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Physicochemical characters of bark exudates of *Lannea coromandelica* and its application as a natural fruit coating

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Abstract
The objective of the present study was to investigate the physicochemical and phytochemical properties of the gum exudates of *Lannea coromandelica,* develop a wax from the exudates to be used as a fruit coating and to check the variation of physicochemical characteristics of a moderate respiratory fruit after applying the waxes. The proximate composition was determined by using AOAC methods. Antioxidant capacity was determined using the free radical 2, 2- diphenyl- 1-picrylhydrazyl (DPPH) method, The total phenolic contents were measured using Folin-Ciocalteu reagent assay according to the method described by Singleton *et al*., (1999) with some modifications. The gum was observed under Scanning electron microscope (SEM) and FTIR was carried out for gum exudates. The effect of waxing was observed by applying the Hik wax on sour banana. Color, hardness, moisture content, titratable acidity and pH variation of sour bananas treated with wax were observed with time (Days).

Keywords: *Lannea coromandelica,* antioxidant capacity, FTIR, SEM, total phenol content

1. Introduction

Lannea coromandelica belonging to family Anacardiaceae, claims for its position among the best medicinal plants in the country. The plant was reported to contain various compounds like Carbohydrates including Gums, Proteins, Glycosides, Terpenoids, Polyphenols. This tree produces an exudates when a damage happens to the tree. The exudate is light brown to dark brown in color. In the early days of oozing the exudate has a liquid nature but with time the exudates become more solid nature.

Biopolymers are constantly grabbing increasing attention as these materials can be used to replace the traditional thermo-stable petrochemical polymers and have been considered for different applications including coatings, adhesives, engineering composites; electrical laminates etc. [1] due to their low toxicity, intrinsic biodegradability and low processing cost [2].

This study was conduct to identify the physicochemical properties of the the gum exudates of *Lannea coromandelica* and to develop a waxy coating from the above exudates to enhance the appearance, keeping quality and insect repellent properties. Initially a proximate analysis of the gum exudates were conducted to determine the moisture content, dry matter content, ash, mineral, crude protein, crude fat, crude fat and carbohydrate content in dry basis. The total polyphenol content and the antioxidant content were also quantified. Then the variation of colour, textural properties, moisture content, titratabe acidity and pH of sour banana treated with above wax were determined with time.

2. Materials and Methods

2.1 Plant material: The gum exudates of *Lannea coromandelica* (Hik gum) were collected from a garden in Kelaniya, Sri Lanka. Combs of *Musa sapientum* (Sour banana) were purchased from local market.

2.2 Preparation of homogeneous samples for proximate analysis

The gum exudates of *Lannea coromandelica* were first cut in to small pieces (approximately 0.5cm x 0.2cm x 0.1cm) and were kept in a LDPE cup at room temperature and was used for the proximate analysis in dry basis.

2.3 Checking the solubility in polar and non- polar solvent

1 g of thoroughly mixed sample was dissolved in each of the solvents: 95% ethanol, Diethyl ether and cold/ hot water. The solution was allowed to stand for 30 min and the solubility of the sample in the different solvents was determined qualitatively.

2.4 Determination of moisture content (Oven drying method)
The moisture content was determined according to the AOAC (Association of Analytical Chemists) official method 925.10.

2.5 Determination of crude protein content (Micro kjeldhal method)
Crude Protein Content was determined according to the AOAC method 978.04.

2.6 Determination of total fat (Majonnier ether extraction method)
Total fat was determined according to the AOAC official method 922.06.

2.7 Determination of crude fiber
The crude fiber content was determined according to AOAC 978.10.

2.8 Determination of Total Ash (Gravimetric Method*)*
Total ash content was determined according to the AOAC official method 923.03.

2.9 Determination of Metals
Dry ashing and the metals were quantified by Atomic Absorption Spectrometer (Thermo Scientific iCE 3000).

2.10 Determination of Carbohydrate
% Carbohydrate content m/m = 100 $100 - (\text{Total fat} + \text{Crude protein} + \text{Crude fibre} + \text{Total ash})$
(Dry basis)

2.11 Analysis of active groups of Hik gum using FTIR spectroscopy
For FTIR analysis, 2 g of Hik gum were dried and crushed. FTIR spectra of Hik gum were recorded on a Digilab Excalibur, series FTS 3000, coupled to an attenuated total reflectance (ATR) accessory equipped with a Zn Se reflection crystal. The spectra were acquired at room temperature with 32 scans/sample in the range of 44000 to 500 cm^{-1} at a resolution of 4 cm^{-1}, using Origin 8.0 software.

2.12 Total phenolic content (TPC)
Using Folin-Ciocalteu reagent assay according to the method described by [3] with some modifications.

2.13 Analysis of antioxidant activity
Using the DPPH method, the procedure followed the method of [4] with some modifications

2.14 Observing the gum samples under scanning electron microscope (SEM)
A hik gum sample, the gel formed by hik hum with hot water were observed under the SEM.

2.15 Preparation of wax from Gum exudates of *Lannea coromandelica* (Hik wax)
The solid exudates was cut in to small pieces by a stainless steel scissor and 3 g of cut pieces were dissolved in 60 mL of distilled water in a 100 mL pyrex beaker by heating water and the exudates mixture heating up to 70 ^{0}C while stirring thoroughly until all the added solid were disappeared. Then the solution was strained using a strainer (mesh size 0.1 mm) to remove undissolved dirt particles. Next the filtered solution was concentrated by slowly evaporating the water keeping the solution on the gas cooker. When the volume was reduced to 20 mL (when the solution is thick) the solution was taken out and allowed to cool to room temperature.

2.16 Applying the waxes to sour banana
Combs of matured sour banana were taken and 12 bananas of one comb are waxed with Hik wax and 5 o bananas from each comb were kept without waxing as controls for the treatment.

2.17 Analysis of properties of waxed sour banana vs. unwaxed sour banana
2.17.1 Color Analysis
Color of the waxed and control banana samples were determined using a reflectance Chroma-Meter (Model CR-400, Konica Minolta Camera Co. Ltd, Osaka, Japan) based on the L* (lightness or brightness), a* (redness/greenness), b* (yellowness/blueness) values, Chroma (C) and hue angle (H^0) according to the procedure described by Bai *et al*., (2013).

2.17.2 Determination of textural properties
Apparatus
Texture Analyzer (Brookfield CT3 50 K).

Method for banana with peel
Compression test was carried out for all the banana samples using probe (TA44). Test parameters were set as below. Test type: Compression test, Pre-test speed: 1.0 mm/s, Test speed: 1.0 mm/s, Posttest speed: 4.5 mm/s, Target type: Distance, Target value: 20.0 mm, trigger force: 5.0 g.

Method for banana flesh
Compression test was carried out for all the flesh of banana samples using probe (TA44). Test parameters were set as below.Test type: Compression test, Pre-test speed: 1.0 mm/s, Test speed: 1.0 mm/s, Posttest speed: 4.5 mm/s, Target type: Distance, Target value: 10.0 mm, Trigger force: 5.0 g.

2.17.3 Moisture Determination
Approximately 1g of thin sliced and small cuts of flesh of bananas (Waxed and control) were kept in the analytical moisture analyzer and the moisture was determined.

2.17.4 Tiitratable Acidity (TA)
Titratable acidity was determined according to the AOAC method [5].

2.17.5 pH value
The digital pH meter (Hanna Instruments HI84435-01 Mini Titrator and pH Meter) was calibrated against standard buffer solutions. The banana juice samples were mixed well to homogenize and the pH values were measured using the calibrated pH meter.

3. Result and Discussion
3.1 Results of proximate analysis of the stem exudates of *Lannea coromandelica*

Table 1: Results of proximate analysis of the stem exudates of *Lannea coromandelica*

Parameter	Hik gum
Moisture %	15.982 ± 0.518
Dry matter %	84.019 ± 0.518
Proximate analysis (g/100 g of dry waste)	
Crude protein %	1.489 ± 1.10
Crude fat %	2.78 ± 0.07
Crude fiber %	0.6 ± 0.22
Ash %	3.6 ± 0.25
Carbohydrates %	75.549 ± 0.35
Minerals (mg/100g) in dry basis	
Na	141.6 ± 0.006
K	305.0 ± 0.377
Ca	1517.5 ± 0.022
Mg	416.0 ± 0.010
Fe	16.0 ± 0.007
Heavy metals (%) in dry basis	
Pb	0.00

3.2 Results of antioxidant activity
DPPH radical scavenging assay: IC50 value for gum exudates of *Lannea coromandelica* was 9.327 ± 0.286 mg of Gallic acid equivalents per mL. According to the DPPH radical scavenging assay, IC_{50} value is the antioxidant concentration in the gums that shows 50% inhibition activity of the DPPH free radical and it is indicated as mg of Gallic acid equivalents per ml of gum. Low IC_{50} value indicates higher antioxidant activity whereas high IC_{50} value indicates low antioxidant capacity.

3.3 Total phenolic content (TPC) of Hik gum
Total phenol content in gum exudates of *Lannea coromandelica* was 0.078 ± 0.0150 ppm

3.4 FTIR result for the Hik gum

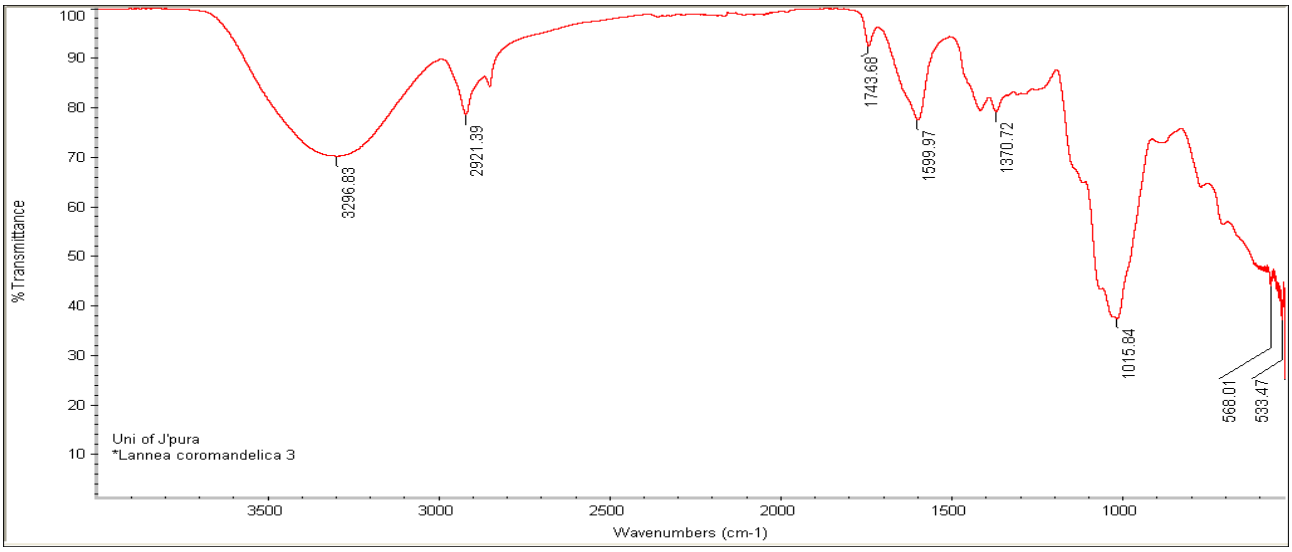

Fig 1: FTIR spectroscopy of Hik gum

The peak around 3200 – 3300 cm^{-1} indicates O-H bonds. The peak around 2900 -3000 cm^{-1} indicates C-H bonds. The peak around 1700 cm^{-1} indicates C=O bonds. The peak around 1000 – 1100 cm^{-1} indicates C-O bonds. By the peaks it can be suggested that Hik gum contains grater amount of carbohydrates.

3.5 Images of Hik gum by scanning electron microscope

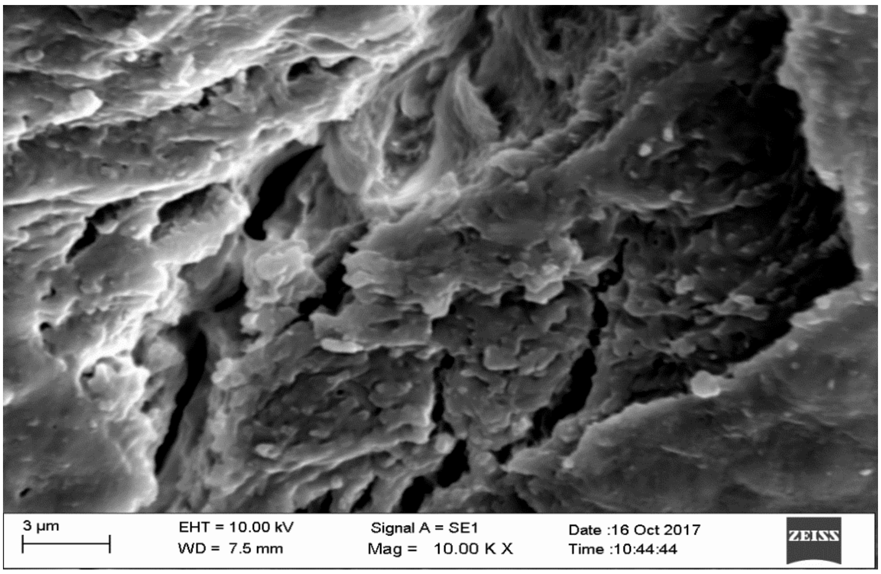

Fig 2: SEM image of Hik gum

SEM was carried out to analyze the microstructure of the gum exudate (Figure 1). When observing the gum surface some cracks were seen on the surface. The Gum consist of compact flake structures which are homogenously distributed and several layers can be seen. Small gaps are present between these layers and during dissolving in hot water, water get absorbed in to these gaps to form the wax. Earlier studies reported the diversity in shape and size of exudates particles [6] (Pachuau *et al*., 2012). In contrast the gum exudates of *Lannea coromandelica* have a layeric nature rather than separated particles.

3.6 Analysis of colour, textural properties, moisture content, titrable acidity and pH of sour banana treated with waxes made by Hik gum

Table 2: Color variation of sour banana waxed with Hik Vs Control:

Sample(Sour banana)	Day	L*	a*	b*	c	Hue angle
control	1	48.43 ± 7.26	-10.06 ± 0.25	29.10 ± 2.42	30.77 ± 2.38	3.9
control	2	49.30 ± 4.48	-8.76± 0.85	31.97 ± 2.83	33.17 ± 2.74	1.8
control	3	56.63 ± 10.15	-2.86 ± 1.51	41.30 ± 8.09	41.40 ± 7.92	0.31
Waxed with Hik	2	47.53 ± 0.56	-8.86 ±1.40	29.87 ± 2.55	31.13 ± 2.62	4.28
Waxed with Hik	3	65.30 ± 1.38	-6.033 ± 0.96	45.53 ± 3.19	45.97 ± 3.29	0.31

L*, a*, b* are measurements of brightness, redness and yellowness, respectively. L* value of sour bananas were increased with time. After 3 days the L* value of the bananas waxed with Hik gum were higher than bananas without waxing. Redness (a)* values of bananas waxed with Hik wax increased slowly than that of controls. b* values of both samples has been increased.

3.7 Variation of hardness of waxed sour banana with peel Vs control

Table 3: Variation of Hardness of waxed sour banana with peel Vs control:

Sample	Day 1	Day 2	Day 3
Control	1405.0g ± 170.0^{B}	1198.3g ± 57.5^{B}	645.0g ± 127.6^{C}
waxed with Hik	1268.3g ± 122.2 ^{B}	1280.0g ± 70.5^{B}	900.0g ± 131.1^{D}

A, B, C, D Values in the same column with different superscripts are significantly different at $P< 0.05$ level.

The results show that the hardness of peel of all the banana samples reduces with time. The hardness of peel of the sour bananas waxed with Hik gum is higher than the hardness of the controls.

3.9 Variation of Hardness of waxed sour banana flesh Vs control

Table 4: Variation of Hardness of waxed sour banana flesh Vs control

Sample	Day 1	Day 2	Day 3
Control	475.67g ± 6.03^{A}	435.00g ± 5.00^{C}	406.67g ± 2.89^{D}
waxed with Hik	475.67g ±6.03^{A}	481.67g ± 10.41^{A}	336.67 ± 2.89^{D}

A, B, C, D, E Values in the same column with different superscripts are significantly different at $P< 0.05$ level.

The results show that the hardness of flesh of all the banana samples reduces with time. The hardness of flesh of the sour bananas waxed with Hik gum is lower than the hardness of the controls. Generally while ripening the firmness of fruits decrease.

3.10 Variation of moisture content of sour banana waxed with Hik gum

Table 5: Variation of moisture content of sour banana waxed with Hik gum

Label	Waxed with Hik	Moisture % in Control
Day 1	59.22% ±0.27^{A}	59.22% ± 0.27^{A}
Day 2	61.30% ± 0.29^{A}	60.17% ± 0.15^{B}
Day 3	62.51% ±0.26^{A}	60.81% ± 0.07^{B}
Day4	64.69% ± 0.16^{A}	61.60% ± 0.31^{B}

A, B, Values in the same raw with different superscripts are significantly different at $P< 0.05$ level.

Moisture content of both waxed bananas and bananas without waxing were increased. Normally in any fruit the moisture content increases during ripening. Moisture content in the waxed bananas are higher than that of controls, because the evaporation of moisture in low in the waxed bananas due to the restriction of stomata due to waxing. Therefore the moisture retains inside the fruit. The reason for this consequence is plant mucilage materials capable to form microfilm over the leaf and this film is capable to act as an additional layer over the leaf while partially or fully covering the stomata. Hence evapotranspiration as well as the rate of respiration of produces can be reduced to a great extent. (Hershko *et al*., 1998).

3.11 Variation of Titratable acidity in sour banana waxed with Hik gum

Table 6: Variation of Titratable acidity in sour banana waxed with Hik gum

	Titratable acidity(mg/100mL)	
Sample	control	Hik waxed
Day 1	0.69 ±0^{A}	0.70 ± 0^{B}
Day 2	1.23 ±0.19^{A}	2.22± 0.20^{B}
Day 3	2.78 ±0^{A}	3.06± 0^{B}
Day 4	2.73 ±0^{A}	3.03±0^{B}

A, B, Values in the same raw with different superscripts are significantly different at $P< 0.05$ level.

A previous study states that the titrable acidity of all sour banana samples was increased with time. The titrable acidity of the waxed bananas shows a higher value than the controls. The fluctuations in TTA values of ripen banana pulp harvested between eight and 13 weeks followed a clear pattern in all portions (Figure 2). Generally, they increased between the eighth to ninth week although it was not significant (P>0.05) in banana from the lower portion. The TTA values were at its lowest at the 1lth week, then, increased drastically in all portions until the harvesting stage at the 13th week (P<0.01) [7].

3.12 Variation of pH in sour banana waxed with Hik Vs control

Table 7: Variation of pH in sour banana waxed with Hik Vs control

	pH	
Label	**Control**	**Waxed with Hik**
Day 1	5.51 ±0.01^{A}	5.51 ±0.01^{A}
Day 2	5.36 ±0.02^{A}	4.86 ±0.02^{B}
Day 3	4.61 ±0.01^{A}	4.53 ±0.005^{B}
Day 4	4.45 ±0.01^{A}	4.52 ±0.03^{B}

A, B, Values in the same raw with different superscripts are significantly different at $P< 0.05$ level.

A previous study states that the pH values were found to be fluctuating but the differences between the respective harvesting stage were highly significant ($P>0.01$). For all portions the values increased in fruits harvested between the eighth to ninth week and the decreased sharply at the 10th week. An increase in pH was observed at 12th week followed by a decrease at the 13th week [7].

4. Conclusion

Lannea coromandelica gum is mainly consist of carbohydrates and soluble in hot water. Waxes made by the gum can be applied on fruit surfaces to bring about several changes in fruits. The experiment is done for sour banana which is a medium respiratory fruit and the wax made by Hik gum gives a shiny appearance to the fruit surface once waxed and dried. The bananas waxed with Hik gum shows a ripening delay when compared with the bananas without waxing. The moisture content of Waxed bananas are always higher than the unwaxed bananas. Titrable acidity and the pH of waxed bananas and bananas without waxing shows a slight difference but the difference in those parameters is not large. Therefore this study confirms that the wax made by the stem exudates of *Lannea coromandelica* can be used to develop a wax that can be applied on fruit surfaces to bring about positive changes of fruits during ripening and no unacceptable outcomes are obtained from fruits by waxing.

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