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Tentative Identification of Phytochemicals and Antioxidant Activities during Fruit-Ripening on *Chamaedorea radicalis* Mart.

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Abstract: This work aims to determine the phytochemical characterization of the pericarp of *Chamaedorea radicalis* Mart. fruit as a non-timber product with potential to obtain phytochemicals with potential applications in the industry. Fruit from *C. radicalis* were grouped in four ripening stages named as S1, S2, S3 and S4, according to maturity; S1 the most unripe stage and S4 the completely ripe stage. Determinations of total phenolic compounds, free radical scavenging activities and total flavonoid contents using spectrophotometric methods were done. Also, the tentative identification of phytochemicals during fruit ripening was done using UPLC-MS-MS. Total phenolic compound (TPC) content ranged from 7.24 to 12.53 mg gallic acid equivalents per gram of fresh weight (mg GAE/g FW). Total flavonoids (TF) contents ranged from 0.163 to 0.23 mg of quercetin equivalents per g FW (mg QE/g FW). Free radical scavenging activity against DPPH and ABTS radicals varied from 40.80 to 53.68 and from 22.29 to 37.76 mmol Trolox equivalents g FW (mmol TE/g FW), respectively. Antioxidant assay in vitro by FRAP (ferric reducing antioxidant power) method showed that S3 was the highest level with antioxidant power while S4 was the lowest with Red ripeness stage showed the lowest contents for all determinations. Mass spectrometry allowed detection of 26 compounds, including phenolics, alkaloids and saponins. Afzelin, Kaempferol 3-neohesperidoside and the four saponins identified were present in all ripeness stages. Preliminary phytochemical identification and the spectrophotometric determinations showed that the pericarp of *C. radicalis* presented antioxidants and compounds related to alkaloids, phenolics and saponins. The presence and abundance of each phytochemical regarding each ripeness stage should be considered.

Keywords: Metabolite identification; palm; free radical scavenging; phenolic compounds; saponins

1 Introduction

Non-timber forest products have the potential to be exploited in several ways, which can be done by means of traditional uses or by innovative applications in the food industry or in medicine, both which

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are associated with the discovery of phytochemicals with bioactive properties according to *in vivo* assays or traditional uses reported by literature [1]. Although many phytochemicals have constitutive accumulation patterns, their diversity and quantity may vary depending on environmental conditions, genetic background and phenology [2,3]. Therefore, the exploration of the phytochemical patterns is a keystone to understanding the physiological processes and to obtain materials that could be used as sources of phytochemicals, that in future could be suggested as bioactive compounds; which could have ecological roles and potential uses [4,5]. In this sense, there are non-timber products of some species that have been considered as a source of bioactive compounds; but they have been little explored [6].

Chamaedorea radicalis Mart., is a small palm that can be considered as an unexplored species about its phytochemicals; this palm has been used only to obtain fresh leaves for the floral ornament industry. Currently, strategies for the management of wild populations and for the establishment of commercial plantations have been applied to reduce the extraction pressure on natural populations [7], which has led to a rebound in its production. The fruit pericarp (pulp and peel) is often obtained during the management of this species, where fruits are used to get seeds, while the pericarp is discarded, due to irritating reactions. In the field, it has been observed that ripe fruits of *C. radicalis* are consumed by some small mammals including foxes and rodents during dry season, remarking the ecological importance for the native fauna [8]. This plant represents a potential reservoir of phytochemicals that has not been studied so far; therefore, its exploration and knowledge could lead to support products generated from *C. radicalis* to get phytochemicals with the potential to be applied for industrial purposes.

Some species of the genus *Chamaedorea* have been used to control bacterial infections, as antitussives, antidiabetics or because of their antidiarrheal effects; all these are based on leaves and aerial parts preparations by traditional practices [9,10]; however, little information is known about the bioactive mechanisms and doses. In this context, it has been especially pointed out that the fruit of *Chamaedorea tepejilote* Liemb. has been related to medicinal effects on skin diseases through indigenous medical practices [11]. This suggests that the fruits of these palms could be sources of compounds with medicinal potential and ecological relevance; therefore, this work aims to the explore phytochemicals of the *C. radicalis* pericarp to contribute with the knowledge of its phytochemicals, antioxidant potential and free-radical scavenging capacity, which has not been reported for this plant. Phytochemicals during ripening of fruits present variation and specific accumulation patterns, which are associated with endogenous roles and ecological interactions, that allow seed dispersion and protection of seeds, however, the differential accumulation of certain phytochemicals could be useful to understand their ecological roles or to conceive industrial applications as highlighted for some commercial fruits that have been studied for decades [12–14]. In the case of unexplored fruits like the case of *C. radicalis*, this remains unknown, but represents a chance to contribute to the knowledge of this natural resource, that in the future, with the correct and deeper studies could lead to applications.

2 Materials and Methods

2.1 Collection of Samples

Fruit samples were obtained from adult individuals of *C. radicalis* grown in an official forest-management unit in the municipality of Gómez Farías, Tamaulipas, Mexico. The *C. radicalis* samples were identified by comparison with specimens of the herbarium (specimen voucher UAT015863) from the Institute of Applied Ecology of Universidad Autónoma de Tamaulipas. Fruits from 100 adult individuals with different ripeness stages were collected and classified according their coloration, obtaining four stages designated as Green (S1), Yellow (S2), Orange (S3) and Red (S4) (Fig. 1). Compound samples of 50 fruits were washed with running water, rinsed with distillate water and processed to separate the pericarps (pulp and peel), which were stored at −20°C until use.

Figure 1: Fruit ripeness stages classification. Green (S1), Yellow (S2), Orange (S3) and Red (S4)

2.2 Preparation of Extracts

The extraction was done by maceration of samples in 50% ethanol in a one: four ratio (w/v) during 30 min at room temperature (28–30°C), followed by centrifugation at 10,000 × g for 10 min. The clarified supernatant was used as a source of phytochemicals for subsequent analyses. Three extractions were done for each ripeness stage.

2.3 Total Phenolic Compounds

TPC contents were determined with the Folin-Ciocalteu reagent according to Singleton et al. [15]. Volumes of 250 µl of each samples or standards were mixed with 125 µl of 1 N Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA), followed by incubation during 5 min, and then 625 µl of 20% Na_2CO_3 (CTR, Monterrey, Nuevo Leon, Mexico) were added. The reaction mixtures were incubated in darkness for 2 h; after this, the absorbance of each reaction was recorded at 750 nm (UV-6000, Metash instruments Co. Ltd., Shanghai, China). The concentration of the samples was determined with a standard curve prepared with a 0.1 mg/ml solution of gallic acid (Sigma-Aldrich, St. Louis, MO, USA) in a range of 1 to 8 µg/ml. Concentrations were expressed in mg GAE/g FW. Detections were done in triplicate.

2.4 Determination of Total Flavonoids

The concentration of flavonoids was determined according to the method suggested by Chang et al. [16], mixing 1 ml of extract, 1.5 ml of ethanol (95%) (CTR, Monterrey, Nuevo Leon, Mexico), 0.1 ml of 10% aluminum chloride (Sigma-Aldrich, St. Louis, MO, USA), 0.1 ml of 1 M potassium acetate (Sigma-Aldrich, St. Louis, MO, USA) and 2.8 ml of sterile distilled water, followed by an incubation during 40 min at room temperature. Absorbance obtained in each reaction was recorded at 415 nm. The standard curve was prepared with quercetin (Sigma-Aldrich, St. Louis, MO, USA) as a standard at concentrations of 10 to 100 µg/ml. The recorded concentrations were expressed in mg QE/g FW. Detections were done in triplicate.

2.5 DPPH Radical Scavenging

The *in vitro* free radical scavenging capacity was determined based on Brand-Williams et al. [17]. For each reaction, a 50 µl aliquot of the standards or extracts were mixed with 0.950 ml of the DPPH reagent

(60 μM) (Sigma-Aldrich, St. Louis, MO, USA) dissolved in methanol (CTR, Nuevo Leon, Mexico) adjusted to 0.7 of absorbance at 517 nm. Each mixture was incubated in darkness at room temperature for 30 min, and then the absorbance was recorded. The standard curve was established with Trolox (Sigma-Aldrich, St. Louis, MO, USA) whose concentration ranged between 100 and 1200 μM. Concentrations were expressed as mmol TE/g FW. Detections were done in triplicate.

2.6 ABTS Radical Scavenging

The free radical scavenging activity against ABTS was determined according to Re et al. [18]. The stock solution was prepared by mixing ABTS 7 mM (Sigma-Aldrich, St. Louis, MO, USA) and potassium persulfate at 2.45 mM (Sigma-Aldrich, St. Louis, MO, USA). This mixture was incubated for 16 h at room temperature in darkness. After this, the working solution was adjusted to 0.7 of absorbance at 732 nm using ethanol. An aliquot of 10 μL of each extract or standard were mixed with 1 ml of ABTS working solution, and its absorbance was recorded after 6 min. The standard curve was generated with Trolox in a range of 100 to 1200 μM. Concentration of activity against ABTS was expressed in mmol TE/g FW. Detections were done in triplicate.

2.7 Antioxidant Capacity Detection Using FRAP

The capacity to reduce ferric ions was measured with a modified version of the method developed by Benzie et al. [19]. An aliquot (100 μl) of extract (with adequate dilution, when necessary) was added to 3 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM TPTZ solution and 1 part of 20 mM $FeCl_3 \cdot 6H_2O$ solution) and the reaction mixture was incubated at 37°C. The increase in absorbance at 595 nm was measured after 60 min. The antioxidant capacity of the extracts was expressed as mM Trolox equivalents per gram of plant material on dry basis. The standard curve was generated with Trolox in a range of 100 to 1200 μM. Detections were done in triplicate.

2.8 Phytochemical Identification

Phytochemicals were extracted in 50% ethanol and then filtered through 0.25 μm; after clarification 0.5 μl of sample were used in each analysis by using an UHPLC system coupled to a TOF/Q-TOF mass spectrometer Agilent G6545A, with a flux of 0.4 ml/min, high-pressure limit of 800 bar, 100% water in channel A, and 100% acetonitrile in channel B, both with 0.1% of formic acid. An Agilent InfinityLab Poroshell 120 EC-C18, 1.9 μm column was used at 40°C. Acquisition software was version 6200 series TOF/6500 series Q-TOF B.06.01 (Agilent Technologies). Detection of the mass spectrum was completed in the positive ion mode with a mass range of 100–1700 m/z. The optimal values for the source parameters were as follows: dry gas temperature 280°C, gas flow 10.0 l/min, nebulizer pressure 3.4 bar, and spectrum velocity 1 Hz. Automated MS/MS (MS^2) assays were done using fixed collision energies of 15 and 20 V with argon as the collision gas and adjustment of the exploration time every 1 s. Tentative identity assignation was done by comparing mass fragmentation patterns. The comparison of the observed mass spectra was done with those found in the MassBank (http://www.massbank.jp/), ChemSpider (http://www.chemspider.com), and PubChem (https://pubchem.ncbi.nlm.nih.gov) [20].

2.9 Statistical Analysis

The one-way ANOVA and comparison of arithmetic means (Tukey $p \leq 0.05$) were carried out with SAS V 9.3 [21].

3 Results

3.1 Phenolic Compounds and Scavenging Capacities during Fruit Ripening

Ripening process of *C. radicalis* fruit includes gradual color variation, starting with green tones in the unripe stage up to reach the red coloration in the ripe stage; this, is accompanied by highly dynamic

metabolic modifications, involving variation in antioxidant, antiradical and phytochemical profiles. In this sense, determination of total phenolic contents and scavenging capacities are preliminary results of polyphenol composition. The hydroalcoholic extract allowed the detection of TPC, TF, free radical scavenging capacities and antioxidant capacity levels in four ripeness stages of *C. radicalis* and statistical analysis allowed to differentiate each ripeness stage (Tab. 1). Regarding the TPC content, it was observed that the concentration between S1-S3 did not show significant differences values, while the S4 was the only statistically different stage and presented the lowest content. TPC values ranged between 7.24 to 12.53 mg GAE/g FW. The contents of TF showed a different behavior, with the highest content in S3 and S2, followed by S1, which were statistically different, while the lowest value was for S4. The TF contents ranged from 0.163 up to 0.23 mg QE/g FW. In relation to the free radical scavenging capacity against DPPH, the highest values were found in S2 and S3, which did not show significant differences, followed by the S1 and the lowest was S4. The free radical scavenging capacity against ABTS showed that the stages with highest capacity were S2 and S3, which showed a slight statistical difference, followed by S1 and finally, S4 which presented the lowest level of activity, both with significant differences. In the case of antioxidant capacity determined by the FRAP assay showed that S1 and S3 were similar in antioxidant capacity; and close to S2 content; while S4 showed the lowest antioxidant capacity. A relation was noticed between the TPC contents in the S1 to S3 stages, which coincides with the superior levels of free radical scavenging capacities, flavonoids and antioxidant capacity, showing that the S4 had the lowest levels on these parameters. This showed that S4 was the ripeness stage with the lowest content for all the detections made. The ripeness stage with the highest concentration for all parameters was S3, and then, all values decreased in the S4. The riper stage presented the lesser contents for all analyzed parameters.

Table 1: Total phenolic compounds, total flavonoids contents and free radical scavenging capacities in the four fruit ripeness stages of *Chamaedorea radicalis*

Ripeness stage	TPC¶	TF°	DPPH*	ABTS*	FRAP*
Green (S1)	11.61 ± 0.58a	0.195 ± 0.003b	46.32 ± 4.69b	28.75 ± 1.81b	8.58 ± 0.54a
Yellow (S2)	12.53 ± 2.05a	0.221 ± 0.010a	52.11± 4.03a	30.11 ± 1.00b	8.41 ± 0.7ab
Orange (S3)	11.75 ± 0.48a	0.230 ± 0.009a	53.68 ± 3.78a	37.76 ± 2.29a	8.57 ± 0.56a
Red (S4)	7.24 ± 0.50b	0.163 ± 0.005c	40.80 ± 4.94c	22.29 ± 1.49c	7.11 ± 0.34b

¶ Expressed in mg GAE/g FW; ° expressed in mg QE/g FW; * mmol TE/g FW. Means values and ± standard error is presented. Different letters indicate significant differences (Tukey, $p \leq 0.05$).

3.2 Metabolite Identification

Regarding the identification of phytochemicals in the fruits of *C. radicalis*, mass spectrometry could detect 26 compounds with a tentative identity assignation, amongst these compounds, the presence of phenolics (Tab. 2), saponins and alkaloids (Tab. 3) was confirmed, which were distributed among the different ripeness stages; some of them, appeared as constitutive compounds due their presence in all ripeness stages; while other ser variable in only some stages. S1 presented 15 compounds of the total detected. S2 had ten compounds of the total, and it was the stage with less phytochemical diversity. S3 had 18 and it was the stage with highest phytochemical diversity; finally, S4 presented 13 compounds. Regarding the phenolics and derivatives, the S2 and S4 showed the lowest diversity with six representatives of this group. S1 presented ten, and S3 showed 11 phenolic compounds. The phenolics that were present in all the stages were afzelin (kaempferol 3-rhamnoside) and kaempferol 3-neohesperidoside. The rest were present in one, two or three stages. Each stage had a pattern of phenolic compounds of unique distribution. For S1 were detected: carpelastofuran and kaempferol 3-(6G-

Table 2: Phenolics and derivatives from *Chamaedorea radicalis* tentatively identified by mass spectrometry

Tentative identification of phenolics and derivatives	RT	Molecular ion	MS^2 Dominant fragment ions	Mass	Molecular formula	Ripeness stages			
						S1	S2	S3	S4
Afzelin	4.532	433.1132	121.0512, 313.0705, 433.1130	432.106	$C_{21}H_{20}O_{10}$	+	+	+	+
Kaempferol 3-neohesperidoside	2.264	595.1654	85.0275, 103.0605, 221.0822, 355.0737, 457.1126, 577.1545, 595.1654	594.1582	$C_{27}H_{30}O_{15}$;	+	+	+	+
2'-Hydroxygenistein 7-(6-malonylglucoside)	6.987	535.1078	147.0654, 281.0514, 415.0337, 535.1076	534.1008	$C_{24}H_{22}O_{14}$	+	+	+	
Kaempferol 4'-glucoside	3.394	449.1077	121.0508, 209.0810, 329.0659, 449.1077	448.1001	$C_{21}H_{20}O_{11}$	+	+	+	
Rhamnocitrin 3-rutinoside	7.066	579.5334	97.1012, 211.0958, 355.0680, 489.0541, 497.1929, 579.5334	608.1732	$C_{28}H_{32}O_{15}$	+		+	+
3-O-Feruloylquinic acid	3.032	369.1184	105.0703, 207.0656, 267.2673, 341.3053, 369.1184	368.1114	$C_{17}H_{20}O_9$	+		+	
Castavinol	12.09	551.1751	121.0644, 227.2087, 303.1229, 381.2921, 551.5003, 551.1751, 565.5658	550.1681	$C_{26}H_{30}O_{13}$	+	+		
Kaempferide 3-glucoside	5.45	463.1236	121.0506, 223.0640, 313.0708, 445.1129, 458.3838, 463.1236	462.1159	$C_{22}H_{22}O_{11}$;	+		+	
Scutellarein	8.298	287.0549	98.9759, 111.1160, 227.0806, 227.0813, 287.0549	286.0477	$C_{15}H_{10}O_6$			+	+
Carpelastofuran	4.85	545.2127	147.0655, 281.0508, 415.0390, 541.2623, 545.2127	522.2242	$C_{30}H_{34}O_8$	+			
Kaempferol 3-(6G-malonylneohesperidoside)	7.302	681.1651	145.0506, 205.0856, 265.1069, 338.3412, 401.1567, 513.2904, 681.1651, 684.2011	680.1578	$C_{30}H_{32}O_{18}$	+			
Kaempferol 7-galactoside	5.056	449.1081	121.0509, 185.1182, 287.0545, 449.1088	448.1008	$C_{21}H_{20}O_{11}$		+		
2',4'-Dihydroxy-4,6'-dimethoxydihydrochalcone	16.72	303.1228	102.1275,188.9850, 303.1228	302.1157	$C_{17}H_{18}O_5$			+	
Isoswertisin 2''-O-beta-arabinoside	6.637	579.1699	177.0526, 271.0595, 423.1326, 433.1122, 551.5017, 579.1699	578.163	$C_{27}H_{30}O_{14}$			+	
Rhamnetin 3-galactoside-3'-rhamnoside	2.795	625.1767	105.0713, 221.0840, 239.2354, 341.3047, 488.1302, 505.1332, 579.5333, 625.1767	624.1693	$C_{28}H_{32}O_{16}$			+	
Davallioside A	2.59	536.1651	105.0183, 281.0509, 341.0180, 415.0364, 503.1070	535.1713	$C_{25}H_{29}NO_{12}$				+
trans-Chlorogenic acid	1.102	355.1017	103.0622, 163.0390, 341.3041	354.0945	$C_{16}H_{18}O_9$				+

+ indicates presence. Ripeness stages: Green (S1), Yellow (S2), Orange (S3) and Red (S4).

malonylneohesperidoside). For S2 it was the kaempferol 7-galactoside, for S3 were 2',4'-dihydroxy-4,6'-dimethoxydihydrochalcone, isoswertisin 2''-O-beta-arabinoside and rhamnetin 3-galactoside-3'-rhamnoside; while for S4 were davallioside A and trans-chlorogenic acid. Regarding saponins and derivatives, four types were identified in the four ripeness stages as a constitutive group.

Table 3: Saponins, alkaloids and derivatives from *Chamaedorea radicalis* identified by mass spectrometry

Saponins and derivatives	RT	Molecular ion	MS^2 Dominant fragment ions	Mass	Molecular formula	Ripeness stages			
						S1	S2	S3	S4
Licoricesaponin E2	7.54	821.3983	167.1061, 369.1729, 515.2307, 741.4414, 821.3983	820.3908	$C_{42}H_{60}O_{16}$	+	+	+	+
Polypodosaponin	8.537	739.4267	102.1273, 269.1884, 381.2983, 577.3714, 739.4254, 739.4267	738.4194	$C_{39}H_{62}O_{13}$	+	+	+	+
Torvoside D	8.096	727.4259	121.0508, 289.2177, 381.2969, 485.6888, 565.3721, 626.4108, 727.4248, 727.4259	726.4186	$C_{38}H_{62}O_{13}$	+	+	+	+
Tuberoside J	7.652	757.4366	121.0510, 289.2157, 451.2685, 513.2867, 595.3833, 675.3396, 757.4366	756.4293	$C_{39}H_{64}O_{14}$	+	+	+	+
Alkaloids and derivatives	**RT**	**Molecular ion**	**MS^2 dominant fragment ions**	**Mass**	**Molecular formula**	**S1**	**S2**	**S3**	**S4**
Abrine (L)	1.339	219.1125	95.0853, 160.0755, 219.1125, 237.1852	218.1052	$C_{12}H_{14}N_2O_2$			+	+
Aniflorine	1.26	352.1658	174.0552, 335.1397, 352.1658	351.1582	$C_{20}H_{21}N_3O_3$			+	+
23-Acetoxysoladulcidine	22.41	496.3405	121.0512, 184.0740, 281.0515, 406.2011, 496.3405, 496.3412	473.3513	$C_{29}H_{47}NO_4$	+			
Etamiphylline	2.953	302.1588	102.1277, 209.1173, 237.1122	279.1696	$C_{13}H_{21}N_5O_2$			+	
Homoarecoline		170.1176	55.0541, 98.9753, 149.1173, 170.1176	169.1104	$C_9H_{15}NO_2$				+

+ indicates presence. Ripeness stages: Green (S1), Yellow (S2), Orange (S3) and Red (S4).

4 Discussion

In this work, phytochemical patterns observed during the pericarp ripening showed that the presence of phenolics and alkaloids was more variable than that observed for saponins. In this respect, the dynamic variation of phytochemical patterns during fruit ripening process should be seed in relation with physiological and ecological phenomena; including maturation of seeds, seed dispersion, protection against pathogen and herbivore attacks [22,23]. The saponin presence during all stages could be related with endogenous processes or defensive roles, as shown for biological effects on some insect pests [24]. By the other hand, the variation on other metabolites could be related with specific developmental stages and specific roles that could temporarily change, like protection against insects of predation during unripe stages or promoting the dispersion of seed by some herbivores [23], as it occurs with consumption of ripe

fruits by foxes and rodents. Also, it is worth to consider that phytochemicals could be implied in additional ecological roles and that knowing their variation during fruit ripening could orientate future uses.

The presence of phenolic compounds, free radical scavenging activity and total flavonoid contents have been reported in the fruits of several palms species (Arecaceae). Particularly, the reported contents obtained from hydroalcoholic extractions are comparable with those found in the *C. radicalis* fruit. The TPC content in the fruit of *Hyphaene thebaica* L. presented contents that varied from 116.26 to 123.36 mg GAE/g dry weight (DW), regarding extractions by agitation or ultrasonic assistance [25]. These values were much higher than levels found on all the ripeness fruits stages of *C. radicalis*, although dry fruits were used in that work. TPC contents have been reported in mature fruits of *Phoenix dactylifera* L. with values from 0.71 to 4.28 mg GAE/g FW [26] and of 0.98 to 1.24 mg GAE/g FW [27], which are lower than those found in all ripeness stages of *C. radicalis*, which were also analyzed as fresh plant material. This shows, that despite of the observed variation in TPC contents of all ripeness stages, these were superior to those observed in the date palm fruit, one of the most consumed palm fruits.

On other hand, the content of flavonoids extracted with ethanolic phases in fruits of *P. dactylifera* L. was 1.5 mg of QE/g FW [28]; which were higher than the *C. radicalis* values; whereas, the flavonoid contents in an additional report, showed that *P. dactylifera* fruits ranged from 0.46 to 1.36 mg QE/g DW [29]. In the case of *C. radicalis*, values obtained indicated to the pericarp as a source of total flavonoids, with lower values considering reports with fresh and dry materials.

Regarding the free radical scavenging capacity against DPPH in the pulp of other palms fruits, it can be mentioned the case of *Butia eriosphata* Martius, where an acidified acetone aqueous extraction was used, and yielded 0.06 mM TEAC/g DW [30], also in the fruits of *P. dactylifera* extracted with 80% acetone, values of 0.8 to 1.1 µM TEAC/g FW were recorded. These values were lower than those observed in *C. radicalis*. The free radical scavenging capacity for ABTS in mature fruits of *P. dactylifera*, showed a wide variation from 0.2 up to 1.4 µM TEAC/g FW [31], which in both cases were lower than the *C. radicalis* results. This shows that *C. radicalis* fruits are also an important source of compounds with free radical scavenging capacity, which was present in all ripeness stages.

Antioxidant capacity also was associated with contents of free radical scavenging capacity and phenolic contents, where S1, S2 and S3 showed superior levels than S4; and it coincides with other reports, where antioxidant capacity varies according the ripening stages and varieties of palm fruits. In such cases, most ripen stage usually presented the lowest level of antioxidant capacity [32–35].

In general, metabolic changes during fruit ripening, specially antiradical and antioxidant status, could be produced to control oxygen reactive species formation and oxidative stress and also to cope with abiotic stress [36,37]; however, more studies are needed to make clear these changes in *C. radicalis*. Up to now, no implications of these conditions could be related with ecological roles or with physiological processes in the fruit of *C. radicalis*; although, the accumulation behavior is similar to other ripe palm fruits.

So far, the pericarp phytochemical characterization of the genus *Chamaedorea* has not been reported, so this study presents the first contribution for the genus, emphasizing that the pericarp of *C. radicalis* possess general phytochemical groups that have been reported in other species of palms. The phytochemicals detected in this work are related to that reported with colorimetric and mass spectrometry detections, including phenolics, alkaloids and saponins [38–41]. The tentatively identified phytochemicals present during the fruit ripening did not correspond to identified compounds in fruits belonging to other palm species such as *Areca catechu* L., *Chamaerops humilis* L., *Euterpe edulis* Mart., *M. flexuosa* and *P. dactylifera*; however, apigenin, kaempferol and quercetin derivatives were observed [42–46]. This indicates that *C. radicalis* could represent a source of phytochemicals not reported in other palms.

Regarding the possible bioactivities of the tentatively identified compounds, it was found that some are related to potential effects for medical applications. Afzelin, was present during all ripeness stages. This is a

flavonoid that has been associated with numerous bioactive properties through in vitro models, highlighting its antibacterial effect, which affects the growth of *Pseudomonas aeruginosa* Migula, responsible for infections in immunocompromised patients [47]. Another potential bioactivity is a gastroprotective effect in the model of acute gastric ulcers, emphasizing that this compound in a mixture with quercitrin, decreased the appearance of ulcers induced by HCl ethanol. It was also associated with the decrease of myeloperoxidase activity, which supported the use of *Solidago chilensis* Meyen as a plant to treat gastric disorders [45]. It has also been reported that this flavonoid can absorb harmful UV radiation and has been linked to the melanogenesis process, which suggests that afzelin may be a protective agent against UV radiation damages [46]; nevertheless, the afzelin showed cytotoxic effects that affected blood cells, and whose effects were concentration dependent [47]. Its potential is evident, but caution must be considered since the pointed-out toxicity potentially could originate health problems.

Another phytochemical common to all ripeness stages was the kaempferol 3-neohesperidoside, which has been associated with stimulating effects for glucose uptake, it does not have a synergistic effect with insulin and favors increases in glycogen within muscle cells, which is linked with antidiabetic properties [48]. This highlights the potential to offer alternative treatments for diabetes applications.

Among the compounds that showed restricted distribution to specific stages and that are associated with reported bioactivities is the scutellarein, which has been related to protective effects against cerebral ischemia in rats, where it attenuated neuronal damage, decreased brain water composition, regulated the accumulation of some amino acids neurotransmitters and raised the activity of Ca_2^+ ATPase and Na^+, K^+ ATPase [49]. In addition, it showed a powerful antiviral potential by inhibiting the helicase of the severe acute respiratory syndrome coronavirus and, also it has been related to inhibition of the HIV reverse transcriptase-1. For this reason, it is believed to be a chemical inhibitor of such viral agents [50]. On the other hand, carpelastofuran, like other bioactive flavonoids, has been associated to have cytotoxic properties [51].

In the case of the alkaloids group, etamiphylline was associated with bronchodilator effects with a recurrent use as a human and veterinary drug, and whose metabolism is well known [52]. Homoarecoline was related with potential pharmacological properties according to silico studies and by some treatment approaches [53,54]. Saponins detected on *C. radicalis* pericarp were not related with reported bioactive effects, and even though the saponins are considered a phytochemical group implied in plant responses against biotic and abiotic stress, no functional roles could be found for those reported here [55,56]. Although, in general, the saponin accumulation has been reported as highly variable during fruit development [57], saponins from *C. radicalis* were present in all ripening process and up to now, we could not relate them to any physiological role.

Although the variation of phytochemical diversity and free radical scavenging capacity is evident, and that this represents a potential source for bioactive compounds, ecological and physiological roles in *C. radicalis* remained unknown. Regarding the potential of several bioactivities in the pericarp of this palm, more studies are urged to determine concentration, accumulation patterns, extraction procedures and biological effects (including toxicity and allergenic issues) in order to optimize strategies to diversity uses of *C. radicalis* fruit.

5 Conclusions

Phytochemical screening of *C. radicalis* pericarp showed that phenolic compounds, alkaloids and saponins were present in all ripeness stages, but they were differentially accumulated. Some were constitutive while others were found only in specific stages. TPC and TF contents and free radical scavenging capacity and antioxidant capacity were different during the ripening process; the ripe stage (S4) presented the lesser content for all the quantified parameters. No endogenous or ecological roles were related to phytochemicals reported here. The *C. radicalis* fruit could be considered as a source of a diverse phytochemicals, which depends on the ripeness stage.

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References

1. Khoo, H. E., Azlan, A., Kong, K. W., Ismail, A. (2016). Phytochemicals and medicinal properties of indigenous tropical fruits with potential for commercial development. *Evidence-Based Complementary and Alternative Medicine,* 1–20.
2. Dhami, N., Mishra, A. D. (2015). Phytochemical variation: how to resolve the quality controversies of herbal medicinal products? *Journal of Herbal Medicine, 5(2),* 118–127. DOI 10.1016/j.hermed.2015.04.002.
3. Dey, P., Dutta, S., Chowdhury, A., Das, A. P., Chaudhuri, T. K. (2017). Variation in phytochemical composition reveals distinct divergence of *Aloe vera* (L.) Burm. F. from other aloe species: rationale behind selective preference of Aloe vera in nutritional and therapeutic use. *Journal Evidence Based Complementary Alternative Medicine, 22 (4),* 624–631. DOI 10.1177/2156587217698292.
4. Tohge, T., de Souza, L. P., Fernie, A. R. (2017). Current understanding of the pathways of flavonoid biosynthesis in model and crop plants. *Journal of Experimental Botany, 68(15),* 4013–4028. DOI 10.1093/jxb/erx177.
5. Lämke, J. S., Unsicker, S. B. (2018). Phytochemical variation in treetops: causes and consequences for tree-insect herbivore interactions. *Oecologia, 187(2),* 377–388. DOI 10.1007/s00442-018-4087-5.
6. Aguirre, G., Loza, M., Gazquez, J. A., Fusco, M., Sosa, A. et al. (2016). The potentiality of non timber forest products. Fruit availability, phytochemical properties of *Rosa rubiginosa* L. rose hips. *American Journal of Plant Sciences, 7(15),* 2272–2287. DOI 10.4236/ajps.2016.715200.
7. Granados-Sánchez, D., Hernández-García, M. A., López-Ríos, G. F., Santiago-López, M. (2004). El cultivo de palma camedor (*Chamaedorea* sp.) en sistemas agroforestales de Cuichapa. *Veracruz Revista Fitotecnia Mexicana, 27,* 233–241.
8. Lara-Villalón, M. (2007). (Universidad Autónoma de Tamaulipas, Ciudad Victoria, Tamaulipas, México). Personal communication.
9. Argüello, M. A. (2016). Palmas medicinales usadas en el Nuevo Mundo. *Enfoque UTE, 7(1),* 91–110. DOI 10.29019/enfoqueute.v7n1.90.
10. Sharma, A., Flores-Vallejo, R. C., Cardoso-Taketa, A., Villarreal, M. L. (2017). Antibacterial activities of medicinal plants used in Mexican traditional medicine. *Journal of Ethnopharmacology, 208,* 264–329. DOI 10.1016/j.jep.2016.04.045.
11. Contreras-Cortés, L. E. U., Mariaca-Méndez, R., Pérez-Farrera, M. A. (2018). Importancia y uso de las palmas entre los mayas lacandones de Nahá. *Chiapas Etnobiología, 16,* 19–30.
12. Howard, L. R., Talcott, S. T., Brenes, C. H., Villalon, B. (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *Journal of Agricultural and Food Chemistry, 48(5),* 1713–1720. DOI 10.1021/jf990916t.
13. Kennedy, J. A., Matthews, M. A., Waterhouse, A. L. (2000). Changes in grape seed polyphenols during fruit ripening. *Phytochemistry, 55(1),* 77–85. DOI 10.1016/S0031-9422(00)00196-5.
14. Bashir, H. A., Abu-Goukh, A. B. A. (2003). Compositional changes during guava fruit ripening. *Food Chemistry, 80(4),* 557–563. DOI 10.1016/S0308-8146(02)00345-X.
15. Singleton, V. L., Orthofer, R., Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteu reagent. *Methods in Enzymology, 299,* 152–178.

16. Chang, C. C., Yang, M. H., Wen, H. M., Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis, 10,* 178–182.
17. Brand-Williams, W., Cuvelier, M. E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 1(1),* 25–30. DOI 10.1016/S0023-6438(95)80008-5.
18. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. et al. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26(9-10),* 1231–1237. DOI 10.1016/S0891-5849(98)00315-3.
19. Benzie, I. F., Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry, 239(1),* 70–76. DOI 10.1006/abio.1996.0292.
20. Torres-Castillo, J. A., Sinagawa-García, S. R., Torres-Acosta, R. I., García-García, L. D., Ramos-Rodríguez, A. G. et al. (2018). Entomochemicals from *Pterophylla beltrani* Bolivar and Bolivar: antioxidants and other metabolites. *Southwestern Entomologist, 43(2),* 369–382. DOI 10.3958/059.043.0208.
21. SAS Institute (2011). *SAS user's guide: statistics, version 9.3*. Cary, NC, USA: Statistic Analysis System Institute.
22. McAtee, P., Karim, S., Schaffer, R. J., David, K. (2013). A dynamic interplay between phytohormones is required for fruit development, maturation, and ripening. *Frontiers in Plant Science, 4,* 79. DOI 10.3389/fpls.2013.00079.
23. Nevo, O., Heymann, E. W., Schulz, S., Ayasse, M. (2016). Fruit odor as a ripeness signal for seed-dispersing primates? A case study on four Neotropical plant species. *Journal of Chemical Ecology, 42(4),* 323–328. DOI 10.1007/s10886-016-0687-x.
24. Singh, B., Kaur, A. (2018). Control of insect pests in crop plants and stored food grains using plant saponins: a review. *LWT-Food Science and Technology, 87,* 93–101. DOI 10.1016/j.lwt.2017.08.077.
25. Aboshora, W., Lianfu, Z., Dahir, M., Qingran, M., Qingrui, S. et al. (2014). Effect of extraction method and solvent power on polyphenol and flavonoid levels in *Hyphaene thebaica* L Mart (*Arecaceae*) (Doum) fruit, and its antioxidant and antibacterial activities. *Tropical Journal of Pharmaceutical Research, 13(12),* 2057–2063. DOI 10.4314/tjpr.v13i12.16.
26. Nematallah, K. A., Ayoub, N. A., Abdelsattar, E., Meselhy, M. R., Elmazar, M. M. et al. (2018). Polyphenols LC-MS2 profile of Ajwa date fruit (*Phoenix dactylifera* L.) and their microemulsion: potential impact on hepatic fibrosis. *Journal of Functional Foods, 49,* 401–411. DOI 10.1016/j.jff.2018.08.032.
27. Souli, I., Jemni, M., Rodríguez-Verástegui, L. L., Chaira, N., Artés, F. et al. (2018). Phenolic composition profiling of Tunisian 10 varieties of common dates (*Phoenix dactylifera* L.) at tamar stage using LC-ESI-MS and antioxidant activity. *Journal of Food Biochemistry, 42(6),* e12634. DOI 10.1111/jfbc.12634.
28. Selim, S., Alfy, S. E., Al-Ruwaili, M., Abdo, A., Jaouni, S. A. (2012). Susceptibility of imipenem-resistant Pseudomonas aeruginosa to flavonoid glycosides of date palm (Phoenix dactylifera L.) tamar growing in Al Madinah, Saudi Arabia. *African Journal of Biotechnology, 11(69),* 416–422. DOI 10.5897/AJB11.4186.
29. Mohamed Lemine, F. M., Mohamed Ahmed, M. V. O., Ben Mohamed Maoulainine, L., Bouna, Z. E. A. O., Samb, A. et al. (2014). Antioxidant activity of various Mauritanian date palm (*Phoenix dactylifera* L.) fruits at two edible ripening stages. *Food Science & Nutrition, 2(6),* 700–705. DOI 10.1002/fsn3.167.
30. Egea, M. B., Pereira-Netto, A. B. (2018). Bioactive compound-rich, virtually unknown, edible fruits from the Atlantic Rainforest: changes in antioxidant activity and related bioactive compounds during ripening. *European Food Research and Technology, 245,* 1081–1093.
31. Al-Turki, S., Shahba, M. A., Stushnoff, C. (2010). Diversity of antioxidant properties and phenolic content of date palm (Phoenix dactylifera L.) fruits as affected by cultivar and location. *Journal of Food, Agriculture and Environment, 8,* 253–260.
32. Allaith, A. A. A. (2008). Antioxidant activity of Bahraini date palm (*Phoenix dactylifera* L.) fruit of various cultivars. *International Journal of Food Science & Technology, 43(6),* 1033–1040. DOI 10.1111/j.1365-2621.2007.01558.x.
33. Eid, N. M., Al-Awadi, B., Vauzour, D., Oruna-Concha, M. J., Spencer, J. P. (2013). Effect of cultivar type and ripening on the polyphenol content of date palm fruit. *Journal of Agricultural and Food Chemistry, 61(10),* 2453–2460. DOI 10.1021/jf303951e.

34. Mohamed, R. M., Fageer, A. S., Eltayeb, M. M., Mohamed Ahmed, I. A. (2014). Chemical composition, antioxidant capacity, and mineral extractability of S udanese date palm (*Phoenix dactylifera* L.) fruits. *Food Science & Nutrition, 2(5),* 478–489. DOI 10.1002/fsn3.123.
35. Odeh, I., Al-Rimawi, F., Abbadi, J., Obeyat, L., Qabbajeh, M. et al. (2014). Effect of harvesting date and variety of date palm on antioxidant capacity, phenolic and flavonoid content of date palm (*Phoenix dactylifera*). *Journal of Food and Nutrition Research, 2(8),* 499–505. DOI 10.12691/jfnr-2-8-11.
36. Jimenez, A., Creissen, G., Kular, B., Firmin, J., Robinson, S. et al. (2002). Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Planta, 214(5),* 751–758. DOI 10.1007/s004250100667.
37. Navarro, J. M., Flores, P., Garrido, C., Martínez, V. (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chemistry, 96(1),* 66–73. DOI 10.1016/j.foodchem.2005.01.057.
38. Rondón, M., Moncayo, S., Cornejo, X., Santos, J., Villalta, D. (2018). Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. *Journal of King Saud University, 30(4),* 500–505. DOI 10.1016/j.jksus.2017.03.009.
39. Bataglion, G. A., da Silva, F. M., Eberlin, M. N., Koolen, H. H. (2014). Simultaneous quantification of phenolic compounds in buriti fruit (*Mauritia flexuosa* L.f.) by ultrahigh performance liquid chromatography coupled to tandem mass spectrometry. *Food Research International, 66,* 396–400. DOI 10.1016/j.foodres.2014.09.035.
40. Srimany, A., George, C., Naik, H. R., Pinto, D. G., Chandrakumar, N. et al. (2016). Developmental patterning and segregation of alkaloids in areca nut (seed of Areca catechu) revealed by magnetic resonance and mass spectrometry imaging. *Phytochemistry, 125,* 35–42. DOI 10.1016/j.phytochem.2016.02.002.
41. Hamed, A. I., Ben, S. R., Al-Ayed, A. S., Moldoch, J., Mahalel, U. A. (2017). Fingerprinting of strong spermatogenesis steroidal saponins in male flowers of *Phoenix dactylifera* (Date Palm) by LC-ESI-MS. *Natural Product Research, 31(17),* 2024–2031. DOI 10.1080/14786419.2016.1274887.
42. Bouhafsoun, A., Yilmaz, M. A., Boukeloua, A., Temel, H., Kaid Harche, M. K. (2018). Simultaneous quantification of phenolic acids and flavonoids in *Chamaerops humilis* L. using LC-ESIMS/MS. *Food Science and Technology, 38 (suppl 1),* 242–247. DOI 10.1590/fst.19917.
43. Barroso, M. E., Oliveira, B. G., Pimentel, E. F., Pereira, P. M., Ruas, F. G. et al. (2019). Phytochemical profile of genotypes of Euterpe edulis Martius-Juçara palm fruits. *Food Research International, 116,* 985–993. DOI 10.1016/j.foodres.2018.09.036.
44. Lotha, R., Sundaramoorthy, N. S., Shamprasad, B. R., Nagarajan, S., Sivasubramania, A. (2018). Plant nutraceuticals (Quercetrin and Afzelin) capped silver nanoparticles exert potent antibiofilm effect against food borne pathogen *Salmonella enterica* serovar Typhi and curtail planktonic growth in zebrafish infection model. *Microbial Pathogenesis, 120,* 109–118. DOI 10.1016/j.micpath.2018.04.044.
45. de Barros, M., Mota, S. L., Boeing, T., Bordignon, S. L., Judah, C. B. et al. (2016). Pharmacological reports about gastroprotective effects of methanolic extract from leaves of Solidago chilensis (*Brazilian arnica*) and its components quercitrin and afzelin in rodents. *Naunyn-Schmiedeberg's Archives of Pharmacology, 389(4),* 403–417. DOI 10.1007/s00210-015-1208-0.
46. Jung, E., Kim, J. H., Kim, M. O., Jang, S., Kang, M. et al. (2016). Afzelin positively regulates melanogenesis through the p38 MAPK pathway. *Chemico-Biological Interactions, 254,* 167–172. DOI 10.1016/j.cbi.2016.06.010.
47. Vellosa, J. C. R., Regasini, L. O., Belló, C., Schemberger, J. A., Khalil, N. M. et al. (2015). Preliminary *in vitro* and ex vivo evaluation of afzelin, kaempferitrin and pterogynoside action over free radicals and reactive oxygen species. *Archives of Pharmacal Research, 38(6),* 1168–1177. DOI 10.1007/s12272-014-0487-1.
48. Tang, X., Olatunji, O. J., Zhou, Y., Hou, X. (2017). *Allium tuberosum*: antidiabetic and hepatoprotective activities. *Food Research International, 102,* 681–689. DOI 10.1016/j.foodres.2017.08.034.
49. Tang, H., Tang, Y., Li, N., Shi, Q., Guo, J. et al. (2014). Neuroprotective effects of scutellarin and scutellarein on repeatedly cerebral ischemia-reperfusion in rats. *Pharmacology Biochemistry and Behavior, 118,* 51–59. DOI 10.1016/j.pbb.2014.01.003.

50. Chledzik, S., Strawa, J., Matuszek, K., Nazaruk, J. (2018). Pharmacological effects of Scutellarin, an active component of Genus *Scutellaria* and *Erigeron*: a systematic review. *American Journal of Chinese Medicine, 46 (02),* 319–337. DOI 10.1142/S0192415X18500167.
51. Dongamanti, A., Aamate, V. K., Devulapally, M. G., Gundu, S., Kotni, M. K. et al. (2015). Synthesis, antimicrobial activity and molecular docking of novel tetracyclic scaffolds incorporating a flavonoid framework with medium sized oxygen heterocycles. *Bioorganic & Medicinal Chemistry Letters, 25(4),* 898–903. DOI 10.1016/j.bmcl.2014.12.066.
52. Lee, H., Song, I., Shin, S. M., Jeong, H. E., Lee, E. K. et al. (2019). Regulatory effect of decreasing therapeutic duplication of respiratory drugs using a prescription database between 2012 and 2015. *Regulatory Toxicology and Pharmacology, 103,* 218–228. DOI 10.1016/j.yrtph.2019.02.003.
53. Peng, W., Liu, Y. J., Zhao, C. B., Huang, X. S., Wu, N. et al. (2015). In silico assessment of drug-like properties of alkaloids from *Areca catechu* L Nut. *Tropical Journal of Pharmaceutical Research, 14(4),* 635–639. DOI 10.4314/tjpr.v14i4.11.
54. Bhat, S. K., Devasya, A., Sarpangala, M. (2017). Arecanut, Areca catechu L. as such is not carcinogenic in normal dose if chewed without tobacco: compilation of research work. *International Journal of Food Sciences and Nutrition, 2,* 46–51.
55. Dowd, P. F., Berhow, M. A., Johnson, E. T. (2011). Differential activity of multiple saponins against omnivorous insects with varying feeding preferences. *Journal of Chemical Ecology, 37(5),* 443–449. DOI 10.1007/s10886-011-9950-3.
56. Szakiel, A., Pączkowski, C., Henry, M. (2011). Influence of environmental abiotic factors on the content of saponins in plants. *Phytochemistry Reviews, 10(4),* 471–491. DOI 10.1007/s11101-010-9177-x.
57. Moses, T., Papadopoulou, K. K., Osbourn, A. (2014). Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. *Critical Reviews in Biochemistry and Molecular Biology, 49(6),* 439–462. DOI 10.3109/10409238.2014.953628.