environment [5] i.e. optimum temperature, moisture, presence of microbial population, etc for the process of degradation and even if they degrade, it produces smaller particles of plastic called micro-plastics [5] which gives shows that even the use of biodegradable plastic is not completely eco-friendly [6]. Therefore, to avoid these problems, use of paper bags is encouraged because it has been scientifically proven that paper bags do not cause as much harm as plastic bags do [7]. Although the rate of biodegradation is slow in landfills with low moisture, but recycling of paper bags requires 91% less energy per pound as compared

to plastic bags and also reduce the production of toxic gases and use of water to a considerable amount [8]. But paper bags have their own limitations. Paper bags are not water resistant due to which the uses of paper bags become very limited. On the other hand, if plastic films are used to laminate the paper bags to make it water resistant then the product becomes non-biodegradable or if they are coated with synthetic compounds like slimicides or lacquer [9] to have additional advantages over plain paper then their degradation might cause water and soil pollution.

So, in an attempt to find suitable organic material which might be able to solve the above-mentioned problems, answers were sought in the nature. Ariel surface of plants contain hydrophobic water proofing wax (bio-wax) on them which provides protection against environmental stresses [10, 11]. In some plants this wax is highly hydrophobic in nature [12]. One example of such plant which possesses leaves having such a layer of hydrophobic bio-wax is *Colocasia esculenta*, a plant belonging to family Araceae [13] which is commonly known as 'taro' is a perennial herb which can grow upto 1.5 meter (4 feet). It is found throughout the tropical and subtropical regions and can grow in a wide range of dry and wet places. These leaves are highly hydrophobic and when water falls on it, the water rolls off in the form of a droplet, a phenomenon known as 'Lotus Effect', which is self-cleaning, water- repellant property found in some plants [14, 15]. So, the bio-wax of the *Colocasia esculenta* leaves, if extracted, it could be used as a coating substance on the surface of paper to attain a layer of hydrophobic bio-wax on the paper. The hydrophobic paper hence attained could be used as a source of raw material for making paper bags. In addition to water resistance or hydrophobic nature, the most important advantage of this product will be that it will be completely biodegradable, and the time required for degradation will also very less due to its organic nature. Moreover, *Colocasia esculenta* is highly abundant in nature. It is positioned at rank fourteenth among the stapled vegetable crops of the world and global production is about 12 million tonnes from approximately 2 million hectares which gives an average yield of 6.5 tonne per hectare [16, 17]. The high abundance of the leaf gives it the potential to be a raw material for industrial uses.

MATERIALS AND METHODS

Isolation of wax from surface of leaves

Fresh leaves of *Colocasia esculenta* were collected. One leaf was cut into fragments. 20ml of Chloroform was taken in a beaker. The leaf fragments were immersed in the chloroform for 3 minutes. Glass rod was used to immerse the leaves completely. After 3 minutes the chloroform was transfer into a new beaker. The chloroform now contains the epicuticular wax present in the leaf fragments. A white cloudy layer of wax can be seen floating on the chloroform [18].

Wax confirmatory test

Wax was extracted from the leaves by solvent extraction method. The solvent (chloroform) is evaporated. The wax was then dissolved in ethanol and transferred into a test tube. Few ml of distilled water was then added to the solution and shaken. The solution turned milky white [19].

Quantitative analysis bio-wax

Fresh leaves were collected and cut into fragments. 1.5g of leaf fragment was measured. The leaf fragments were then dipped into 10ml of chloroform for 3 minutes. The chloroform was then discarded and the remaining chloroform on the leaf fragments were then allowed to air dry. The weight of the leaf fragments were then measured again. The process was repeated for 2.5g and 3.5g with 15ml and 20ml of chloroform respectively. By subtracting the weight of the leaves after the chloroform treatment from the weight of the fresh leaves the amount of wax can be calculated. The amount of bio wax present in 1gm of fresh leaves can be calculated by the following formula:

$$\textbf{Average weight of wax per gram of leaf} = \frac{\textbf{Total weight of epicuticular wax (in gram)}}{\textbf{Total weight of the leaf fragments (in gram)}}$$

Test for hydrophobicity

Bio-wax was isolated from leaves by solvent extraction method. The chloroform containing the wax was poured into a Petri dish and the solvent was allowed to evaporate. After the solvent has evaporated the wax coating on the Petri plate left was again dissolved in 3ml of chloroform to get high concentration of wax. The solvent containing wax was then poured on a rectangular piece of filter paper using a micropipette. Test for hydrophobicity was then done by dropping water on to it using pipette and was compared to a filter paper without the wax coating. The water resistance was evaluated by observing the time till which the paper shows hydrophobicity.

Test for anti-bacterial property

Nutrient agar media was prepared by dissolving 8.4gm of nutrient agar media in 300ml of distilled water and was then autoclave [20, 21]. It is then poured into 12 Petri plates and was allowed to solidify. *E.coli* and *Streptococcus* species were then inoculated onto 6 Petri plates for each species one Petri plate of each are control. To the other five Petri plates of each species, disc containing sample of varying concentration are added. The plates were incubated in an incubator at 35ºC. The radiuses of zone of inhibition were then measured for each species.

Heat sensitivity test

Wax was extracted in different Petri plates using chloroform. The solvent was then allowed to evaporate at room temperature. Different Petri plate was then subjected to different temperature ranging from 30ºC to 120ºC for 2 minutes. The retention of hydrophobic property and integrity of the wax was then observed.

Removal of pigment from the wax

The extracted wax contains greenish pigment in it which is due to the presence chlorophyll. To make the wax free of any pigment the following method was used:

The sample leaf was exposed steam in a water bath for 30 minutes. The temperature was set at 100ºC because chlorophyll degrades at high temperature [22]. The colour of the leaf turns into brownish-green and becomes soft. The steam treated leaf was then treated with chloroform to extract the wax. The chloroform containing the wax was then poured into a Petri plate and was left to evaporate. After the solvent gets evaporated a white layer of bio-wax can be seen. The Petri plate containing the wax was then kept in oven for then minutes at 60ºC to remove water molecules.

RESULTS

Isolation of wax from surface of leaves

Fig 1: Cloudy layer of bio-wax on chloroform.

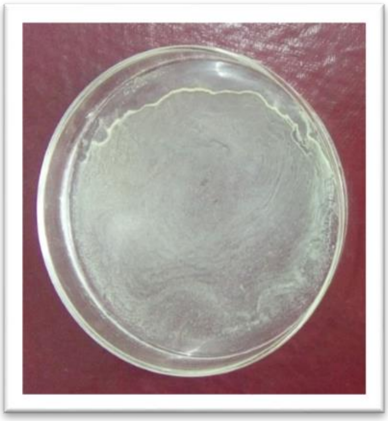

Fig 2: Solvent free layer of bio-wax on a Petri plate.

Wax confirmatory test

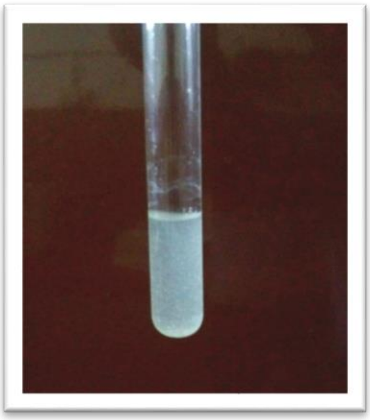

Fig 3: Wax confirmatory test

Quantitative analysis bio-wax

Sl. No.	Weight of leaf fragments before chloroform treatment (in gram) A	Weight of leaf fragments after chloroform treatment (in gram) B	Amount of epicuticular wax (in gram) A-B
1	1.5	1.28	0.22
2	2.5	2.20	0.30
3	3.5	3.15	0.35

Table 1: Comparative table of weight of the fresh leaves and chloroform treated leaves

Calculation:

$$\text{Average weight of wax per gram of leaf} = \frac{\text{Total weight of epicuticular wax (in gram)}}{\text{Total weight of the leaf fragments (in gram)}}$$

$$\text{Average weight of wax per gram of leaf} = \frac{0.22 + 0.30 + 0.35}{1.50 + 2.50 + 3.50} = \frac{0.87}{7.50} = 0.116\text{grams}$$

Test for hydrophobicity

SL. NO.	Object for Test	Time (in minutes)
1	Paper Coated with Bio-wax	30+
2	Paper without bio-wax coating	0

Table 2: Comparative table of duration of water resistance

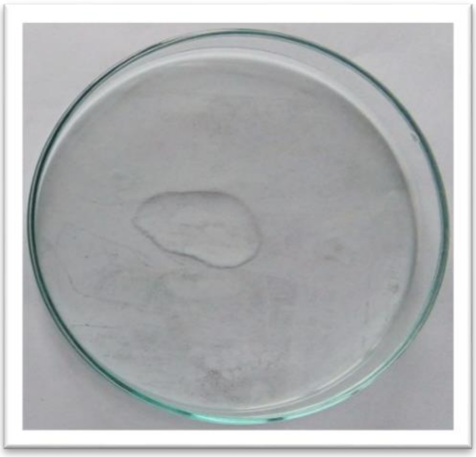

Fig 4: Water on Petri plate without bio-wax

Fig 5: Water on Petri plate with bio-wax layer

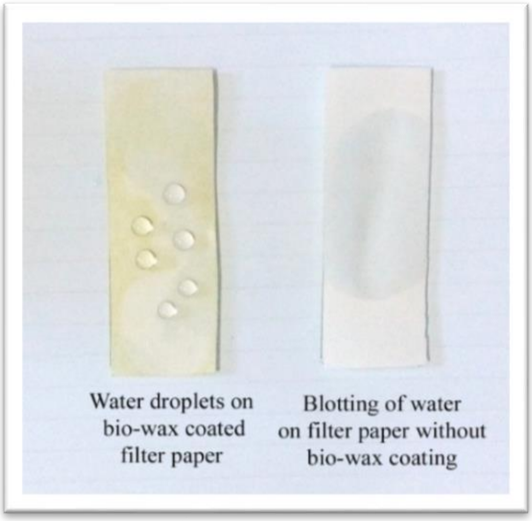

Fig 6: Comparison between bio-wax filter paper and normal filter paper when water droplets are dropped on them.

Test for anti-bacterial property

Sl. No.	CONCENTRATION OF WAX (in mg)	Radius of zone of inhibition for *E. Coli* (in mm)	Radius of zone inhibition for *Streptococcus* (in mm)
1	Control	None	None
2	50	0	2
3	100	3	3
4	150	3.5	4
5	200	4	5
6	250	4.3	5.1

Table 3: Radius of zone of inhibition at 24 hrs

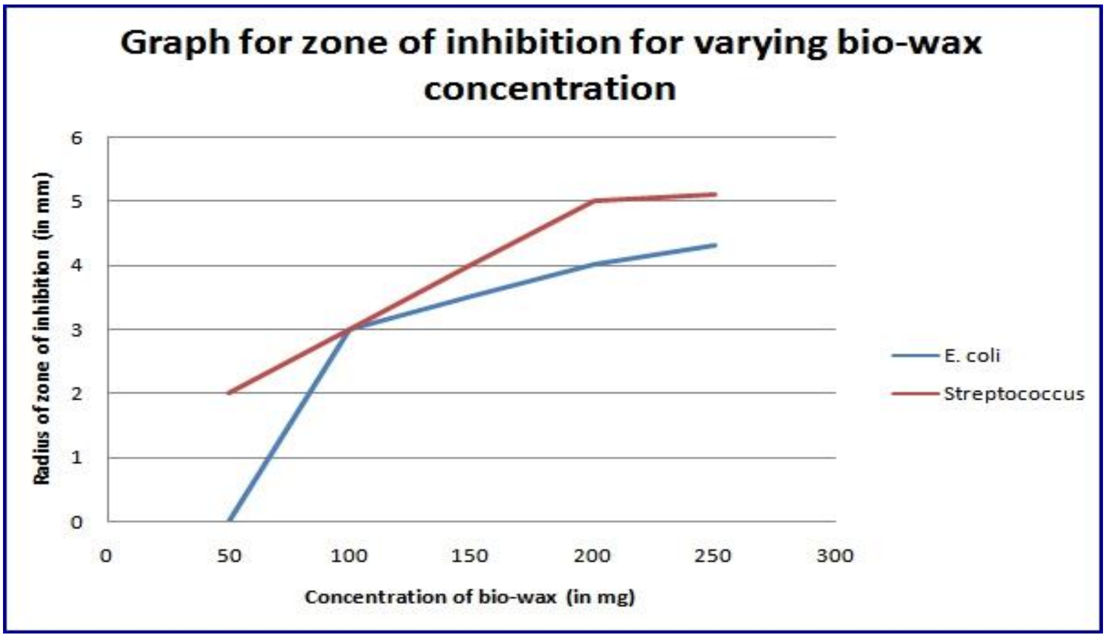

Fig 7: Graph for zone of inhibition

Heat sensitivity test

Sl. No.	TEMPERATURE (in °C)	HYDROPHOBIC PROPERTY	WAX INTEGRITY
1	30	Present	Intact
2	40	Present	Intact
3	50	Present	Intact
4	60	Present	Intact
5	70	Present	Intact
6	80	Present	Intact
7	90	Present	Intact
8	100	Present	Intact
9	110	Present	Starts to degrade
10	120	Present	Becomes semi-solid

Table 4: Hydrophobic property and integrity of wax at different temperatures

Removal of pigment from the wax

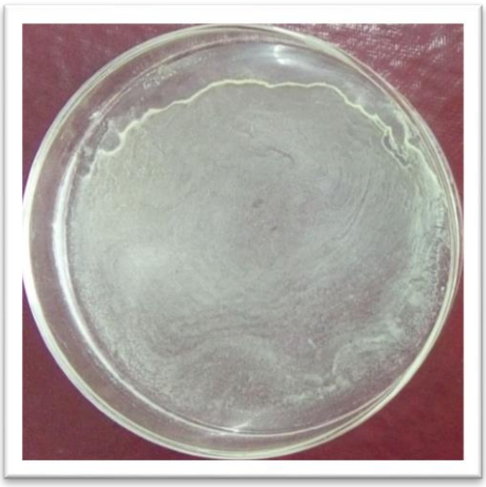

Fig 8: Normal solvent free bio-wax

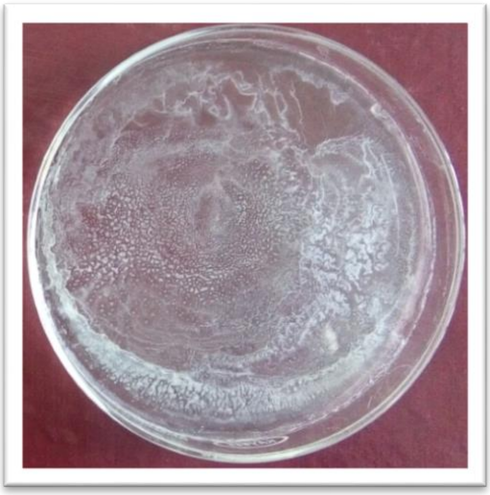

Fig 9: Solvent free bio-wax extracted from steam treated leaves

DISCUSSION

The present study shows that the plant *Colocasia esculenta* which is abundantly found not only in Assam and many parts of India but also all over the world possesses a highly hydrophobic layer of bio-wax on its leaves. The bio-wax can be extracted by from the leaves by organic solvent extraction method which in this case is chloroform. During the course of study quantitative analysis shows that the amount of bio-wax present per gram of leaf is equal to 0.116 gram approximately.

The extracted bio-wax shows hydrophobic property even when it is exposed to high temperatures which is around 95-100 degree Celsius. These characteristics make the bio-wax of *Colocasia esculenta* a suitable substance for coating papers to make them hydrophobic. The hydrophobic papers thus created may be then used to make biodegradable hydrophobic paper bags.

It is observed that the bio-wax extracted by the process of organic solvent extraction possesses a greenish pigment which might be unsuitable for future industrial use. The pigment was successfully removed by treating the leaves with steam (at 100°C for 30 minutes) prior to the process of extraction. The bio-wax extracted after steam treatment exhibited white colour rather than green which proved that the green pigment was successfully eliminated by the process of steam treatment. It is due to degradation of chlorophyll at high temperature provided by steam. The white coloured wax was subjected to a temperature of 60°C for 15 minutes to remove any water molecule that may have got trapped into the wax due to steam. The wax hence extracted was free from water molecules and it even loosed its white colour and became almost transparent. The heat did not denature the wax since it is stable even at 100 degrees Celsius.

The present study also shows that the bio-wax also possesses anti-bacterial property against *E.coli* and *Streptococcus* which can be an added advantage (All though the actual resistance against the bacteria of the bio-wax might be more than the result shown is the experiment.)

CONCLUSION

From the study it can be concluded that since the leaves of the plant *Colocasia esculent* (L.) is abundantly available and its bio-wax possesses high hydrophobic property, therefore the bio-wax could be used as a surface coating substance for papers which might be used for making biodegradable hydrophobic paper bags which not only will reduce the use of plastic bags up to a great extent but also will help in reducing pollution in the environment.

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