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Chapter

The Phytochemical Composition of Medicinal Plants: Brazilian Semi-Arid Region (Caatinga)

Iago Almeida da Ponte, Murugan Muthuvel, Sudarsini Saravanabhavan and Stephen Rathinaraj Benjamin

Abstract

Carnauba wax, the most important vegetable wax under the economic and extracted from the leaves of the carnauba (*Copernicia prunifera* (Miller) H. E. Moore), is extensively applied in food due to its physiochemical characteristics with a majority of esters. *p*-Methoxycinnamic acid diesters obtained from the ceriferous powder of carnauba wax (PCO-C) have been associated with biological actions. However, being a versatile product, many types of research have been carried out seeking to expand the possibilities of applications of this raw material. Furthermore, different experimental studies on the pharmacological activities have also been undertaken in recent years and have tested various biological activities, such as hypolipidemic, hypocholesterolemic and hypoglycemic effects in mice. Therefore, in this book chapter, it is reviewing the development of a process of extraction of 4-hydroxycinnamic acid diesters of carnauba wax powder and investigates their biological actions and physical and chemical characteristics.

Keywords: Caatinga, *Copernicia prunifera*, *p*-methoxycinnamic acid, phytochemistry, biotechnological uses

1. Introduction

Caatinga is a Brazilian biome with a semi-arid climate, vegetation with small leaves and adapted to dry periods, as well as great biodiversity. This biome is found in areas of northeastern Brazil, in the states of Maranhão, Piaui, Ceará, Rio Grande do Norte, Paraiba, Pernambuco, Alagoas, Sergipe, Bahia and part of Minas Gerais. This whole area covers about 844,000 km², or 11% of the Brazilian territory [1]. This ecosystem is very important from the biological point of view because it has unique fauna and flora, formed by vast biodiversity, rich in genetic resources and vegetation consisting of species, woody, herbaceous, cactus, and bromeliads. It has 932 species of plants, 148 mammals and 510 birds, for example, and many of these species occur only in the Caatinga.

The main characteristics of the Caatinga are: Strong presence of shrubs with twisted branches and deep roots; Presence of cacti and bromeliads; Shrubs usually lose their leaves almost completely in times of drought (property used to prevent evaporative water loss). The leaves of this vegetation type are small in size; Caatinga soil has low fertility and is stony. Caatinga biodiversity supports various economic activities aimed at agroforestry and industrial purposes. Despite its importance, Caatinga vegetation is a type of vegetation adapted to the aridity of the soil and the scarcity of water in the region. They are classified depending on the natural conditions of the areas and different characteristics like strata: arboreal: with species ranging between 8 and 12 m in height; shrub: with species ranging between 2 and 5 m in height; herbaceous: with species below 2 m in height [2]. *Copernicia prunifera* (Miller) H. E. Moore (Arecaceae) family, a typical desert flora animal categories and exclusively located of areas through the Caatinga biome [3]. It is also known as "Tree of life", *carnauba*, *carandauba*, *carnaba*, *carnaubeira*, *caranaiba*, *carnaúva*, among others.

The carnauba is a palm tree very common in the northeast region whose main feature is its height, which can reach 15 m. The stem is straight and cylindrical, with a diameter that can vary from 10 to 20 cm and has thorns at the bottom. The tree provides fruits from November to March. They are greenish when young and turn purple when they mature. Its fruits are well used to feed farm animals. According to Brazilian specialized guidelines characterize the "pó de olho" and "pó de palha" wax powder as category A and B, respectively [4–6]. Meanwhile, the apical leaves have found lower chlorophyll content, type A wax has a pigmentation that shifts from white to light yellow and has a higher incentive than category B, which has a greenish-gray pigmentation.

Carnauba wax is derived besides the leaves regarding the *Copernicia prunifera* tree (**Figure 1**) and is made principally out of long-chain wax esters (80%), 20% contained fatty acids, fatty alcohols, and hydrocarbons [7–9]. Carnauba wax has the most maximum melting point conditions of all vegetable waxes and has been utilized in an assortment of items, including cosmetic and food products, nourishment items, and the paper area [9]. Additionally, this material is widely used in folk medicine, including the treatment of rheumatism and syphilis. However, carnauba



Figure 1. Copernicia prunífera *tree (from Fortaleza, Brazil)*.

wax is utilized as a stabilizer to different waxes, for example, beeswax to improve the melting point, taking into account expanded utilization of these waxes [10].

Despite that, a great deal of research has been carried out into an attempt to extend the chances of potential outcomes of utilizations of this crude material. With this goal, analysis has been finished streamlining customary applications and examining advancements, for example, the utilization of wax for the microencapsulation of flavors and as a wellspring of molecules following up on the avoidance and treatment of, diabetes, dyslipidemia, and others. The wax is an item to show the level of local consumption with extraordinary potential for use all through the Brazilian food production chain. Along these lines, it is imperative to experts in the food area to more likely comprehend this crude material so as to misuse its maximum capacity. In this way, this review talks about the utilization of carnauba wax in food ranging from the nutritional, phytochemical evaluation, ethnobotanical and biotechnological applications.

2. Nutritional and chemical composition

Carnauba wax consists of complex mixture regarding long-chain fatty acids, free alcohols, esters, aromatic acids, aliphatic acids, triterpene diols, cinnamic acids, proteins, and hydroxy acids and ω -hydroxycarboxylic free acids [10–14]. Recently, one triterpene carnaubadiol was also isolated and identified present in the leaves were reported. The inorganic compounds existing such as aluminum, copper, magnesium, zinc, manganese, calcium, iron, and sodium [15]. Recent studies continued to assess more genetic resource of carnauba wax while revealing a more extensive variety in the nutritional composition as described in the following sections.

2.1 Pectin

Paim et al. [16] extracted the pectin from the aqueous pulp extracts (APE) of *Copernicia prunifera* analyzed by chromatographic and spectroscopic methods. From this study, the pectin substance acquired from the pulp of unripe fruits of *C. prunifera* demonstrated an estimation of 2.9%. Additionally, the pectin was observed by using the absorption spectra by demonstrating several carbonyl groups in the form of esterified and carboxylate compounds. Furthermore, the thin layer chromatography (TLC) technique identified galactose, galacturonic acid patterns, and arabinose compounds, respectively. By using, ¹³C NMR spectroscopy analysis method, various forms of polymers were recognized in the pectic polysaccharides chain compounds including D-galacturonic acid (major signs), D-galactose (lower signs) and the peak molar mass (*M*pk) was determined by gel permeation chromatography of 0.6×105 g mol⁻¹. All these studies highlighted that pectin presence of higher molecular weight and a igher degree of esterification displaying improved performances.

2.2 Triterpenes

Almeida et al. [11], explored phytochemical investigation of hexane and ethanolic extracts carnauba wax (types 1 and 4) was analyzed and identified 16 dammarane-type triterpenes, with 13 newly categorized as $(24R^*)$ -methyldammara-20,25-dien- 3α -ol and a mixture of alkyl (24R^{*})-methyldammar25-en-20-ol- 3β -carboxylates, and 3 triterpenes such as carnaubadiol,(24R^{*})-methyldammara-20,25-dien- 3β -ol and (24R^{*})-24-methyldammara-20,25-dien-3-one. Furthermore, fatty alcohols such as docosanol, eicosanol, and hexacosanol, tetracosanol as well as four sterols

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(campesterol, cholesterol, sitosterol and stigmasterol) were detected and identified. These finding isolated compounds were characterized by using Infrared (IR) spectra and confirmed by classical chromatographic techniques such as gas chromatographyflame ionization detections (GC-FID), ¹H and ¹³C nuclear magnetic resonance (NMR) methods.

¹H and ¹³C NMR spectroscopy techniques have been applied for structural elucidation of dammarane triterpenoids [basic skeleton as carnaubadiol] in carnauba wax powder obtained from the leaves of *Copernicia cerifera* [13]. Totally four types of triterpenes were identified from hexane extract of carnauba wax. Four of these compounds were, structure1, (24RŁ)-24-methyldammara-21,25- diene-3[°]-ol, structure of 2 and 3 was distinguished as (24RŁ)-24-methyldammara-25-ene-3-one. Furthermore, the structure of 4, illustrated as (E)-25-hydroperoxydammar-23-ene-3[°],20-diol. The chemical composition analyzed after successive column chromatography using silica gel hexane followed by ethanol at room temperature, respectively.

2.3 Proteins

Cruz et al. [12] isolated the wax protein from "Carnauba" wax and the samples accomplished by SDS-Tricine-gel electrophoresis technique. It showed relative molecular masses of 26,000 (β -1,3-glucanase) and 24,000 Da (class III chitinase), respectively. However, these proteins have been involved in the resistance systems of plants against insects and pathogens. In addition, the authors found that proteins segregated from the different portions of carnauba wax have antifungal enzymatic action. These chemicals, chitinase and β -1,3-glucanases, can hinder early development of organisms and modify hyphal (threadlike fibers like mycelium of parasites) morphology of growths developing within the proteins.

2.4 Ethnobotanical study of carnauba wax

2.4.1 Antioxidant activity

Phenolic bioactive and polyphenolic compounds occur normally and significant segments of the human diet due to their antioxidant capacity that decreases oxidative stress-inducing cellular damage associated with severe pathologies such as cardiovascular, neurodegenerative diseases and cancers [17]. The simplest bioactive phytochemicals containing a single substituted phenolic ring, like cinnamic acid and caffeic acid. Cinnamic acid is a naturally proceeding organic acid in plants, has low toxicity and a broad spectrum of biological activities. However, cinnamic acid derivatives comprise a series of trans-3-phenylpropenoic acids which differ in their substituents on the aromatic ring. The presence of a benzene ring and a low unsaturated hydrocarbon chain determines its low polarity and solubility in water. The most common cinnamic acid derivatives in plants are p-coumaric, caffeic, and chlorogenic acids and hydroxybenzoic and hydroxycinnamic acids, respectively [18].

Claisa et al. [19] studied the antioxidant activity by ABTS and FRAP methods and *in-vivo* cellular antioxidant activity assay. The antioxidant activity of ethyl acetate and hexane extracts of *p*-methoxy cinnamic diester (**Figure 2**) (PCO-C) showed the values $107.27 \pm 3.92 \mu$ M Trolox/g and $73.3 \pm 1.83 \mu$ M iron sulfate/g, respectively. From these results showed significant antioxidant activity values due to the presence of derivative of cinnamic acid compounds [20]. In addition, the *in-vivo* antioxidant activity showed lower ROS values in PCO-C alone (50 and 250 μ g/ mL). Accordingly, PCO-C did not produce any cellular oxidation significantly it produces low level of ROS, because of the oxidation of lymphocytes endured with

 H_2O_2 . However, PCO-C had a best antioxidant effect in high dose level (250 µg/mL) similar of Trolox (80 µM) and found an oxidation inhibition capacity in human peripheral blood lymphocytes (HPBLs). According to the authors, it is revealed that antioxidant activates arise from *p*-methoxy cinnamic diesters presence of phenolic compounds PCO-C. Therefore, the presence of these excellent antioxidant potentials of produce reflects its ability to deliver bioactive substances that neutralize reactive oxygen species (ROS) and scavenge free radicals produced by oxidative stress.

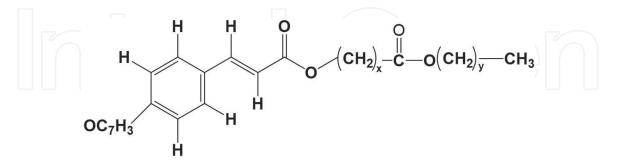


Figure 2.

Chemical structure of p-methoxy cinnamic acid diesters.

Rufino et al. [21] reported that significant antioxidant activities of polyphenolrich extracts from tropical fruits and dry fruits especially carnauba, both DPPH, ABTS, FRAP, β -Carotene oxidation methods and total phenolic contents were performed. The antioxidant activity of the methanolic and ethanolic extracts of fresh fruits of carnauba found decreased values DPPH values 3549 ± 184 g fruit/g DPPH and increased ABTS values $10.7 \pm 0.2 \mu mol Trolox/g$, FRAP values $15.5 \pm 0.4 \mu mol$ Fe₂SO₄/g and high β -Carotene bleaching values found 87.7 ± 2.7(% O.I) and extractable polyphenols values 830 ± 28.3 mg GAE/100 g, respectively. Additionally, the bioactive compounds values (mg/100 g fresh matter) such as, vitamin-C (78.1 ± 2.6) , total anthocyanins (4.1 ± 0.1) , yellow flavonoids (66.4 ± 2.3) , and total carotenoids (0.6 ± 0.2) , chlorophyll (4.2 ± 0.2) , respectively. According to the authors, this study provides an adaptation of ABTS, DDPH, FRAP and β -carotene bleaching methods, along with an evaluation of the compounds related to antioxidant potential. The results showed promising perspectives for the exploitation of non-traditional tropical fruit species with considerable nutritional properties and antioxidant capacity.

2.4.2 Anti-microbial and anti-fungal activities

The prevention of the decomposition and assurance of the food safety can be attained by the use of compounds that act as preservatives of foodstuffs, by presenting antimicrobial properties, preventing the degradation by enzymatic and non-enzymatic reactions. The identification of new sources of compounds and increased demand for the prospection that has antimicrobial properties. However, its realization depends on some conditions, including solubility of the food and pH. Cinnamic acid derivatives (CAD) such as trans, hydroxy and methoxy cinnamic acid, 4-chlorocinnamate, cinnamic acid derived from oxazoline ions, antimicrobial and antifungal activities. These substances showed strong activity against Grampositive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative bacteria (*Escherichia coli* and *Proteus vulgaris*).

Gonçalves et al. [22] studied the effects on different concentration of carnauba wax (1, 2, 3 and 4.5%) on the brown rot, produced by *Monilinia fructicola* (G. Wint.) and Rhizopus rot, developed by *Rhizopus stolonifer* (Erhenb.:Fr.) *in vitro*

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evaluation on infection of nectarine and plums. The authors, distinguished that no mycelial development of *M. fructicola* at any wax concentrations in post-contamination tests, however, *R. stolonifer* was totally restrained via by carnauba wax at all concentrations except at 1%. Additionally, *in vitro* evaluation for both *M. fructicola* and *R. stolonifera* no germination occurred of spores at any carnauba wax concentrations. There was 50% inhibition observed in spore germination for *M. fructicola* by utilizing 9% carnauba wax concentrations and covered with nectarines 90% for *R. stolonifera*. The carnauba wax concentrations (4.5% and 9%) were applied to the protections with essentially reduced frequencies of both diseases in nectarines and plums. Nevertheless, the utilization of wax control was ineffective after infection by both diseases.

According to Jo et al. [23] studied quality and microbial safety of Fuji apples coated with CSW/LO (Carnauba-shellac wax nanoemulsion containing lemongrass oil). In this work, carnauba wax incorporated into shellac wax (Carnauba-shellac wax) with essential oils like lemongrass oil coating formulations and their effects on the coating and shelf life of the Fuji apples were evaluated. Total soluble solid content to titratable acid ratio, hardness, weight loss and color, sensory quality and microbial growth of fresh Fuji apples were studied during 5 months of storage at room temperature. According to the authors, results showed that carnauba extracts incorporated to shellac wax-based coatings together with lemongrass oil successfully maintained the firmness and color of coated freshly harvested apples in comparison with uncoated control samples, which presented severe texture softening. During storage conditions, the hardness of the uncoated apples exhibited the lowest conditions by 3.3 N and the weight loss was found by 7.7%. Interestingly, the weight loss was found to be 5.2% and the hardness of the coated apples did not change at any conditions, respectively. The total soluble solids and titratable acidity revealed that not significantly different between coated and uncoated apples.

Hence, the application of CSW-LO coated apples had better sensory scores with the sensory acceptability threshold for any attributes evaluated. In addition, the total aerobic bacteria population on the coated apples were deteriorated (1.4 log CFU/g) compared with uncoated apples after 5 months of storage. Additionally, the population of yeast and molds of the uncoated apples were found 2.2 log CFU/g after 5 months of storage, although yeast and molds were not detected on the coated apples, respectively. The results achieved demonstrate the feasibility of the addition of carnauba wax coating formulations for increasing the nutritional value of fresh apples without compromising their fresh-like quality attributes.

2.4.3 Antifungal activity

Different kinds of antimicrobial proteins have been purified from plants such as, β -1,3-glucanases, chitinases, ribosome-inactivating proteins, thionins, and defensins. In this case, β -1,3-glucanase and chitinase separated from type B wax of *Copernicia cerifera*, has revealed antifungal activity against phytopathogenic fungi medium [12]. Based on the results, the yeast *Saccharomyces cerevisiae* showed the patterns of growth for *Fusarium oxysporum* and *S. cerevisiae* in the presence of different fractions obtained from "Carnauba" wax and in control medium. Plant chitinases and β -1,3-glucanases are known as antifungal hydrolases since they inhibit fungal growth in model experiments by using on agar plates and in liquid media. The presence of isolated proteins by using SDS-Tricine-gel electrophoresis, and showed inhibit early growth of all fungi in their fractions in agar plates. Based on these results, defense proteins like chitinase and glucanases which appear to inhibit the early growth of all fungi and cause hyphal morphological alterations for fungi growing in the presence of these proteins (relative molecular masses of 26,000 and 24,000 Da) as compared with growth on control medium. According to the authors, *Copernicia cerifera* wax contains defense proteins ability to inhibit fungal growth. Moreover, the fungal cell walls together with β -1,3-glucans, recommend a protective role for these hydrolases.

2.4.4 Hypercholesterolemic activity

Paim et al. [24] first time studied *in vivo* study of the antihypercholesterolemic effect of the aqueous pulp extracts (APE) from the C. prunifera (APE 150 and 300 mg/Kg b.w./day) were directed to hyperlipidemic mice for 90 days. It showed that APE was promising results with lipidemic alterations were effective in both models causing significant changes in the values of total cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C and triglycerides in serum. Nevertheless, it showed no renal toxicity and liver toxicity parameters (enzyme AST) and renal metabolites (urea and creatinine) to animals. Additionally, APE in high doses showed no renal and liver toxicity to animals. Despite the fact that the histological results bring about liver of mice treated with APE shows that doses (150 and 300 mg/Kg b.w./day) were not ready to alter the inflammatory procedure contrasted with the standard diet (SD) fed mice, all things considered, that better reaction opposing the hypercholesterolemic diet (HD). Besides, it was recognized the reduced intensity of inflammation in higher dose receiving present in the group. According to these results revealed that aqueous fruit pulp extracts of carnauba reduced hypercholesterolemia showing a potential preventive effect against cardiovascular diseases without side effects cause.

Furthermore, in this investigation, Filho et al. [25] revealed that the extract of PCO-C (100 mg/kg) found that productive in decreasing total cholesterol (TC) and triglyceride (TG) levels in both dyslipidemia induction models in hypercholesterolemic mice. This effect ascribed to the presence of high dietary and crude fiber content and antioxidant potential of PCO-C. Histological investigations demonstrated that PCO-C has no hepatotoxic impact and diminishes hepatic steatosis in animals that expended hyperlipidemic ration. In this manner, it was inferred that PCO-C separated from *Copernicia prunifera* may be helpful in the treatment of hyperlipidemia and atherosclerosis. Additionally, the authors highlighted that the results obtained in animals treated with PCO-C were pivotal compound had therapeutic potential in the prevention and treatment of diseases related with the metabolism of carbohydrates and lipids.

2.4.5 Hypoglycemic activity

Rodrigues et al. [26] studied that oral administration of *Copernicia cerifera* in glibenclamide diabetic mice at doses of 100 and 150 mg/kg bodyweight for 21 days.

According to the authors, the findings of this study indicated that 10% isopropanol in heptane leaf extract of Carnauba powder extract had antidiabetic activity when using therapeutic doses (100 and 150 mg/kg body weight (b.w.)). However, after treatment with 150 mg/kg b.w dose was found to be effective in significantly controlling blood glucose levels (p < 0.05), when compared to the reference drug glibenclamide. The observed hypoglycemic activity could be associated with the phytochemicals present in carnauba wax powder. These finding results suggested that PCO-C leads to diabetes by protecting beta-cells from oxidative damage. Indeed, the presence of the antioxidant effect of PCO-C may improve the pancreatic beta-cells to inhibit glucagon secretion and release more insulin levels. Finally, this study clearly shows that the leaf extract of carnauba wax powder possesses possible hypoglycemic activity in alloxan-induced diabetic mice.

2.4.6 Antiprotozoal activity

Almeida et al. [22] identified antiprotozoal metabolites from the Copernicia prunifera (Miller) showed in vitro action against promastigote and amastigote types of Leishmania infantum, trypomastigote forms of Trypanosoma cruzi. Among the separated dammarane-type triterpenoids, from the hexane and ethanolic extracts 'carnauba' wax (type 1 and 4) indicated antiprotozoal activity against promastigotes of Leishmania *infantum*, which showed the values of IC_{50} of 46.2 mM in tested extract 1. Besides, considering the positive controls miltefosine and benznidazole, the obtained results recommended that the impact of tested extract 1 against L. infantum is less noticeable than that observed against trypomastigotes of Trypanosoma cruzi. The intracellular amastigotes of L. infantum were sensitive to three types of triterpenoids, with IC₅₀ estimations of 7.8, 37.6 and 51.9 μ M, individually. Regardless of triterpenoid 2 and 3 exhibited absence of activity against the extracellular promastigotes, they killed the intracellular structures with selective index (SI) esteems more than 5.3 and 3.8, respectively, proposing a conceivable commitment of macrophages at the end of parasites. Notwithstanding, the tested extract 1 and 2 were less effective than standard drug miltefosine, which showed an IC_{50} of 16.4 μ M. Finally, this study provided useful information about the antiprotozoal activity of 'carnauba' (C. prunifera) wax as well as the identification of compounds responsible to this potential.

2.5 Pharmaceutical processing

Carnauba wax has a wide scope of utilizations and, as a result, is industrially accessible in an assortment of blends. Carnauba wax utilized in fruit and vegetable coating is constantly connected as a microemulsion made with unsaturated fatty acids and an essential counterion [27]. These produce an anionic emulsifier where the carnauba wax is dispersed. In addition, various types of unsaturated fats utilized incorporate oleic, linoleic, palmitic, myristic or lauric acids. The fundamental counterion may be hydroxides of sodium, potassium salts, ammonium, morpholine [28] and triethanolamine [29]. Since carnauba wax is just utilized as a fruit coating in the mix with different substances, the adequacy and consistence of different substances should likewise be considered.

Nart et al. [30] studied carnauba wax demonstrates a pivotal reinforcement to support the sustained release of high soluble medications in relationship with Ethocel[™] (EC) and Kollicoat[®] SR 30D utilizing reservoir and matrix systems, respectively. However, melt granulation of the medication with carnauba wax was connected as an intermediary of the key to sustained release mini-tablets, utilizing captopril (6.25 mg/mini-tablet) and metformin hydrochloride (15.0 mg/minitablet) as profoundly soluble model drugs. In addition, investigating the impacts of carnauba wax as a granulating excipient in the arrangement of mini-scale tablets, unmistakably the excipient diminished the contact of the drug particles with the disintegration medium, decreasing the release rate of the drugs and submitting the disintegration of the smaller than usual tablets. In this manner, it was seen that the melt granulation technique with carnauba wax improved the rheology of the considered drugs. The carnauba wax added to diminish the diffusion rate of the drug to the medium by expanding the hydrophobicity and lessening the disintegration rate of the structure of the measurements, impeding water dispersion a while later. The blend of carnauba wax with the EC at 50% indicated promising profiles for sustained release formulations.

Neto et al. [31] developed methionine microencapsulated with lipid matrix using carnauba wax by the melt emulsification technique. Different compositions of

carnauba wax: methionine (MEM 2:1 and MEM 4:1) were prepared and compared with pure methionine. In addition, scanning electron micrograph results showed no invade by ruminal microorganisms of both formulations after *in situ* testing. Taking into account that carnauba can apply an impact of protective on amino acids by covering their degradation in the rumen due to its hydrophobic distinguishing. In addition, it is a characteristic result of low degradability because of its concoction structure in unsaturated fats, and it is easy to obtain. Notwithstanding, carnauba wax sustained its thermal degradation temperatures and typical melting after the microencapsulation procedure, this diminishing in thermal stability of methionine is not because of its collaboration with the wax however most likely is because of the forces of intermolecular level (presences of hydrogen bridges) among the methionine particles. Finally, the formulation MEM 4:1 showed that promising results of the lower level of thermal degradation and higher yield and efficiency of microencapsulation.

2.5.1 Post-harvest storage

The valuable role of carnauba wax is outstanding for improving shelf life and supporting postharvest quality of a few fruits, for example, mango [32], avocado [33] and mamey sapote organic product [34]. Barmen et al. [35] studied pomegranate (Punica granatum L., cv. Mridula) fruits were treated with putrescine, carnauba wax and putrescine + carnauba wax combination prior at 2°C cold storage temperature. Further, carnauba wax is additionally stated to reduce the improvement of chilling injury (CI) manifestations. Respiration rate of stored fruits has been discovered expanded with the progression of the capacity period under every one of the medicines. Up to the fifteenth day of capacity, there was no critical contrast in breath rate in the organic products treated with polyamine like putrescine (PUT), carnauba wax and their mix. The low breath rate in carnauba wax treated organic product ascribed because of diminished gas exchange and thusly low oxygen accessibility to the natural product tissues for breath. The utilization of carnauba wax gave higher maintenance of fruit solidness, most likely because of the less drying out happened and furthermore to a slower degradation of cell divider segments. In this way, in control group and carnauba wax treated pomegranate fruits, the expansion in juice recuperation after 30th day of storage capacity may be ascribed to CI intervened activities of cell degrading enzymes such as pectin methylesterase and polygalacturonase. In addition, the utilization of carnauba wax covering in blend with PUT may have applied synergistic impact which aided in keeping up higher juice recuperation by diminishing loss of moisture from the fruits.

Germano et al. [36] studied, a galactomannan-carnauba wax-based coating improved the guava fruit in postharvest quality and storability over preservation of firmness in ambiental conditions (25°C). The authors prepared edible coating galactomannan (0.75%) and carnauba wax (0.9%) were treated with guava fruits (Paluma). At day 15, coated and refrigerated (FR) guava fruits were showed a climacteric rise in 59.3 mg CO₂ kg⁻¹ h⁻¹ and however, coated guava at ambient (FA) showed a diminished value 168.6 mg CO₂ kg⁻¹ h⁻¹ at 15 days of storage and firmness of 14.3 N attributed to lower lipid peroxidation and cell wall hydrolysis. In addition, no increase values of control refrigerated fruit (CR) and no further evaluation of control-uncoated 'Paluma' guava stored at ambient (CA) for 9 days. Additionally, coating improves increased antioxidant enzymes CAT and SOD activities refrigerated samples presented 35% lower H₂O₂ levels (p < 0.05) while compared to uncoated control samples. However, symptoms of chilling injury (CI) inhibition of softening and respiratory peaks are exhibited in refrigerated uncoated fruits. According to the authors, galactomannan carnauba wax coating was effective in guava postharvest quality and maintaining firmness and color, also preventing chilling symptoms under refrigerated conditions, respectively.

3. Conclusion and future perspectives

Despite the current recognition of *Copernicia prunifera* as a quintessential Brazilian plant with growing interest of research and a boost in its commerce and industrial application for the formulation of therapeutic products, it can be safely postulated that its therapeutic potentials have not been fully explored. At present, researches and commercial interest on carnauba wax are skewed toward its cosmeceutical, food and pharmaceutical applications which have dwarfed research interest in its potential as a remedy for other diseases. Thus, further researches on its pharmacological activity recommended with the end-goal of unraveling the pharmacodynamics, pharmacokinetics and clinical relevance. In addition, toxicity risk assessment studies of both the bioactive extracts and isolated constituents need to be given more attention. Nevertheless, further studies and long-term human trials should be carried out in order to clarify the relationship between the consumption of 4-methoxy cinnamic acid diesters and its derivatives such as isolated pectin and their benefic impact on the human body, there seems to be certainly a promising future for new investigations.

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Conflict of interest

The authors declare no conflicts of interest to disclose in relation to this book chapter.

Acronyms and abbreviations

PIP TLC	precipitation index permits thin layer chromatography
Mpk CSW	peak molar mass carnauba-shellac wax
LO	lemongrass oil
APE	aqueous pulp extracts
LDL-C	low-density lipoprotein cholesterol
ТС	total cholesterol
TG	triglyceride
HD	hypercholesterolemic diet
GC-FID	gas chromatography-flame ionization detections
IR	infrared spectroscopy
PCO-C	4-methoxy cinnamic acid diesters
b.w	body weight

DPPH EC ABTS	2,2-diphenyl-1-picrylhydrazyl effective concentration 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammo-
	nium salt
FRAP	ferric reducing antioxidant power
NMR	nuclear magnetic resonance spectroscopy
SDS	sodium dodecyl sulfate
AST	aspartate transaminase
ROS	reactive oxygen species
PG	polygalacturonase
PUT	putrescine
CI	chilling injury
PPO	polyphenol oxidase
SI	selective index

Author details

Iago Almeida da Ponte¹, Murugan Muthuvel², Sudarsini Saravanabhavan³ and Stephen Rathinaraj Benjamin^{1*}

1 Department of Biotechnology and Molecular Biology, State University of Ceará, Fortaleza, Brazil

2 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Sriramachandra Institute of Higher Education and Research, Chennai, Tamilnadu, India

3 Department of Ayurveda and Siddha, Trichy Ayurvedic Union Pvt. Ltd., Tiruchirappalli, Tamilnadu, India

*Address all correspondence to: biotechstephen@gmail.com

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