

Developing Hydrophobic Paper as a Packaging Material Using Epicuticular Wax: A Sustainable Approach

Jyoti Yadav, Manali Datta, and Vinod Singh Gour*

There is an urgent need for a biodegradable, hydrophobic material that can be used in developing packaging materials. In this preliminary study, epicuticular wax has been extracted from the leaves of *Calotropis procera* and *Alstonia scholaris* using various solvents (*i.e.*, ethanol, methanol, benzene, and acetone). The highest wax amounts were found to be 0.54 $\mu\text{g}/\text{cm}^2$ and 0.13 $\mu\text{g}/\text{cm}^2$ from *Alstonia scholaris* and *Calotropis procera*, respectively. The highest hydrophobicity (29.57%) was found to be in paper discs coated with epicuticular wax extracted with benzene from the adaxial surface of *Calotropis procera*.

Keywords: *Calotropis procera*; *Alstonia scholaris*; Organic solvents; Water gain

Contact information: Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, India;

*Corresponding author: vkgaour@jpr.amity.edu

INTRODUCTION

Easy biodegradability and an eco-friendly nature make paper a very good packaging material for food as well as non-food items. However, the hydrophilic nature of paper due to the presence of -OH groups limits its application in various sectors. Paper can absorb water from the environment or from food and become hydrated (Khwalidia 2010). Moisture migration can occur in paper by the diffusion of water vapor through the void spaces, as well as in condensed form through the fiber cell walls (Bandyopadthay *et al.* 2002).

In order to improve hydrophobicity, paper is often coated with various polyolefin materials. This coating results in a loss of biodegradable potential, and the paper becomes difficult to recycle. To overcome these problems and ensure sustainable development, there is an urgent need for a natural hydrophobic substance that can provide hydrophobicity to the paper at a low price. Various biodegradable natural components have been studied for this purpose, such as chitosan or chitosan/carnauba wax (Despond *et al.* 2005; Kjellgren *et al.* 2006) and whey-protein (Han and Krochta 2001). A cost-effective and efficient natural hydrophobic coating has yet to be found.

Plant epicuticular waxes are complex mixtures of long-chain aliphatic compounds, including primary alcohols, aldehydes, fatty acids, and alkyl esters (Walton 1990). The wax composition of various plant species varies with the season and age of the plants. The wax acts as a mechanical barrier between the environment and leaf tissue. Studies also found that higher wax content in cabbage heads reduced the effect of three species of insect pests (Znidarcic *et al.* 2008). Epicuticular wax present on the outer surface of plants acts as a protective barrier against biotic and abiotic loss of water and against UV radiation, bacteria, and fungi (Jurczak 1998). Plants have adopted this protective coating to survive in a terrestrial environment.

Epicuticular waxes from plant extracts are also used in the food and food-grade material industries. The principal roles of food packaging are to maintain food quality and to protect food products from outside influences and damage in order to maintain quality (Coles 2003). The goal of food packaging is to pack food in a cost-effective manner that satisfies industrial requirements and consumer desires, maintains food safety, and ensures a healthy and sustainable environment.

Calotropis procera (Ait.) R. Br. belongs to Asclepiadaceae family and is a wild perennial shrub that grows up to a height of 2 to 3 m in drought prone areas. Its leaves are 10 to 13 cm wide and 17 to 19 cm long (Rathore and Meena 2010). *C. procera* plant grows as a weed in hot and arid environments and survives due to the presence of hairs and a thick layer of wax on the leaf surface. It has medicinal properties, so it is used in the treatment of several diseases and disorders including eczema, asthma, digestion problems, vomiting, diarrhea, and coughing (Das 1996). The leaves of this plant are available throughout the year, but are reduced during summer (May-June) since leaves are shed as a xerophytic adaptation. The other plant is *Alstonia scholaris* (L.) R. Br. (Apocynaceae), a large evergreen tree 12 to 25 m in height having leaves arranged in whorls. Due to the presence of various secondary metabolites, this plant is considered a medicinal plant and is used in treatment of malaria, toothache, dysentery, and bacterial infection (Kaushik *et al.* 2011). Being a common evergreen, leaf availability is not a problem in this plant. Since leaf biomass is easily available, *C. procera* and *A. scholaris* were selected for the present investigation.

It is well known that surface wax of plant leaves play an important role in reducing the rate of transpiration. The leaf surface properties and composition may vary with environmental conditions (Dutta and Laskar, 2009). Considering this, preliminary experiments were designed and carried out to evaluate and compare the hydrophobicity of paper discs coated with epicuticular wax extracted from leaves of *C. procera* and *A. scholaris*. The epicuticular wax from leaf samples was extracted using organic solvents (benzene, acetone, methanol, and ethanol) to provide the hydrophobic coating on the paper discs.

EXPERIMENTAL

Materials

Leaf samples of *Calotropis procera* and *Alstonia scholaris* (Figs. 1a, b, c, and d) were collected in June 2013 from plants growing on the campus of Amity University Rajasthan (Jaipur, India). Analytical-grade pure solvents, namely acetone, benzene, methanol, and ethanol, were used to extract epicuticular wax from leaf surface.

Methods

Leaf weight and surface area

Leaf weight was recorded before wax extraction. The leaf area was calculated using graph paper.

Wax extraction

Epicuticular wax was extracted by exposing the adaxial surface to 10 mL of benzene for 60 sec at 25 °C. The epicuticular wax was then extracted from the abaxial surface in a separate petri plate by the same method. Benzene was allowed to evaporate,

and the wax was weighed with a BS323S analytical balance (Sartorius INISCO, New Delhi). Similarly, epicuticular wax from fresh leaves was extracted with other solvents (*i.e.*, acetone, methanol, and ethanol), collected, and weighed.

Quantification of wax

The amount of wax value was obtained using the following formula:

$$\text{Concentration of wax (w/A)} = \text{weight of wax/area of leaf} \times 100 \quad (1)$$

Hydrophobicity test

The dried wax was dissolved in each respective solvent used to extract the wax to prepare a solution of 8.0 µg/µL concentration. Five discs of Whatman filter paper No. 1 (78.5 mm²) were cut, weighed, immersed in 250 µL of a wax solution (concentration of 8.0 µg/µL), and again weighed. The difference in weight between the discs before wax loading (control) and after wax loading provided the total wax load. Since the water load was relatively small for each disc, five discs were batched and weighed and the load adjusted on a per disc basis. The wax-coated discs were dipped in water for 5 min and weighed, and compared with the water load in a disc without a wax coat. This comparison directly gives the hydrophobicity attained by the wax coated discs,

$$\% \text{ hydrophobicity} = \frac{W_1 - W_2}{W_1} \times 100 \quad (2)$$

where W_1 and W_2 are the water gained by untreated paper discs (g) and by wax-loaded paper discs (g), respectively.

RESULTS AND DISCUSSION

Wax Study in Leaves of *Calotropis procera*

Wax was extracted from the adaxial and abaxial surfaces of *Calotropis procera* leaves (Table 1). The wax concentration was found to be highest (0.13 µg/cm²) with the ethanol extraction on the adaxial surface, followed by benzene (0.07 µg/cm²). In the case of the abaxial surface extractions, the wax content in benzene, acetone, ethanol, and methanol were the same (0.03 µg/cm²).

The wax content varied from 0.07 µg/cm² to 0.13 µg/cm² on the adaxial surface, while on abaxial surface it was found to be 0.03 µg/cm² in *C. procera*. The wax content was found to be higher with chloroform extract than with n-hexane in *Mandevilla guanabara* (Cordeiro *et al.* 2011). The amount of wax extract varied from species to species in the genus *Mandevilla*. The mean wax content per unit of leaf area in the n-hexane extract was about 13 to 30 µg/cm² in *M. guanabara*. This is quite a high amount of wax in comparison to our present results, probably due to the exposure of both leaf surfaces to solvent at a time and extraction under gentle agitation.

According to Premachandra *et al.* (1993), the average epicuticular wax load was 0.014 µg/cm² and 0.009 µg/cm² for the adaxial and abaxial surfaces of *Sorghum bicolor*, respectively. Similar results have been reported in *Sorghum bicolor* (Premachandra *et al.* 1993), where epicuticular wax load measured on the adaxial surface was greater than on the abaxial surface at all three leaf positions in line bm-22.

Table 1. Concentration of Wax Extracted from Leaves of *Calotropis procera* and *Alstonia scholaris*

Plant sources			<i>Calotropis procera</i>	<i>Alstonia scholaris</i>
Sample No.	Surface	Solvent (10 mL)	$\mu\text{g}/\text{cm}^2$	$\mu\text{g}/\text{cm}^2$
1	Adaxial surface	Benzene	0.07	0.03
2		Acetone	0.03	0.03
3		Ethanol	0.13	0.06
4		Methanol	0.03	0.03
5	Abaxial surface	Benzene	0.03	0.54
6		Acetone	0.03	0.08
7		Ethanol	0.03	0.03
8		Methanol	0.03	0.06

Study in Leaves of *Alstonia scholaris*

Wax was extracted from the leaves of *Alstonia scholaris* separately from adaxial and abaxial surfaces and quantified (Table 1). The wax content was found to be highest ($0.06 \mu\text{g}/\text{cm}^2$) in ethanol extract from the adaxial surface, while the remaining extracts contain a wax content of $0.03 \mu\text{g}/\text{cm}^2$. On the abaxial surface, the highest wax amount was $0.54 \mu\text{g}/\text{cm}^2$ in benzene extract, followed by acetone ($0.08 \mu\text{g}/\text{cm}^2$), methanol ($0.06 \mu\text{g}/\text{cm}^2$), and then ethanol ($0.03 \mu\text{g}/\text{cm}^2$) extract. There is no earlier report on quantification of epicuticular wax from *A. scholaris*; however, composition of this wax has been reported by Dutta and Laskar (2009). According to them it contains 18 identified long chain (C17 -C34) n- alkanes.

Hydrophobic Surface Preparation Using Epicuticular Wax

The highest hydrophobicity was observed using wax extracted by benzene from the adaxial surface of *Calotropis procera* (Fig. 2). This was followed by the acetone extract from the adaxial and abaxial surfaces of *Calotropis procera* and acetone extract from the adaxial surface of *Alstonia scholaris*. An increase in the load of wax on the paper discs may lead to a more hydrophobic paper, which is suitable for commercial packaging. The epicuticular wax derived from leaves of *Digitaria sanguinalis* and *Festuca arundinacea* have been reported to be degraded by enzymes secreted by *Curvularia eragrostidis* conidia (Wang *et al.* 2008). This biodegradable nature of epicuticular wax will help in developing bioplastic.

Global demand and dependency on plastic is increasing (Bernard 2014). Its increased use has led to human and animal health concerns and environmental implications. Epicuticular wax yield can be improved by using adhesive treatment combined with solvent extraction (Buschhaus *et al.* 2007) from these plants.

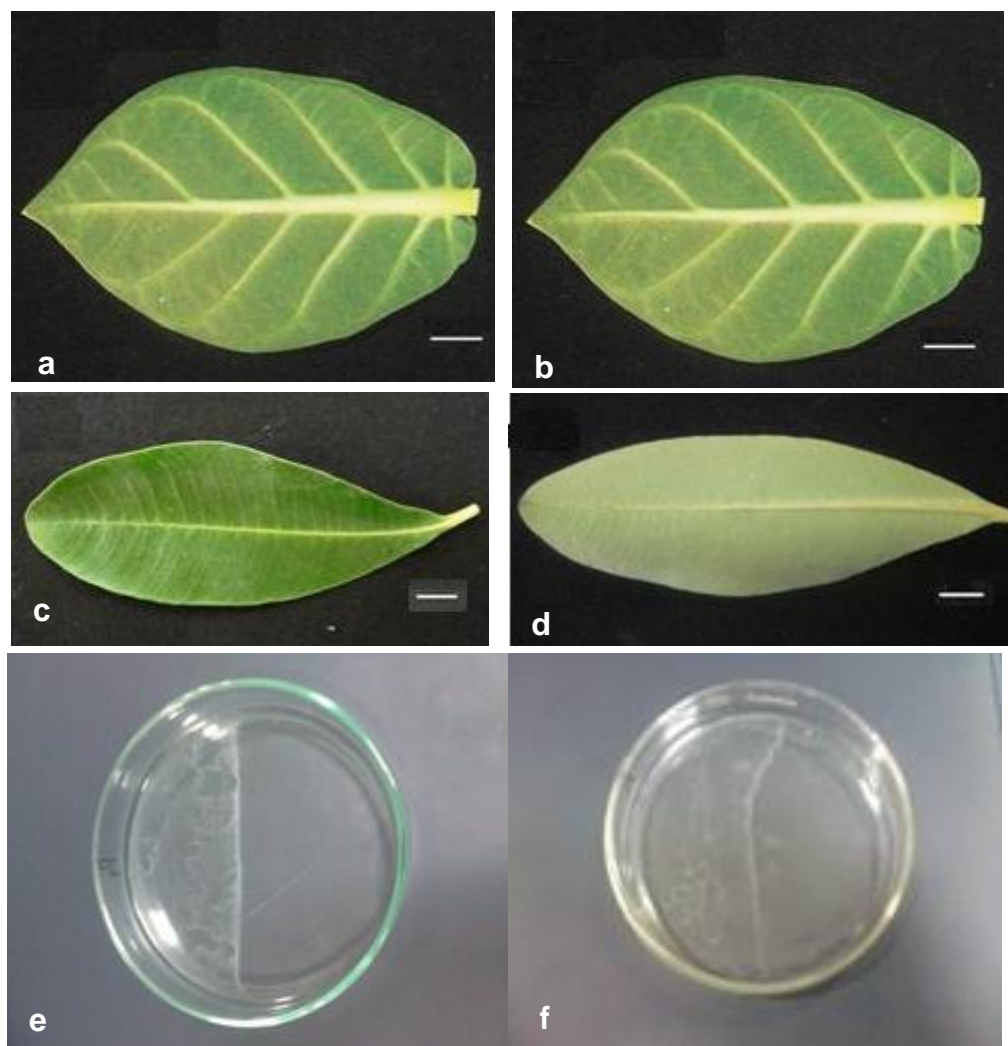


Fig. 1. (a) Adaxial surface of leaf of *Calotropis procera*, (b) Abaxial surface of leaf of *Calotropis procera*, (c) Adaxial surface of leaf of *Alstonia scholaris*, (d) Abaxial surface of leaf of *Alstonia scholaris*, (e) Wax extracted from adaxial surface of *C. procera* in benzene, and (f) Wax extracted from adaxial surface of *A. scholaris* in acetone. Scale bars in each image represents 1 cm

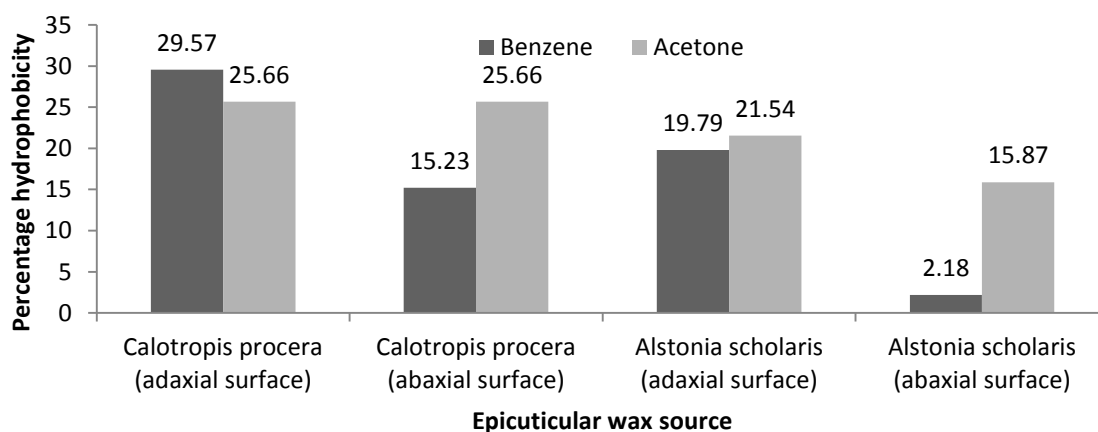


Fig. 2. Hydrophobicity attained by paper discs loaded with various wax extracts from leaves of *Calotropis procera* and *Alstonia scholaris*

Further study of chemical composition using chromatography and spectroscopy will help in understanding the nature of substances including toxicity in wax (Buschhaus *et al.* 2007; Olubunmi and Gabriel 2010). This information will lead to development of an economical, novel, biodegradable, and sustainably available source of hydrophobic material for the packaging industry.

CONCLUSIONS

1. In this preliminary study, the wax content was found to be highest ($0.13 \mu\text{g}/\text{cm}^2$) in ethanol from the adaxial surface of the *Calotropis procera* leaf, followed by benzene ($0.07 \mu\text{g}/\text{cm}^2$).
2. In the abaxial leaf surface of *Calotropis procera*, the wax quantity was found to be similar in all solvents used ($0.03 \mu\text{g}/\text{cm}^2$).
3. The abaxial surface of *Alstonia scholaris* has the highest amount of wax in the benzene extract ($0.54 \mu\text{g}/\text{cm}^2$).
4. Epicuticular wax isolated from the adaxial surface of *Calotropis procera* leaves in benzene provided 29.57% hydrophobicity to the paper surface.
5. Better yield of epicuticular wax with more hydrophobicity was found from *Calotropis procera* in comparison to *Alstonia scholaris*.
6. Further experiments should be performed to optimize the wax extraction, determine the chemical composition of the waxes extracted from *C. procera* and *A. scholaris*, and better analyze the hydrophobicity of the waxes. This will help ensure the sustainability of hydrophobic material for the packaging industry.

ACKNOWLEDGMENTS

The authors are grateful to Amity University Rajasthan, Jaipur for providing infrastructure facilities.

REFERENCES CITED

- Bandyopadthay, A., Romarao, B. V., and Ramaswamy, S. (2002). "Transient moisture diffusion through paper board materials," *Colloids Surf. A*. 206(1-3), 455-467.
- Coles, R. (2003). "Introduction," in: *Food Packaging Technology*, R. Coles, D. McDowell, and M. J. Kirwan (eds.), Blackwell Publishing, CRC Press, London, UK, pp. 1-31.
- Cordeiro, S. Z., Simas, N. K., Arruda, R. D. D., and Sato, A. (2011). "Composition of epicuticular wax layer of two species of *Mandevilla* (Apocynoideae, Apocynaceae) from Rio de Janeiro, Brazil," *Biochem. Syst. Ecol.* 39(3), 198-202.
- Das, B. B. (1996). *Rasraj Mahodadhi*, Khemraj Shri Krishnadas Prakashan, Bombay.
- Despond, S., Espuche, N., Cartier, N., and Domard, A. (2005). "Barrier properties of paper-chitosan and paper-chitosan-carnauba wax films," *J. Appl. Polym. Sci.* 98(2), 704-710.

- Dutta, M., and Laskar, S. (2009). "Hydrocarbons in the surface wax of the leaves of *Alstonia scholaris* (Linn.) R. Br.," *Orient. J. Chem.* 25(2), 437-439.
- Han, J. H., and Krochta, J. M. (2001). "Physical properties and oil absorption of whey-protein-coated paper," *J. Food Sci.* 66(2), 294-299.
- Jurczak, M. G. (1998). "Conifer epicuticular wax as a biomarker of air pollution: An overview," *Acta societatis botanicorum poloniae* 67 (3-4), 291-300.
- Kaushik, P., Kaushik, D., Sharma, N. and Rana, A. C. (2011). "*Alstonia scholaris*: It's phytochemistry and pharmacology," *Chronis of Young Scientists* 2(2), 71-78.
- Khwaldia, K. (2010). "Water vapor barrier and mechanical properties of paper-sodium caseinate and paper-sodium caseinate-paraffin wax films," *J. Food Biochem.* 34(5), 998-1013.
- Kjellgren, H., Gällstedt, M., Engström, G., and Järnström, L. (2006). "Barrier and surface properties of chitosan-coated greaseproof paper," *Carbohydr. Polym.* 65(4), 453-460.
- Premachandra, G. S., Hahn, D. T., and Joly, R. I. (1993). "A simple method for determination of abaxial and adaxial epicuticular wax loads in intact leaves of *Sorghum bicolor* L.," *Can. J. Plant Sci.* 73(2), 521-524.
- Rathore, M., and Meena, R. K. (2010). "Potential of utilizing *Calotropis procera* flower biomass as a renewable source of energy," *J. Phytol.* 2(1), 78-83.
- Walton, T. J. (1990). "Waxes, cutin and suberin," in: *Methods in Plant Biochemistry*, P. M. Dey and J. B. Harborne (eds.), Academic Press, San Diego, CA, pp. 105-158.
- Wang, F., Zhang, P., Qiang, S., Zhu, Y.-Z., and Xu, L.-L. (2008). "Effects of epicuticular wax from *Digitaria sanguinalis* and *Festuca arundinacea* on infection by *Curvularia eragrostidis*," *Australasian Plant Pathology* 37(1), 43-52.
- Znidarcic, D., Valič, N., and Trdan, S. (2008). "Epicuticular wax content in the leaves of cabbage (*Brassica oleracea* L. var. capitata) as a mechanical barrier against three insect pests," *Acta Agric. Slovenica* 91(2), 361-370.

Article submitted: March 29, 2014; Peer review completed: May 14, 2014; Revised version received: June 12, 2014; Accepted: June 13, 2014; Published: July 10, 2014.