

Comparative Analysis of Medicinal Plant Cuticular Waxes as Natural Fruit Coatings

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ABSTRACT

This study investigated the potential of plant cuticular wax, extracted from the leaves of Teak (*Tectona grandis* L.f.) and Oregano (*Plectranthus amboinicus* (Lour.)), to form an edible coating for the post-harvest preservation of tomato (*Solanum lycopersicon* L.). The cuticular wax was extracted efficiently using chloroform, and subsequent characterization was performed. Gravimetric analysis revealed that the *P. amboinicus* cuticular wax extract yielded a significantly high content of gravimetric Assessment of native hydrophobicity by contact angle measurement indicated that *T. grandis* leaves were superior, exhibiting a contact angle of on the abaxial surface. Gas Chromatography Mass Spectrometry (GCMS) analysis identified compounds in the *T. grandis* extract and compounds in the *P. amboinicus* extract, highlighting distinct chemical profiles. The edible coating suspensions were successfully formulated using the extracted cuticular wax along with pectin as a gelling agent and then applied to fresh tomatoes. Post-storage assessment of quality parameters, including weight loss, pH, and estimation of protein and carbohydrate content, demonstrated that the *P. amboinicus* coated tomatoes yielded better preservation results compared to the *T. grandis* coated and uncoated control groups. These findings confirm the viability of utilizing plant cuticular wax as a novel, natural, and effective coating material for extending the shelf life of fresh produce.

Keywords: *Tectona grandis* L., *Plectranthus amboinicus*, cuticular wax, Edible coatings

INTRODUCTION

The global challenge of food waste necessitates continuous innovation in post-harvest technologies. It is estimated that a substantial portion of fresh produce is lost between harvest and consumption, primarily due to spoilage, microbial degradation, and physiological deterioration. For climacteric fruits like the tomato (*Solanum lycopersicon* L.), quality and nutritional value diminishes rapidly after harvest due to high respiration and transpiration rates, leading to a severely limited shelf life and substantial economic loss for both producers and retailers. Extending the shelf life of fresh produce is therefore crucial for optimizing the supply chain, reducing waste, and ensuring consumer access to high-quality food. Traditional preservation methods often rely on refrigeration or chemical treatments, which can be energy intensive or raise concerns regarding chemical residues. Edible coatings offer an appealing, biodegradable, and environmentally friendly

alternative. These coatings are thin layers of material applied to the food surface, designed to function as selective barriers that regulate moisture loss and gas exchange (specifically and). By modifying the fruit's internal microenvironment, they effectively slow down respiration, delay ripening, minimize weight loss, and provide a protective shield against microbial attack. The efficacy of an edible coating hinges on the barrier properties of its constituent materials. Plant cuticular wax is a natural biopolymer covering the epidermis of leaves and fruits, serving as the plant's primary defense against non-stomatal water loss and environmental stressors. This intrinsic, highly hydrophobic nature makes cuticular wax an ideal raw material for developing sustainable and highly effective moisture-barrier films. This research focuses on extracting and utilizing cuticular wax from two distinct plant sources: Teak (*Tectona grandis* L.f.) and Oregano (*Plectranthus amboinicus* (Lour.)). These plants were selected for their robust structures and potential for yielding waxes with varied chemical

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

compositions and functional properties. The core objective of this study was to extract, characterize, and compare the physicochemical properties of the CW from these two species and subsequently evaluate the superior formulation's efficacy in preserving the critical quality attributes of tomatoes during extended storage.

MATERIALS AND METHODS

Sterilization of glass wares

Glass wares were soaked overnight in cleaning solution and washed thoroughly with running tap water. They were then cleaned with detergent solution, rinsed several times with tap water and finally in distilled water. It was sterilized in an autoclave at 15 psi for 20 minutes at 121 degrees Celsius.

Collection of plant sources

Healthy, mature leaves were selected and washed gently with distilled water to remove surface debris. All glassware utilized for the experiments was sterilized using autoclave and the collected leaves were transported to laboratory under aseptic conditions. The leaves of *Plectranthus amboinicus* were collected from Ashok Nagar, Chennai, Tamil Nadu. The leaves of *Tectona Grandis* were collected from Uthukottai, Tiruvallur, Tamil Nadu.



Figure 1: Leaves of *P.amboinicus*



Figure 2: Leaves of *T. grandis*

Physicochemical Characterization of Extracts

Hydrophobicity and Thermal Stability

The hydrophobicity of the native leaf surfaces was assessed by measuring the contact angle. A micro syringe was used to place a standardized micro droplet of distilled water onto both the adaxial (upper) and abaxial (lower) leaf surfaces. The angle formed between the edge of the droplet and the leaf surface were measured using a goniometer and image analysis software.

A heat sensitivity test was conducted to determine the thermal stability of the dried cuticular wax extracts by subjecting them to a temperature gradient ranging from to, monitoring for any signs of melting or loss of integrity.

Gravimetric Analysis

Gravimetric analysis was used to determine the total mass yield of the extracted cuticular wax. The solvent was completely evaporated from the filtered extracts, and the residual solid wax was weighed precisely using a calibrated electronic balance. This technique provided a direct, quantitative measure of the amount of wax successfully removed from the leaves over time.

Colorimetric Assay

The colorimetric assay method was used to quantify the extracted cuticular wax based on chemical oxidation. The wax sample was reacted with the oxidizing agent potassium dichromate, which causes a colour change. The resulting colour intensity was measured using a spectrophotometer and referenced

against a standard curve to confirm the accurate concentration of the wax.

CHEMICAL PROFILING

Phytochemical Screening

The chloroform extract of *Plectranthus amboinicus* and *Tectona grandis* were freshly prepared and various chemical constituents were analysed. The different chemical constituents tested include carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, terpenoids, phenols, steroids.

Test for Carbohydrate (Molisch's Test)

The leaves extract of about 2ml was taken in a clean dry test tube and 1 ml of Molisch's reagent was added to it. Few drops of concentrated sulphuric acid were added along the sides of the test tube. Presence of purple or reddish colour indicates the presence of carbohydrates.

Test for Tannins (Alkaline Ne Reagent Test)

The leaves extract of 1ml was taken in a test tube and 2 ml of 0.7 M NaOH, few drops of Folin Denis reagent was added to it. Formation of dark blue or greenish black colour indicates the presence of tannins.

Test for Saponins (Froth Test)

The leaves extract of 2 ml was taken in test tube and equal volume of distilled water was and shaken for 15 minutes. Foam formation indicates the presence of saponins.

Test for Flavonoids (Alkaline Reagent Test)

To 2 ml of leaves extract, 1 ml of 2 N sodium hydroxide was added. Yellow colourations observed in each extract indicate the presence of flavonoids.

Test for Alkaloids (Mayer's Test)

The leaves extract of 2 ml was taken in a test tube. 2 ml of concentrated Hydrochloric acid and few drops of Mayer's reagent was added. Greenish colour was observed which indicate the presence of alkaloids.

Test for Quinones

To 1 ml of extract, 1 ml of concentrated sulphuric acid was added. Red colour formation indicated the presence of quinones.

Test for Terpenoids (Salkowski Test)

The leaves extract of 0.5 ml was taken in a test tube. 2 ml of Chloroform and few drops of concentrated sulphuric acid was added. Appearance of reddish-brown colour at interface indicated presence of terpenoids.

Test for Phenols

The leaves extract of 1 ml was taken in a test tube. Few drops of Phenol Denis reagent and 2 ml of 15% sodium carbonate solution was added. Formation of green colour indicated the presence of phenols.

Gas Chromatography Mass Spectrometry (GCMS)

Gas chromatography Mass spectrometry (GC MS) an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated, identified and quantified. The cuticular wax samples were analysed using 7890B GC and 7000 MS. After the standard for the analysis was determined, the samples were injected into the GC-MS analyser. The graphs generated from this analysis were saved and interpreted to determine the possible compounds present in each sample.

Thin Layer Chromatography (TLC)

Thin layer chromatography is a semi-quantitative method and is based on the principle of separation. The separation depends on the relative affinity of compounds toward stationary and mobile phase. The compounds under the influence of mobile phase travel over the surface of the stationary phase. During this movement, the compounds with higher affinity to stationary phase travel slowly while the others travel faster and hence separation is achieved. After separation, the individual components are visualized as spots at a respective level on the plate. The use of thin layer chromatography (TLC) plate for extracts profiling, is to indicate the presence of numerous chemical constituents represented by different retention factor (RF) values, without revealing the



exact names for each compound. TLC plates were prepared by the application of a uniform layer of adsorbent (silica gel) on to 25mmX75mm glass slide. The plates were heated at 100°C for 15 minutes to activate the silica gel. After that, a capillary pipette was used to spot 1 to 2 ul of the sample on one end of the TLC plates leaving 1.5 cm from the bottom of the plates. The plates were inserted into the beaker containing the mobile phase (chloroform). After the development of the chromatogram, the compounds were located and the retention factor for each compound was calculated using the following formula:

$$\text{Rf value} = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the solvent}}$$

EDIBLE COATING FORMULATION AND APPLICATION

Pectin Extraction

Pectin, which served as the hydrophilic gelling agent, was extracted from orange peels using an acid hydrolysis method followed by precipitation with ethanol. The extracted pectin was dried and stored for use in the coating preparation.

Coating Suspension Preparation

The film-forming edible coating suspension was prepared by dispersing the extracted cuticular wax in an aqueous solution containing the pectin. The mixture was homogenized and stirred continuously to ensure the formation of a stable emulsion, resulting in a ready-to-use coating solution (CW-pectin film).

Sample Preparation and Coating Application

Fresh, uniform-sized tomatoes (*S. lycopersicon* L.) were selected at the mature green to breaker stage. The fruits were washed, surface-sterilized with a dilute sodium hypochlorite solution, and air-dried. The tomatoes were then divided into three experimental groups: Uncoated Control, *T. grandis* CW-Pectin Coated, and *P. amboinicus* CW-Pectin Coated. The coating was applied by briefly dipping the tomatoes into the respective suspensions, followed by air-drying under ambient conditions.

RESULTS AND DISCUSSION

Contact Angle Measurement

Contact angles (°) were measured on both sides of the leaves and it was found that contact angle on the abaxial surface was significantly higher than the contact angle of adaxial surface. Table indicates that abaxial surface of *T. grandis* has highest degree of contact angle and this may be due to the presence of trichomes on leaf surfaces. As the values of $\theta > 90^\circ$, the leaves are non-wettable.

Table 1: Contact angles of water on Adaxial and Abaxial leaf surfaces

LEAF SAMPLES	CONTACT ANGLE	
	Adaxial Surface	Abaxial Surface
<i>T. grandis</i>	98	110
<i>P. amboinicus</i>	97	106

Gravimetric Analysis

Cuticular wax extracted from teak leaves appeared as cloudy layer at top of the test tube. In *Oregano* leaf wax extract, two layers were separated and the transparent viscous layer at the top indicates the presence of cuticular wax.

Table 2: Determination of amount of cuticular layer extracted at Adaxial and Abaxial leaf surface

Leaf wax extracts	Adaxial surface of leaf	Abaxial surface of leaf
<i>T. grandis</i>	0.188	0.179
<i>P. amboinicus</i>	0.227	0.203

Colorimetric Analysis

In colorimetric analysis, the extracted cuticular wax content due to the colour change produced by reaction of leaf cuticular wax with acidic $\text{K}_2\text{Cr}_2\text{O}_7$ was found to be high in *Oregano* leaves.

Table 3: Cuticular wax content as determined by colorimetric method of analysis

Leaf wax extracts	O.D at 590 nm
<i>T. grandis</i>	0.145
<i>P. amboinicus</i>	0.198

Heat Sensitivity Test



Leaf cuticular wax of teak and oregano retains hydrophobic property and remains intact up to 110 F after which it starts to degrade.

Table 4: Hydrophobic property and Cuticular wax integrity at different Temperatures

Sr. No	Temperature (F)	Hydrophobic Property		Leaf cuticular Wax Integrity
		<i>T.grandis</i>	<i>P.amboinicus</i>	
1	30	Present	Present	Intact
2	50	Present	Present	Intact
3	70	Present	Present	Intact
4	90	Present	Present	Intact
5	110	Present	Present	Start to Degrade

Phytochemical Screening

The Phytochemical characteristics of the Cuticular wax investigated are summarized in the Table. The result reveals the presence of Carbohydrates, Tannins,

Flavonoids, Terpenoids, Phenols, Glycosides, Alkaloids, Steroids and also the Saponins in the Cuticular layer of wax. Phytochemicals are known as secondary plant metabolites and provide health benefits to humans.

Table 5: Phytochemical analysis of leaf cuticular wax extracts

Sr. NO	PHYTOCHEMICALS	INFERENCE	OREGANO EXTRACT	TEAK EXTRACT
1.	CARBOHYDRATES	Layer at the bottom of the tube	Positive	Positive
2.	TANNINS	Appearance of Bluish black color	Positive	Negative
3.	FLAVONIDS	Appearance of yellow color	Positive	Positive
4.	TERPENIDS	Appearance of red color ring	Positive	Positive
5.	PHENOLS	Appearance of dark green color	Positive	Positive
6.	QUINONES	Appearance of blood red color	Negative	Positive
7.	ALKALOIDS	Appearance of Yellow precipitate	Positive	Negative
8.	STEROIDS	Appearance of Brown precipitate	Positive	Positive
9.	SAPONINS	Foam formation	Positive	Positive

Gas Chromatography/ Mass Spectrometry Analysis

The present study was aimed to find out the bioactive compounds present in the chloroform extract of *T.grandis* and *P.amboinicus* leaf cuticular wax by using Gas chromatography and Mass spectroscopy. The peaks in the chromatogram were integrated and compared with the database of spectrum of known components stored in the GC MS library. The active

compounds with their retention time (RT) and chemical structure shows the presence of 12 bioactive compounds in the chloroform extract of *T.grandis* and 6 bioactive compounds in the chloroform extract of *P.amboinicus*. The compound 2,4-Di-tert-butylphenol has the highest match score of 75.3 in teak extract and the compound Phenol, 2-methyl-5-(1-methylethyl)- has highest match score of 92.6 in oregano extract. p-Cymene has match score of 91.9 in oregano extract.

Table 6: Compounds in cuticular wax Oregano extract by GCMS analysis

Sr. No.	Compound Name	Rt (Min)	Molecular Formula	Area	Match Score
1.	Octadecane, 3-ethyl- 5-(2-ethylbutyl)-	6.1924	C ₂₆ H ₅₄	2073203	61.8
2.	p-Cymene	6.4188	C ₁₀ H ₁₄	14306781	91.9
3.	gamma.-Terpinene	6.6786	C ₁₀ H ₁₆	7614358	83.4



4.	Phenol, 2-methyl-5- (1-methylethyl)-	8.3521	C ₁₀ H ₁₄ O	21682272	92.6
5.	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	9.6917	C ₁₅ H ₂₄	6177613	80.1
6.	1H- Cyclopropa[e]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl [1aR(1a.alpha.,7.alpha., 7a.beta.,7b.alpha.,)]-	10.5340	C ₁₅ H ₂₄	1937751	64.1

Thin Layer Chromatography

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel Gas stationary phase. The chromatogram developed with chloroform revealed the presence of three major compound at R_f value of 0.73, 0.61 and 0.34 in case of teak extract and presence of two major compound at R_f value of 0.81 and 0.69 in oregano extract. TLC profile of the leaf extract of cuticular wax from *T. grandis* R_f values: 0.73, 0.61 and 0.34.

ASSESSMENT OF TOMATOES QUALITY

Weight Loss

The weights of the samples were measured on every second day of storage period at room temperature. The overall weight loss percentage of uncoated tomato was found to be 0.043%, 0.0342 for teak extract coated tomato and 0.0297 for oregano extract coated tomato. The results indicate that oregano coated tomato has low weight loss percent compared to teak and control.

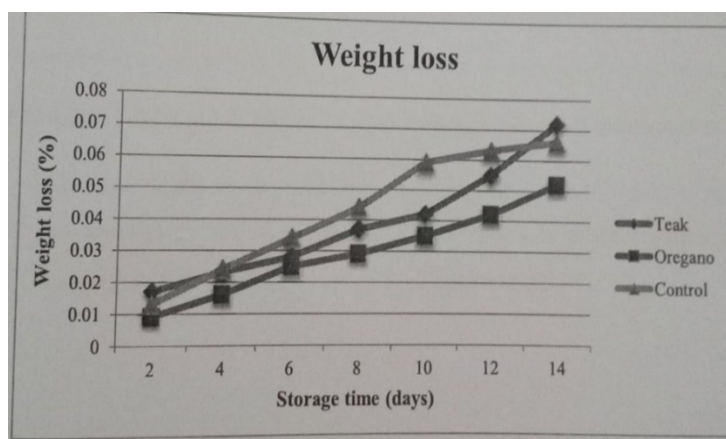


Figure 3: Percentage weight loss of tomato during storage

pH Analysis

In control, as the storage time increased pH also gets increased but in case of teak and oregano coated

tomato the pH is maintained constant during storage period which shows that coating is helpful in maintaining PH.

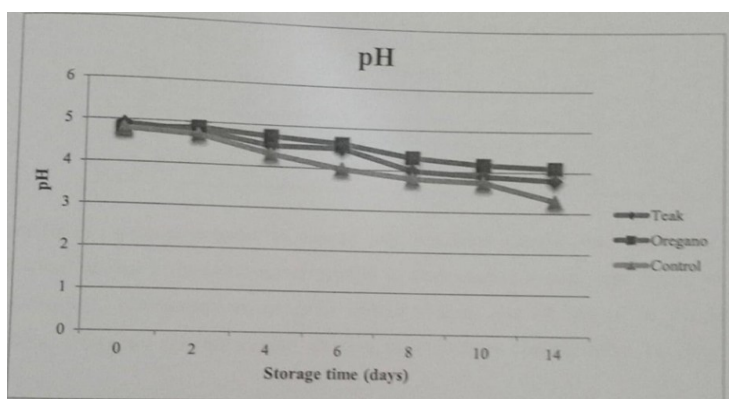


Figure 4: percentage weight loss of tomato during storage



Protein Estimation

Table 7: protein estimation of coated and uncoated tomato by Bradford method

Concentration of protein (μL)		Volume of distilled water (μL)	Bradford Reagent (mL)	Absorbance
Blank	-	1000	3	0
S1	100	900	3	0.01
S2	200	800	3	0.02
S3	300	700	3	0.036
S4	400	600	3	0.047
S5	500	500	3	0.052
TT	100	900	3	0.116
OT	100	900	3	0.156
UT	100	900	3	0.098

Amount of protein present in coated and uncoated tomato was estimated by Bradford assay. Concentration of protein in teak coated tomato was found to be 116.8μg/ml, 156.8μg/ml in oregano

coated tomato and 98.8μg/ml in uncoated tomato. Oregano coated tomato contain high protein content after 14 days of storage.

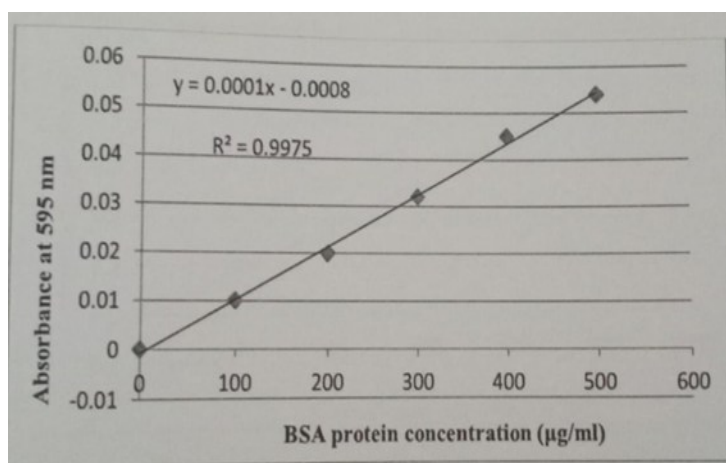


Figure 5: Standard curve for BSA

Carbohydrate Estimation

Table 8: Carbohydrate estimation of coated and uncoated tomato by Phenol Sulphuric acid method

Concentration of protein (μL)		Volume of distilled water (μL)	Volume of Phenol (mL)	Volume of sulphuric acid (mL)	Absorbance
Blank	-	1000	1	5	0
S1	100	900	1	5	0.21
S2	200	800	1	5	0.34
S3	300	700	1	5	0.52
S4	400	600	1	5	0.72
S5	500	500	1	5	0.96
TT	200	800	1	5	0.678
OT	200	800	1	5	0.932
UT	200	800	1	5	0.790

Amount of carbohydrate present in coated and uncoated tomato was estimated by Phenol sulphuric acid method. Concentration of carbohydrate in teak coated tomato was found to be 36.03µg/ml, 49.4µg/ml

in oregano coated tomato and 41.93µg/ml in uncoated tomato. Oregano coated tomato contain high carbohydrate content after 14 days of storage.

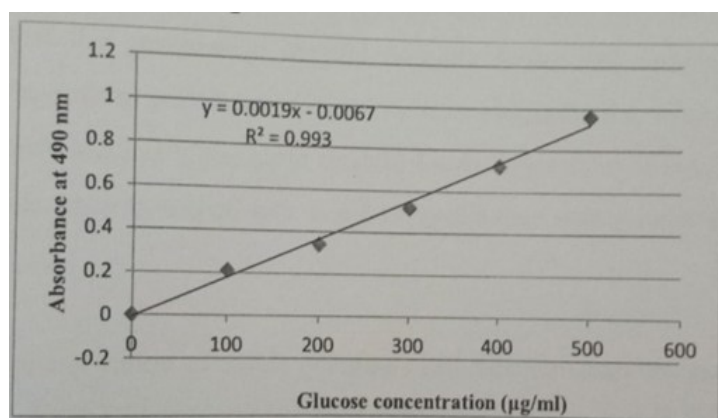


Figure 6: Glucose standard curve for carbohydrate estimation

CONCLUSION

This research successfully established a protocol for extracting and characterizing cuticular wax from *Tectona grandis* and *Plectranthus amboinicus* leaves, confirming their potential as source materials for edible coatings. The study concludes that the cuticular wax extracted from *Plectranthus amboinicus*, when formulated with pectin, is a highly promising natural coating material for extending the shelf life of fresh tomatoes. Its application resulted in the least weight loss and the highest retention of critical quality parameters (pH, protein, and carbohydrate) compared to the *T. grandis* formulation and the control. These findings provide strong evidence supporting the utilization of natural plant by-products, specifically cuticular wax, to create sustainable and effective post-harvest preservation solutions.

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HOW TO CITE: Jerin Joshna J, Niranjana G, Hanisha R, Joyce Hellen Sathya D, Comparative Analysis of Medicinal Plant Cuticular Waxes as Natural Fruit Coatings, J. Pharm. Sci., 2025, 1 (11), 589-597. <https://doi.org/10.5281/zenodo.17605040>

