# Antioxidant fractions and phenolic constituents from leaves of *Pluchea carolinensis* and *Pluchea rosea*

**Wilmer Hervet Perera Córdova[1]*, Jean-Noël Wauters[2], Claire Kevers[1], Michel Frédérich[2], Jacques Dommes[1]**

*[1]Plant Molecular Biology and Biotechnology Unit, Institute of Botany B22, University of Liège, Sart Tilman, BE-4000 Liège, Belgium, [2]Laboratory of Pharmacognosy, Institute of Pharmacy B36, University of Liège, Sart Tilman, BE-4000 Liège, Belgium*

## ABSTRACT

**Objective:** The objective is to evaluate the antioxidant potential of several polar fractions of *Pluchea carolinensis* and *Pluchea rosea* as well as pure chemicals, some of them quantified in both species by high-performance liquid chromatography (HPLC). **Methods:** The antioxidant potential of polar fractions and pure chemicals were assayed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical potential methods. The phenolic content was performed by using Folin-Ciocalteu's reagent. Specific phenolic acids and flavonoids were quantified by diode array detector-reversed phase-HPLC. **Results:** The highest DPPH antioxidant potential expressed in milligrams of trolox equivalents per gram of dry extract (mg TE/gDE) were frequently measured in fractions from *n*-butyl alcohol, i.e., 2 (192.1 ± 0.3); 6 (181.0 ± 0.1) of *P. carolinensis* and in fraction 7 (188.1 ± 5.5) of *P. rosea* while for oxygen radical scavenging capacity (mg TE/gDE) assay fraction 2 (543.0 ± 64.6) and 4 (501.4 ± 49.7) of *P. carolinensis* and 3 (401.3 ± 16.1) and 6 (401.3 ± 16.1) of *P. rosea* showed the best results. Some flavonoids and phenolic acids were also assayed; all of them showed highest oxygen radical absorbance capacity values. **Conclusion:** We report the antioxidant potential of polar fractions, as well as of some pure phenolics responsible of the antioxidant potential. Some phenolics were identified and quantified for the first time in both species. Apparently, caffeoylquinic acid derivatives contribute more significant to the total antioxidant potential of the extracts.

**Keywords:** Antioxidant potential, caffeoylquinic acid derivatives, cinnamic acids, flavonoids, phenolic profile

## INTRODUCTION

The genus *Pluchea* belongs to one of the most diverse botanical family, Asteraceae. *Pluchea* counts about 80 species of small herbs and shrubs[1] and a large number of these taxa (30-40) thrive in tropical regions. In Cuba, three species are reported: *Pluchea carolinensis* (Jacq.) G. Don., *Pluchea odorata* (L.) Cass. and *Pluchea rosea* Godfrey.

*Pluchea* is frequently identified as a source of antioxidants,[2] of antimicrobial compounds,[3] of fungicides,[4] of insecticides,[5] of anti-inflammatory chemicals[6] and of allelopathic compounds[7] among others pharmacological properties. The antioxidant potential of some plants has been described in literatures.[8,9] Cuban species of *Pluchea* has recently been evaluated. In these species, high antioxidant capacity has been correlated with the content of phenolic compounds.[10] Hence, the Cuban *Pluchea* species are considered as an interesting source of chemicals for preparing functional foods.

Many of the *Pluchea* species are known as aromatic plants with a characteristic scent produced by complex mixtures of volatile terpenoids. Studies based on the identification or isolation of secondary metabolites suggested that terpenoids are the most widespread metabolites in the genus.[11,12] Eudesmane derivatives are a sesquiterpene group widely distributed in the genus.[13,14] Phenolic compounds are the second most widespread metabolites in *Pluchea*.[15] Several flavonols mainly of the quercetin, kempferol and quercetagetin types have been identified.[15-17] In addition,

***Corresponding address:**
Dr. Wilmer Hervet Perera Córdova, Plant Molecular Biology and Biotechnology Unit, Institute of Botany B22, University of Liège, Sart Tilman, BE-4000 Liège, Belgium.
Phone: +3243663899; Fax: +3243663872.
E-mail: Wilmer.Perera@gmail.com

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a few flavonol aglycones have been previously isolated from *P. carolinensis*,[18] no report from *P. rosea* and various flavonoids have been identified from *P. odorata*.[16] In addition, polyphenols including flavonoids have been reported to exhibit a wide range of biological activities and their effects are mainly attributed to the antioxidant properties.[19]

As far as we know, the chemical composition of phenolic compounds of the Cuban *Pluchea* species and thus, the phytochemicals responsible of the antioxidant capacity of the crude extracts are still unknown. Hence, the present study aim to determine the major phenolic phytochemicals in the ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) extracts of *P. carolinensis* and *P. rosea*. The antioxidant potential of several fractions was evaluated by a phenolic screening by high-performance liquid chromatography (HPLC). Furthermore, the antioxidant potential of various phenolic identified and other analogous compounds was assayed using 2,2-diphenyl-1 picrylhydrazyl (DPPH) and oxygen radical scavenging capacity (ORAC) assay.

## MATERIALS AND METHODS

### Plant material

*P. carolinensis* (voucher HAC 41725) was collected in Sierra del Rosario, Pinar del Río, Cuba (Longitude: 82° 56' 57" and Latitude: 22° 50' 56") in March 2008 during their flowering stage and *P. rosea* (voucher LS 16648) in Ciénaga de Zapata, Matanzas (Longitude: 87° 10' 47" and Latitude: 22° 22' 89") in 2006. All the vouchers were authenticated by MSc. Ramona Oviedo Prieto and Dr. Pedro Herrera Oliver, and deposited at the HAC herbarium of the "Instituto de Ecología y Sistemática."

### Chemicals

The flavonoids standards: Quercetin, kempferol, myricetin, isorhamnetin, quercetagetin, quercitrin, casticin, herbacetin, hyperoside, spiraeoside, apigenin, luteolin, daidzein, daidzin, genistein, genistin, ononin, rutin, naringenin, taxifolin, quercetin-3-*O*-glucopyranoside, kaempferol-3-*O*-rutinoside were purchased from Extra synthèse (France). The phenolic acids: Ferulic acid, ellagic acid, rosmarinic acid, vanillic acid, and p-hydroxy benzoic acid were purchased from Aldrich, as well as the reagent 2',2-azobis(2-amidinopropane) dihydrochloride. Gallic acid, chlorogenic acid, syringic acid, p-coumaric acid, o-coumaric acid, sinapic acid, gentisic acid, caffeic acid, salicylic acid were purchased from Sigma, as well as DPPH and Trolox. Folin-Ciocalteu reagent was purchased from BDH.

### Preparation of the extracts and fractionation

A total of 1293 g and 895 g of dried, powdered leaves of *P. carolinensis* and *P. rosea* respectively, were extracted using ethanol: $H_2O$ (7:3 v/v) macerations. Ethanol was evaporated under reduced pressure. The resulting aqueous solutions were successively fractioned with solvent of increasing polarity: *n*-hexane, chloroform ($CHCl_3$), EtOAc and *n*-BuOH.[10] Five grams of EtOAc extracts previously lyophilized were subjected to chromatography on a 100 cm × 5 cm column containing 500 g of silica gel 60 Merck (70-230 mesh).

The elution was made using a gradient of increasing polarity with *n*-hexane; *n*-hexane-$CHCl_3$ from 1% to 90% in $CHCl_3$; $CHCl_3$; $CHCl_3^-$ ethanol from 1% to 75% in ethanol; ethanol (98%). Solvent in each fraction was evaporated until dryness under vacuum and the extract was lyophilized to yield 14 and 13 fractions for *P. carolinensis* and *P. rosea* respectively. In general 100 mL of fraction was collected except for eluted from $CHCl_3$− ethanol from 1% to 2% in ethanol where 75 mL were collected.

One gram of the *n*-BuOH leaf crude extract of both species was separately subjected to the chromatography column using 100 g of Sephadex LH-20, Pharmacia. Successive elutions through a conventional column (80 cm × 2 cm) with methanol were performed, and 20 mL fractions were collected. Solvent in each fraction was evaporated until dryness under vacuum and extract was lyophilized to yield six and seven fractions for *P. carolinensis* and *P. rosea* respectively. Silica gel 60 F254 Thin layer chromatography plates were used for determining the end of the fraction collected. They were visualized under 254 and 365 nm ultraviolet light and subsequently sprayed with a solution of cerium sulfate (IV) in sulfuric acid (65%).

### Antioxidant potentials and total phenolic compound measurement

The amount of total phenolic compounds of the different crude extracts was determined using Folin-Ciocalteu's reagent and gallic acid as standard. The absorbance at 765 nm was recorded.[20] Results of phenolic determination were expressed in milligrams of gallic acid equivalents per gram of dry extract (mg GAE/gDE). The radical scavenging capacity was examined by the reduction of DPPH free radical in methanol.[21,22] The ORAC assay was also assayed.[23] Results of the antioxidant potential of fractions were expressed in milligrams of Trolox equivalents per gram of dry extract (mg TE/g DE), whereas antioxidant potential of pure compounds was expressed in μmol TE/mmol. Each sample was analyzed in triplicates.

### Flavonoid analyses

HPLC analyses were conducted with a Merck Hitachi La Chrom Elite liquid chromatography equipped with an L-2455 photodiode array detector. 10 μL of each sample was injected in an analytical Grace Davison Grace Smart RP C-18 column (250 mm × 4.6 mm, 5 μm) at 30°C.

Mobile phase A consisted in 0.05% trifluoracetic acid (TFA) and mobile phase B was acetonitrile ($CH_3CN$). The gradient elution was performed from 0% B to 65% B in 40 min at flow rate of 1 mL/min. Results were expressed in milligrams per gram of dry weight.

### Phenolic acids

The mobile phase for chromatographic separation consisted of the solvent A (acetic acid 2%) and solvent B ($CH_3CN$) in the gradient. The flow rate was 0.5 mL/min and the gradient was the following: Until 32 min (95% A, 5% B); 35-40 min (100% A, 0 % B); 63 min (70% A, 30% B), 73 min (0% A, 100% B).

### Caffeoylquinic acid (CQA) derivatives

Mobile phase A consisted in TFA (0.05%) and mobile phase B was $CH_3CN$. The flow rate was 1.0 mL/min and the gradient was the following: 0-5 min (100% A); 5-45 min (35% A, 65% B). The absorption wavelength was monitored at 328 nm. Six CQA derivatives: 3-caffeoylquinic acid (3-CQA), 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, 3,4,5-tricaffeoylquinic acid, 1,3,4,5-tetracaffeoylquinic acid (tetra-CQA) were quantified. 3-CQA was purchased from Sigma and di and tri-CQA derivatives from Biopurity Phytochemicals Limited, while tetra-CQA was purified previously by HPLC.[24]

### Statistical analyses

Values were expressed as means of three replicates determinations ± standard deviation. Variance analysis, using Turkey HSD's post-test $P < 0.05$, was applied.

## RESULTS

### Evaluation of the antioxidant potential of fractions from polar extracts

The *in vitro* antioxidant potential and the estimation of phenolic content of several fractions obtained by fractionation from EtOAc and *n*-BuOH leaf crude extracts of *P. carolinensis* and *P. rosea* were measured, and they are shown in Table 1.

In general, the estimation of phenolic content was considerably higher in fractions from both *n*-BuOH extracts than those obtained from EtOAc ones. In EtOAc extracts of *P. carolinensis*, the highest phenolic contents were found in the fractions (11-14) varying from 5.6 ± 0.2 to 21.0 ± 0.2 mg GAE/gDE. In *P. rosea,* phenolics were only detected in the most polar fractions (10-13) from 1.1 ± 0.1 to 4.0 ± 0.2 mg GAE/g DE. In both extracts, the highest contents were found in the most of polar fractions eluted (fraction 14 of *P. carolinensis* and fraction 13 for *P. rosea*).

Phenolic content in *n*-BuOH extracts from *P. carolinensis* varied from 13.8 ± 0.2 to 159.5 ± 3.7 mg GAE/g DE in different fractions, with the highest content in fractions 2, 4 and 6 and in *P. rosea* from 2.6 ± 0.2 to 180 ± 10.5 mg GAE/g DE with the highest contents in fraction 3, 5, 6 and 7.

DPPH scavenging activity of the different fractions showed a similar trend to those obtained from phenolic compound assays. Fractions eluted from *n*-BuOH extracts showed in general, considerably higher antioxidant potential than those obtained from EtOAc extracts. Generally, the fractions with the highest concentrations in phenolic compounds were also those with the highest antioxidant potential. In EtOAc extracts, the highest level of antioxidants was also detected in the most polar fractions in the range of 15.8 ± 0.5-26.5 ± 0.7 mg TE/g DE for *P. carolinensis* and for *P. rosea*.

The ORAC assay revealed the highest antioxidant potential in fraction 11-14 and 8-13 from EtOAc extracts of *P. carolinensis* and *P. rosea,* respectively. The highest antioxidant potential from *n*-BuOH extracts were measured in fractions 2, 4 and 6 for *P. carolinensis* and in fractions 3, 6 and 7 for *P. rosea*, the same fractions as revealed with DPPH.

### Phenolic profile in polar fractions of *P. carolinensis* and *P. rosea*

The fractions from EtOAc and *n*-BuOH extracts of the species *P. carolinensis* and *P. rosea* were analyzed by reversed phase-HPLC and results were expressed in mg of phenolic/gDW (Table 2).

### Antioxidant potential of pure phenolic compounds

As an attempt to correlate the antioxidant potential to specific flavonoids and phenolic acids detected in Cuban *Pluchea* species we evaluated the DPPH and ORAC antioxidant potential of each compounds and compared with the standard Trolox (Figure 1).

**Table 1: Antioxidant capacity (DPPH and ORAC) and total phenolic content of fractions from 5 g of EtOAc and 1 g of *n*-BuOH leaf extracts of *P. carolinensis* and *P. rosea***

| Fraction | Phenolics (mg GAE/gDE) | DPPH (mgTE/gDE) | ORAC (mgTE/gDE) | Phenolics (mgGAE/gDE) | DPPH (mgTE/gDE) | ORAC (mgTE/gDE) |
|---|---|---|---|---|---|---|
| | *P. carolinensis (EtOAc)* | | | *P. rosea (EtOAc)* | | |
| 1 | t | 11.3±3.6$^{d}$ | 7.0±1.1$^{g}$ | - | - | - |
| 2 | t | t | 10.2±2.6$^{f}$ | - | - | - |
| 3 | t | t | 14.0±0.5$^{f}$ | - | - | - |
| 4 | t | t | 13.8±0.1$^{f}$ | - | - | - |
| 5 | 1.1±0.1$^{f}$ | 10.5±0.1$^{d}$ | 4.3±0.5$^{h}$ | - | - | - |
| 6 | t | t | t | - | - | - |
| 7 | t | t | 2.7±0.8$^{i}$ | - | - | - |
| 8 | 1.6±0.1$^{f}$ | 2.0±0.1$^{f}$ | 5.2±1.2$^{h}$ | - | - | 16.1±0.9$^{c}$ |
| 9 | 2.1±0.1$^{e}$ | 2.1±0.1$^{f}$ | 23.9±1.7$^{e}$ | - | - | 1.2±0.1$^{e}$ |
| 10 | 1.6±0.1$^{f}$ | 2.0±0.2$^{f}$ | 12.5±1.5$^{f}$ | 2.4±0.1$^{c}$ | 2.7±0.2$^{c}$ | 13.5±2.0$^{c}$ |
| 11 | 5.6±0.2$^{d}$ | 7.4±0.4$^{e}$ | 50.0±0.6$^{c}$ | 3.6±0.2$^{b}$ | 6.4±0.6$^{b}$ | 21.2±0.9$^{b}$ |
| 12 | 10.3±0.2$^{c}$ | 15.8±0.5$^{c}$ | 36.5±1.2$^{d}$ | 1.1±0.1$^{d}$ | 1.7±0.1$^{d}$ | 8.0±0.3$^{d}$ |
| 13 | 13.4±0.3$^{b}$ | 20.7±0.1$^{b}$ | 90.4±9.4$^{b}$ | 4.0±0.2$^{a}$ | 8.7±0.4$^{a}$ | 57.0±2.6$^{a}$ |
| 14 | 21.0±0.2$^{a}$ | 26.5±0.7$^{a}$ | 107.1±5.2$^{a}$ | Nf | Nf | Nf |
| Fraction | *P. carolinensis* (*n*-BuOH) | | | *P. rosea* (*n*-BuOH) | | |
| 1 | t | t | t | 2.6±0.2$^{f}$ | 3.7±0.1$^{f}$ | 19.8±0.6$^{e}$ |
| 2 | 125.4±2.8$^{c}$ | 192.1±0.3$^{a}$ | 543.0±64.6$^{a}$ | 24.5±0.9$^{e}$ | 38±0.2$^{e}$ | 171.3±11.6$^{c}$ |
| 3 | 29.1±1.3$^{d}$ | 54.8±0.3$^{c}$ | 121.2±7.1$^{c}$ | 98.4±2.1$^{c}$ | 156.3±0.1$^{b}$ | 401.3±16.1$^{a}$ |
| 4 | 150.1±1.4$^{b}$ | 162.4±0.6$^{b}$ | 501.4±49.7$^{a}$ | 70.2±1.8$^{d}$ | 91.7±0.3$^{c}$ | 120.9±13.8$^{d}$ |
| 5 | 13.8±0.2$^{e}$ | 26.5±0.1$^{d}$ | 36.4±1.1$^{d}$ | 89.8±6.2$^{c}$ | 82.3±0.3$^{d}$ | 162.9±14.7$^{c}$ |
| 6 | 159.5±3.7$^{a}$ | 181.0±0.1$^{a}$ | 383.1±20.6$^{b}$ | 109.1±5.2$^{b}$ | 156.7±0.2$^{b}$ | 415.4±15.3$^{a}$ |
| 7 | Nf | Nf | Nf | 180±10.5$^{a}$ | 188.1±5.5$^{a}$ | 336.3±45.9$^{b}$ |

Nf: No fraction was eluted, -: No detection, t: ≤1 mg TE or GAE/gDE (dry extract). The antioxidant capacity for each individual technique was compared between fractions of the same species. Different letters indicated significant statistical differences. Coefficients of variance for total phenolics, DPPH and ORAC were lower than 10%. DPPH: 2,2-diphenyl-1 picrylhydrazyl, ORAC: Oxygen radical scavenging capacity, mg GAE/g DE: Milligrams of gallic acid equivalents per gram of dry extract, mg TE/g DE: Milligrams of Trolox equivalents per gram of dry extract, EtOAc: Ethyl acetate, *n*-BuOH: *n*-butanol, *P. carolinensis: Pluchea carolinensis, P. rosea: Pluchea rosea*

**Table 2: Quantification of phenolic compounds in *P. carolinensis* and *P. rosea* by HPLC-diode array detector**

| Classification | Phenolics | *P. carolinensis* | *P. rosea* |
|---|---|---|---|
| Cinnamic acids | Caffeic acid | 0.29±0.03$^{h}$ | 0.28±0.01$^{f}$ |
| | Ferulic acid | *0.11±0.01$^{k}$ | Nd |
| | Rosmarinic acid | 0.69±0.02$^{f}$ | Nd |
| Caffeoylquinic acid derivatives | 3-CQA | 1.49±0.21$^{e}$ | 0.30±0.01$^{f}$ |
| | 3,4-diCQA | 8.99±0.30$^{b}$ | 1.20±0.05$^{e}$ |
| | 3,5-diCQA | 3.84±0.31$^{c}$ | 3.95±0.09$^{d}$ |
| | 4,5-diCQA | 21.2±0.4$^{a}$ | 25.6±0.2$^{a}$ |
| | triCQA | 2.42±0.09$^{d}$ | 8.92±0.32$^{b}$ |
| | tetra-CQA | 1.44±0.14$^{e}$ | 5.79±0.22$^{c}$ |
| Flavonoids | Casticin | Nd | *0.19±0.01$^{g}$ |
| | Herbacetin | 0.01±0$^{j}$ | Nd |
| | Isorhamnetin | 0.13±0.04$^{i}$ | Nd |
| | Kaempferol | 0.11±0.04$^{i}$ | Nd |
| | Luteolin | 0.11±0.01$^{i}$ | Nd |
| | Myricetin | 0.43±0.03$^{g}$ | Nd |
| | Quercetin | 0.12±0.01$^{i}$ | Nd |
| | Quercitrin | 0.06±0.01$^{j}$ | Nd |
| | Quercetagetin | 0.02±0$^{j}$ | *0.24±0.04$^{g}$ |
| | Rutin | Nd | 0.4±0.02$^{f}$ |

Nd: Not detected, 3-CQA: 3-caffeoylquinic acid, 3,4-diCQA: 3,4-dicaffeoylquinic acid, 3,5-diCQA: 3,5-dicaffeoylquinic acid, 4,5-diCQA: 4,5-dicaffeoylquinic acid, triCQA: 3,4,5-tricaffeoylquinic acid, tetra-CQA: 1,3,4,5-tetracaffeoylquinic acid. In each column, different letters mean significant difference at $P \leq 0.05$. Results are expressed as milligrams of phenolic per gram of dry weight. *($10^{-2}$). DAD: Diode array detector, HPLC: High performance liquid chromatography

The majority of the flavonoids showed lower values of DPPH antioxidant potential than Trolox. The group of isoflavones, apigenin and casticin (the most methoxylated flavonols) did not react with the DPPH. On the other hand, luteolin, herbacetin, hyperoside and quercetagetin showed the highest values of antioxidant potential (Figure 1a). Ferulic acid, caffeic acid and rosmarinic acid displayed lower antioxidant potential than Trolox (Figure 1b). Tri and tetra-CQA derivatives showed the best antioxidant potential. All the phenolics assayed, exhibited a higher ORAC antioxidant activity than Trolox. The biggest differences were observed for the flavonols herbacetin, quercitrin and spiraeoside that showed an antioxidant potential about 8 times higher than Trolox (Figure 1a and b). The DPPH and ORAC antioxidant potential of phenolic compounds showed very low correlation coefficients.

In our study, several phenolic compounds were identified, therefore, we decided to estimate the contribution of the total phenolic content as well as of each individual pure chemical quantified to the total antioxidant potential of the crude extracts. The sum of DPPH and ORAC antioxidant potential of each individual phenolic identified by HPLC was computed. The contributions to the total antioxidant potential of the crude extract are shown in Table 3.

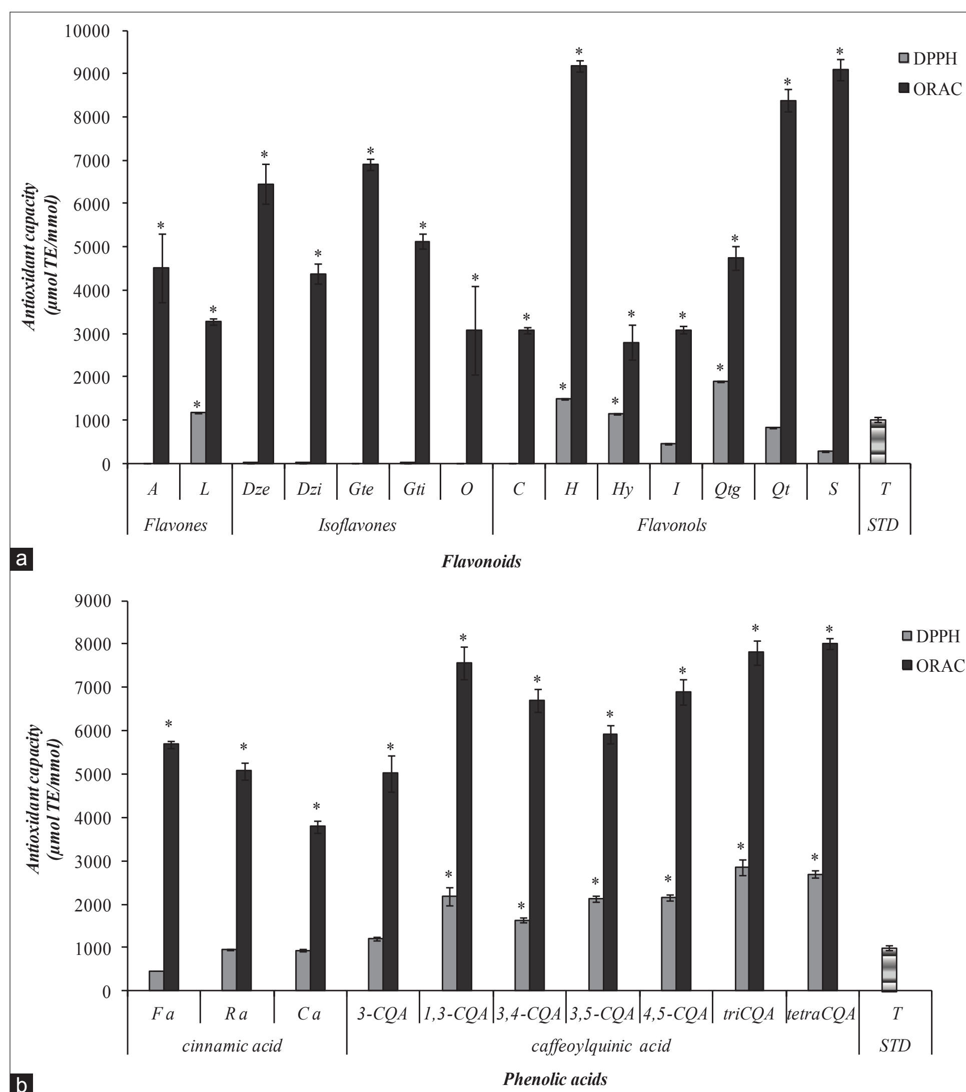


**Figure 1:** Antioxidant capacity of phenolic pure compounds: a (flavonoids) and b (phenolic acids). Results for DPPH and ORAC were expressed in µmol TE/mmol. All the analyses were performed in triplicate. Abbreviations: Apigenin (A), Luteolin (L), Daidzein (Dze), Daidzin (Dzi), Genistein (Gte), Genistin (Gti), Ononin (O), Casticin (C), Herbacetin (H), Hyperoside (Hy), Isorhamnetin (I), Quercetagetin (Qtg), Quercitrin (Qt), Spiraeoside (S), Ferulic acid (F a), Rosmarinic acid (R a), caffeic acid (C a), 3-caffeoylquinic acid or chlorogenic acid (3-CQA), 3,4-dicaffeoylquinic acid (3,4-CQA), 3,5-dicafeoylquinic acid (3,5-CQA), 4,5-dicaffeoylquinic acid (4,5-CQA), 3,4,5-tricaffeoylquinic acid (triCQA), 1,3,4,5-tetracaffeoylquinic acid (tetraCQA), Trolox (T), standard (STD). * indicate positive significant statistical differences compared to the standard. Tukey HSD's post hoc test $P < 0.05$, $n = 3$

The estimation to the contribution of the antioxidant potential of the phytochemicals identified to the total antioxidant potential (DPPH and ORAC) of the leaf extracts from *P. carolinensis* and *P. rosea* (without forgetting we are not taking into account the intermolecular interactions as well, only the sum of the antioxidant potential of each individual phenolic) are shown in Figure 2.

The contributions of all the phytochemicals (expressed in percent) to the DPPH antioxidant potential in the leaf crude extracts of *P. carolinensis* was 38.7% and in *P. rosea* 49.7%. Additionally, the ORAC contribution in *P. carolinensis* was 53.2% and in *P. rosea* 52.4%. The phenolic compounds identified and quantified in both species contribute significantly to the total antioxidant potential of the crude extracts of both species. Three phytochemical groups were mainly identified in both species: Cinnamic acids, CQAs and flavonoids (flavones and flavonols). The contribution of each groups to the total antioxidant potential of the leaf extracts of *P. carolinensis* were calculated independently (Figure 3).

## DISCUSSION

The highest antioxidant potentials and phenolic content were previously reported in polar EtOAc and *n*-BuOH leaf

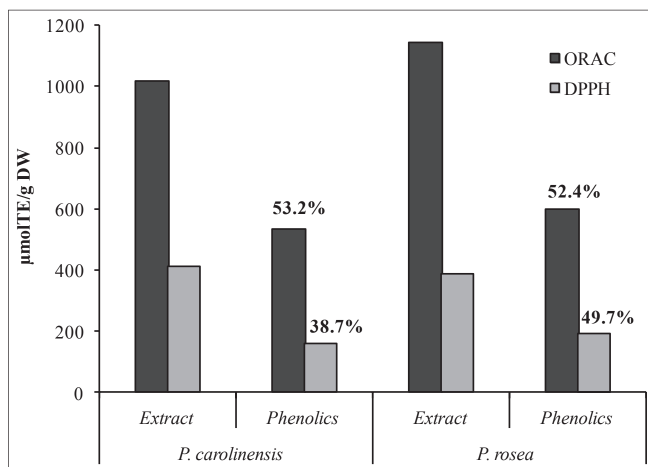


**Figure 2:** Estimation of the contribution of the antioxidant potential of all the phenolics to the total antioxidant potentials (2,2-diphenyl-1 picrylhydrazyl and oxygen radical scavenging capacity) of the leaf extracts of *Pluchea carolinensis* and *Pluchea rosea*

**Table 3: Determination of the contribution to the antioxidant capacity (2,2-diphenyl-1 picrylhydrazyl and oxygen radical scavenging capacity) of each pure compound identified in *Pluchea carolinensis***

| Chemicals | Phenolics identified | Concentration (mg/gDW) | µmol TE/g DW | |
|---|---|---|---|---|
| | | | DPPH | ORAC |
| Cinnamic acid | Caffeic acid | 0.29 | 1.53 | 6.16 |
| | Ferulic acid | 0.0011 | 0 | 0.03 |
| | Rosmarinic acid | 0.69 | 1.84 | 9.72 |
| CQAs | 3-CQA | 1.49 | 5.08 | 27.19 |
| | 3,4-diCQA | 8.99 | 28.44 | 116.59 |
| | 3,5-diCQA | 3.84 | 15.80 | 43.91 |
| | 4,5-diCQA | 21.17 | 88.69 | 282.38 |
| | triCQA | 2.42 | 10.17 | 27.88 |
| | tetra-CQA | 1.44 | 6.22 | 20.13 |
| Flavonoids | Herbacetin | 0.01 | 0.04 | 0.24 |
| | Isorhamnetin | 0.13 | 0.19 | 1.27 |
| | Kaempferol | 0.11 | 0.10 | 0.72 |
| | Luteolin | 0.11 | 0.45 | 1.26 |
| | Myricetin | 0.44 | 0.8 | 1.55 |
| | Quercetin | 0.12 | 0.14 | 0.42 |
| | Quercetagetin | 0.02 | 0.12 | 0.3 |
| | Quercitrin | 0.06 | 0.11 | 1.12 |
| Phytochemicals | | 41.34 | 160 | 541 |
| Crude extract | | | 413 | 1016 |

Values of the antioxidant capacity for quercetin, kaempferol, myricetin previously determined by Tabart *et al.* (2009) were used to estimate the contribution of these molecules to the total antioxidant of crude extracts. CQAs: Caffeoylquinic acids, DPPH: 2,2-diphenyl-1 picrylhydrazyl, ORAC: oxygen radical scavenging capacity, 3-CQA: 3-caffeoylquinic acid, 3,4-diCQA: 3,4-dicaffeoylquinic acid, 3,5-diCQA: 3,5-dicaffeoylquinic acid, 4,5-diCQA: 4,5-dicaffeoylquinic acid, triCQA: 3,4,5-tricaffeoylquinic acid, tetra-CQA: 1,3,4,5-tetracaffeoylquinic acid, ORAC: Oxygen radical scavenging capacity

crude extracts of the species *P. carolinensis* and *P. rosea*.[10] As a first step for identifying the major antioxidant constituents, the EtOAc and *n*-BuOH extracts of both species were fractionated. We correlated the antioxidant

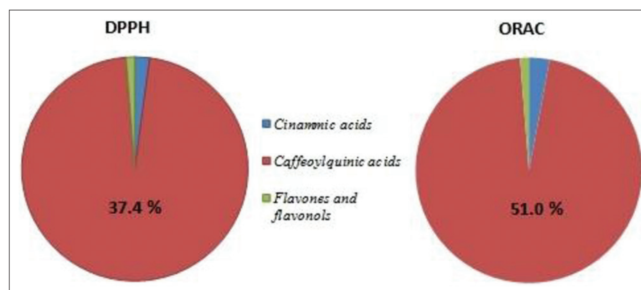


**Figure 3:** Contribution of each phytochemical group to the 2,2-diphenyl-1 picrylhydrazyl and oxygen radical scavenging capacity antioxidant potential of the leaf extracts of *Pluchea carolinensis*

potential and the phenolic content in *n*-BuOH fractions of both species. The highest correlation coefficients were observed in *P. carolinensis* and *P. rosea* for DPPH versus. total phenolics with $r^2 = 0.9365$ and $r^2 = 0.8793$ respectively. Correlation coefficients of ORAC vs. phenolic were lower with $r^2 = 0.8715$ and $r^2 = 0.511$ for *P. carolinensis* and *P. rosea* respectively.

Various analytical methods were developed to identify and quantify some abundant phenolic compounds in plant kingdom as well as others previously describe in the genus *Pluchea*.[16,25,26] Nineteen phenolics were identified in the fractions: Three cinnamic acids, six CQAs, a flavone and nine flavonols including seven aglycone and two glycoside forms. In general, the CQAs were detected in higher concentrations than the other phenolic acids and flavonoids measured. The 3,4-CQA isomer was the compound prevalent in both species. Seven of the flavonoids identified were flavonol aglycones, two flavonol glycosides (quercitrin and rutin) and one flavone aglycone (luteolin). These results are consistent with those observed in the genus *Pluchea* in which the occurrence of flavonoid aglycones is more frequently reported than flavonoid glycosides.[15-17,25,26] Some of the phytochemicals were identified for the first time in *P. carolinensis* and in *P. rosea* and the antioxidant potential was assayed. These results are in accordance with those observed for flavonoids and phenolic acids.[27]

The antioxidant potential of plant organs from Cuban species of *Pluchea* has been recently assayed. Leaves followed by inflorescences were the plant organs with highest *in vitro* antioxidant potential.[28] It's well-known that a crude extract is composed by a mixture of several phytochemicals, sometimes with insufficient structural knowledge. Moreover, intermolecular interactions trigger antagonistic or synergistic effects in the antioxidant properties of natural mixtures. The understanding of this phenomenon is not very clear until today thus it is difficult

to predict the behavior of a mixture of various chemicals. Hence, the contribution to the antioxidant potential of each pure chemical to the total antioxidant potential of the crude extracts is very difficult to establish. Thus, results showed that CQAs contribute more significantly to the total antioxidant potentials of the crude extract. Cinnamic acids and flavonoids contributed very slightly. This fact suggests that the antioxidant potential found in *P. carolinensis* is due, at least partially, to the CQAs.

## CONCLUSION

The DPPH and ORAC antioxidant potential of several fractions from polar EtOAc and *n*-BuOH extracts of the species *P. carolinensis* and *P. rosea* were evaluated. Frequently, fractions from *n*-BuOH extracts showed higher antioxidant potential than those from EtOAc extracts. In general, highest correlation between antioxidant potential and phenolic content by Folin-Ciocalteu assay were computed. Various antioxidant compounds were identified in leaf extracts, almost all the phytochemicals were reported for the first time in Cuban *Pluchea* species. Additionally, rosmarinic acid, ferulic acid, quercetagetin, herbacetin and quercitrin were identified for the first time in *Pluchea* genus. The phenolic identified contributes significantly to the total antioxidant potential of the crude extracts (around 50%). Apparently, CQA derivatives are the metabolites which contribute more significantly to the total antioxidant potential of the crude extracts.

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