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Phenol content and antioxidant activity of different young and adult plant parts of tobacco from Tunisia, dried at 40 and 70 ºC

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ABSTRACT

Different parts of tobacco plants were dried at 40 and 70 ºC. Some of them were also dried at room temperature. Dried plant material was extracted by sonication for obtaining hydro-alcoholic extracts (70%). Total phenol and total flavonoids were determined as well as antioxidant activities which were evaluated through different methods (capacity for scavenging DPPH, ABTS, superoxide and hydroxyl radicals; capacity for preventing lipid peroxidation using egg yolk as substrate; and reducing power). In young and adult plants, leaves generally had higher amounts of phenols (14.46-23.05 mg GAE g^{-1}) than the remaining parts of the plant, independent on the temperature used. Generally, roots had lower amounts of phenols (1.56-4.63 mg GAE g^{-1}). Leaves and flower had significantly higher concentrations of flavonoids (3.08-4.17 mg QE g^{-1} and 1.17-2.12 mg QE g^{-1}, respectively) than the remaining parts. The antioxidant activity was generally higher in leaf extracts, although stalk ones had also a good capacity for scavenging hydroxyl radicals. Generally, young plants had the best capacity for scavenging DPPH and hydroxyl free radicals which may be related with the phenol content. Concerning drying temperatures, the results were not conclusive.

INTRODUCTION

Nicotiana tabacum L. belongs to Solanaceae family. It is a perennial herbaceous plant from South America. Such species is one of the most commercially valued agricultural crops in the world due to its application in cigarette industry. Nevertheless in China, it can also be used as anesthetic, diaphoretic, sedative and emetic agent in folk medicine (Chen *et al.*, 2012). Beyond alkaloids, such properties have been attributed to several other compounds, such as sesquiperepnoids, diterpenoids, phenols and their homologous, essential oils, and proteins (Petterson *et al.*, 1993; Shinozaki *et al.*, 1996; Wei *et al.*, 2005; Feng *et al.*, 2010; Chen *et al.*, 2012; Zhang *et al.*, 2012). Over 20% of tobacco resources are discarded as processing waste, polluting the environment. Knowing that leaves are rich in bioactive compounds as referred above, such may be utilized as a good resource of those natural constituents (Ru *et al.*, 2012).

According to Torras-Claveria *et al*. (2012), the levels of phenylpropanoid compounds increase under water stress or senescent conditions, with the exception of hydroxycinnmaic acid amides which decrease in senescent samples, and 4-*O*-*p*-coumaroylquinic acid and trihydroxycinnamic acid-*O*-glucoside, which did not exchange.

The antimicrobial and antioxidant of leaf tobacco were already reported by some authors (Wang *et al.*, 2008; Ru *et al.*, 2012).

Since some authors (Torras-Claveria *et al.*, 2012) have already reported the importance of the stage of tobacco leaves on the type and concentration of phenols as well as the antioxidant activity of leaves (Wang *et al.*, 2008; Ru *et al.*, 2012), in the present work we intended to study the importance of plant part used from young and adult plants and drying temperatures on the total content of phenols and antioxidant activity measured through several methods.

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MATERIAL AND METHODS

Plant material

Young and adult plants of *Nicotiana tabacum* were collected at Cap-Bon (northeast Tunisia). In the laboratory, the different parts of plants were separated and each one was divided in three sets: One of them was dried in a forced-draught oven at 70 ºC, the second one was dried at 40 ºC and the last one was left in a fresh, dark place of the laboratory and maintained at 20 ºC. After dried and before extraction, plant material was reduced to powder.

Extraction of phenols

Extraction of phenols was performed by sonication on ice bath for 6 min using a VC300 Vibracell sonicator with a 20 kHz frequency. One gram of dried powder in 10 mL of a hydro-alcoholic solution (70%) was used. After sonication, the samples were centrifuged for 5 min, at 2,000 g at 20 ºC and the supernatant was removed and kept at −20 ºC until determination of total phenols and antioxidant activities. Gallic acid was used as standard for the construction of calibration curve. The results are presented as mg Gallic Acid Equivalent g-1 extract (mg GAE g-1).

Determination of total phenols (Folin-Ciocalteau)

The extracts total phenol content was determined using the Folin-Ciocalteau reagent and gallic acid as standard as described by Slinkard and Singleton (1977). The sample (0.5 mL) and 2 mL of sodium carbonate (75 g L^{-1}) were added to 2.5 mL of 10% (v/v) Folin-Ciocalteau reagent. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm.. Tests were carried out in triplicate.

Determination of total flavonoids

Total flavonoid content was quantified as described by Ahn *et al*. (2007). Briefly, 0.5 mL of 2% $AlCl_3$-ethanol solution was added to 0.5 mL of sample or standard. After 1 h at room temperature, the absorbance was measured at 420 nm. Quercetin was used as standard for the construction of calibration curve. The results are presented as mg Quercetin Equivalent g^{-1} extract (mg QE g-1).

ANTIOXIDANT ACTIVITY

Thiobarbituric acid reactive species (TBARS)

The ability of samples to inhibit lipid peroxidation was determined by using a modified thiobarbituric acid reactive species (TBARS) assay. Egg yolk homogenates were used as a lipid-rich medium obtained as described by Dorman *et al*. (1995). The absorbance of the organic upper layer was measured at 532 nm. All of the values were based on the percentage antioxidant index (AI%), whereby the control was completely peroxydized and each oil demonstrated a degree of change; the percentage inhibition was calculated using the formula $[(A_o-A_1/A_o)\times100]$, where A_o was the absorbance of the blank sample and A_1 was the absorbance of the sample, was plotted against sample concentrations and IC_{50} was determined (concentration of extract able to prevent 50% lipid oxidation).

$ABTS^+$ free radical-scavenging activity

The determination of $ABTS^{\bullet+}$ radical scavenging was carried out as reported by Dorman and Hiltunen (2004). The values of IC_{50} were determined as reported above. Tests were carried out in triplicate.

Free radical scavenging activity (DPPH)

A hydro-alcoholic stock solution (50 µL) of each sample at different concentrations was placed in a cuvette, and 2 mL of 60 µM methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was added (Brand-Williams *et al.*, 1995). Absorbance measurements were made at 517 nm after 60 min of reaction at room temperature. The values of IC_{50} were determined as reported above. Tests were carried out in triplicate.

Superoxide anion scavenging activity (non-enzymatic method)

Measurements of superoxide anion scavenging activity of samples were based on the method described by Soares (1996). Superoxide anions were generated in a non-enzymatic phenazine methosulfate nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by reduction of nitroblue tetrazolium (NBT). Tests were carried out in triplicate. The sample concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against extract concentrations.

Hydroxyl radical scavenging activity

The assay of OH-scavenging activity was developed according to Chung *et al*. (1997) with small modifications. Briefly, the reaction mixture was prepared with 10 mM $FeSO_4{\cdot}7H_2O$, 10 mM EDTA, 10 mM 2-deoxyribose, 0.1 M phosphate buffer and sample in a test tube to give a total volume of 1.8 mL. Finally, 200 µL of H_2O_2 was added to the mixture, which was incubated at 37 ºC for 4 h. After that, 1 mL trichloroacetic acid (2.8%) and 1 mL thiobarbituric acid (1%) were added to the test tube, which was boiled for 10 min. After cooling, its absorbance was measured at 520 nm.

The OH-scavenging activity (%) was calculated using the following equation: Inhibition (%) $[(A_o-A_1)/A_o] \times 100$, where A_o is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample. Tests were carried out in triplicate. The sample concentration providing 50% inhibition (IC_{50}) was obtained by plotting the inhibition percentage against extract concentrations.

Reducing power determination

The reductive potential of the samples was determined according to the method of Oyaizu (1986). The assay was carried out in triplicate. Each sample was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A

portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 2,000 g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm.

STATISTICAL ANALYSIS

Statistical analysis was performed with the SPSS 18.0 software (SPSS Inc.). Statistical comparisons were made with one-way ANOVA followed by Tukey multiple comparisons. Paired Student t test were used in some tests to determine differences at 5% and 1% significance.

RESULTS AND DISCUSSION

Phenol content

The phenol (mg GAE g^{-1}) and flavonoid (mg QE g^{-1}) concentrations are depicted in Table 1. In young and adult plants, leaves generally had higher amounts of phenols (14.46-23.05 mg GAE g^{-1}) than the remaining parts of the plant, independent on the temperature used. One exception was observed in those samples dried at 40 ºC (4.54 mg GAE g^{-1}). In this case, plantlets had higher amounts of total phenols (8.64 mg GAE g^{-1}). This exception is difficult to explain. Generally, roots had lower amounts of phenols (1.56-4.63 mg GAE g^{-1}), although in some cases such difference was not significantly different ($p<0.05$) from those found for petioles or stalks, mainly when samples were dried at 40 ºC (4.02 and 4.98 mg GAE g^{-1}, respectively).

Differences between young and adult plant in what concerns the total phenol content were observed but dependent on the part of plant used. For example, comparing the same part of plant dried at the same temperature (70 ºC), it was observed that roots and petioles ($p<0.01$; t-Student) and leaves ($p<0.05$; t-Student) of young plants had higher total phenol content than the corresponding parts in adult plants (Table 1). At 40 ºC, roots and leaves from adult plants had higher amounts of phenols than the corresponding young plants, nevertheless we did not prefer to compare the results of leaves dried at 40 ºC, because we consider it an anomalous value (Table 1).

Generally, in young plants, the levels of phenols were higher when dryness was made at 70 ºC and/or room temperature (roots, stalks and leaves). In plantlets, such difference was not significant (Table 1). However, those differences were not observed in adults' plants, in which the levels of phenols were independent on the drying temperature. The sole exception was roots, in which the concentration of phenols was higher in those samples dried at 40 ºC (4.63 mg GAE g^{-1}) than in those maintained at 70 ºC (1.56 mg GAE g^{-1}) ($p<0.01$, t-Student).

The concentrations of phenols found in leaves in the present work were significantly lower than those reported by Karabegović *et al*. (2011), nevertheless different solvents and sonication time were used. For extracting phenols, the authors used methanol and acetone; and the extraction procedure was sonication as we do, but for 30 minutes, whereas in our case only 6 minutes of sonication were used. In our case the extraction solvent was a hydro-alcoholic solution (70%).

Beyond extraction solvent, type and/or time of sonication and temperature, the type of plant material is also important on the total phenol content (Karabegović et al., 2011). The concentrations of phenols detected in the present work are closer to those reported by Wang *et al.* (2008) who found 23.6 mg GAE g^{-1} in tobacco leaves. The plant material dried at 40 ºC was not submitted to the analysis of flavonoids. Roots had very low amounts of flavonoids, mainly in adult plants. Such compounds were not found in this plant part. Leaves and flower had significantly higher concentrations of flavonoids (3.08-4.17 mg QE g^{-1} and 1.17-2.12 mg QE g^{-1}, respectively) than the remaining parts (Table 1).

Differences between young and adult plant in what concerns the flavonoid content were only observed in stalks ($p<0.01$; t-Student), when comparing the same part of plant dried at the same temperature (70 ºC). Young plant presented higher concentrations of flavonoids than adult ones (Table 1). In the remaining parts of plants, significant differences were not found.

In adult plants, extracts obtained from petioles dried at 70 ºC had higher amounts of flavonoids (1.37 mg QE g^{-1}) when compared to those dried at 40 ºC (0.40 mg QE g^{-1}). The inverse was observed for leaf extracts: samples dried at 40ºC presented higher concentrations of flavonoids (4.77 mg QE g^{-1}) than those samples dried at 70 ºC (3.16 mg QE g^{-1}) or at room temperature (3.76 mg QE g^{-1}) (Table 1). In the remaining parts of plants, significant differences were not found (Table 1). Explanations for such results are quite difficult to find.

The amounts of flavonoids are significantly inferior to those of total phenols (Table 1). In the present work, the identification of compounds was not performed; nevertheless other authors demonstrated that the predominant compounds in tobacco plants were neochlorogenic and cryptochlorogenic acids, that is, hydroxycinnamoylquinic acids. In addition, the levels of these compounds along with other ones also identified by the authors were highly dependent on the development phase (senescent and young tissue) of the plant and stress conditions (water stress) in which they had been submitted (Torras-Claveria *et al*., 2012). In this work, rutin and kaempferol-7-*O*-neohesperidoside were the predominant flavonoids found in tobacco tissues, although in much lower concentrations than hydroxycinnamoylquinic acids.

Antioxidant activity

Phenol compounds have been reported as possessing antioxidant activity among other biological properties, acting as scavenging free radicals or preventing their formation. Several methods may be used for evaluating in vitro antioxidant activity of phenolic extracts. They may be divided in two main groups: those that evaluate lipid peroxidation and those that measure free radical scavenging ability (Sánchez-Moreno, 2002). In the present work, Trolox Equivalent Antioxidant Capacity (TEAC) or ABTS method, 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods, capacity for scavenging superoxide and hydroxyl radicals, reducing power and capacity for preventing lipid peroxidation in the presence of

egg yolk as lipid substrate were assayed in some extracts of tobacco samples. Lipid peroxidation inhibition by the Thiobarbituric Acid Reactive Species (TBARS) measures the malondialdehyde (MDA) formed after lipid hydroperoxide decomposition, which forms a pink chromophore with thiobarbituric acid (TBA). This coloured complex absorbs at 532 nm and results from the condensation of two equivalents of TBA and one equivalent of MDA in an acid environment (Aazza *et al.*, 2011). By this method, young plant material dried at 70 ºC revealed that leaves and petioles had the best capacity for preventing oxidation, although at 40 ºC, differences in the activities among samples were not found. In adult plants, the extracts of leaves dried at 70 ºC had the highest antioxidant activity, whereas at 40ºC, leaves and stalks had similar capacity for preventing lipid oxidation (Table 2). Overall, leaves presented the best capacity for preventing lipid peroxidation which may be related to the phenol content (Table 1).

The developmental stage of the plant did was not influenced by the drying temperature (70 ºC, 40 ºC or room temperature) (Table 2). At 70 ºC, the IC_{50} values of young and adult leaf extracts were 57.56 ± 3.85 µg mL^{-1} and 44.21 ± 4.14 µg mL^{-1}, respectively, whereas at 40 ºC, IC_{50} values were 68.24 ± 10.45 and 69.23 ± 2.95 µg mL^{-1}, respectively. At room temperature, such values were 24.89 ± 0.84 and 32.24 ± 1.52 µg mL^{-1}, respectively. Therefore, comparing young and adult plants for every temperature, the IC_{50} values were not significantly different ($p<0.05$, t-Student). In both young and adult plants, drying leaves at room temperature revealed to be more adequate for obtaining extracts with higher capacity for preventing lipid peroxidation (Table 2).

In adult plants, extracts obtained from leaves dried at 70 ºC and room temperature had better antioxidant activity that when dried at 40 ºC. In the remaining extracts, the temperature of dryness did not affect the antioxidant activity (Table 2).

In young plants, leaves dried at room temperature produced extracts with better capacity for preventing lipid peroxidation than in the remaining temperatures of dryness (Table 2). In adult plants, leaves dried at room temperature and 70 ºC produced the best antioxidant activities (Table 2).

Oven drying is simple but has the disadvantage that many changes may occur before the material is free of water. Some authors consider that oven drying, even at 100 ºC, is a process that essentially involves “moist incubation” of the tissue. This drying procedure may give rise to autolysis of the material which may be so great that its final composition in secondary metabolites may be quite different when compared to that of fresh material (Marur and Sodek, 1995). In our case, we did not compare antioxidant activities of dried material with those of fresh plant material, but different drying temperatures influenced the antioxidant activity which may be an indication of alteration of chemical composition of extracts.

Maximum allowable temperatures depend on the chemical composition of the secondary metabolites. For example, for glycosides, maximal temperature allowed is 100 ºC, whereas for mucilage species, temperature cannot overpass 65 ºC and for aromatic plants, the maximal temperature permitted is 35-45 ºC (Müller and Heindl, 2006). Although these roles, modifications may occur and be responsible for the diverse antioxidant activities found.

Concerning the capacity for scavenging free radicals, particularly ABTS ones, leaves and flowers independent on the drying temperatures had the best capacity for scavenging those radicals (Table 2). In young plants, leaf extracts also had the best capacity for scavenging those free radicals, along with plantlets.

Young and adult leaves dried at 70 ºC did not present significant differences ($p<0.05$, t-Student) in the capacity for scavenging ABTS free radicals (4.76 ± 2.03 µg mL^{-1} and 4.24 ± 5.01 µg mL^{-1}, respectively). The same happened when both young and adult leaves were dried at room temperature (5.17 ± 6.08 µg mL^{-1} and 6.34 ± 0.57 µg mL^{-1}, respectively) (Table 2). In young plants, drying leaves at 70 ºC or room temperature did not produce significant differences in the antioxidant activity. Different drying temperatures (70 ºC, 40 ºC or room temperature) used in adult samples did not produce extracts with significant differences in the capacity for scavenging ABTS free radicals (Table 2).

DPPH is a stable free radical that accepts one electron or hydrogen radical to become a stable diamagnetic molecule. The reduction ability of DPPH radicals formation was determined by the decrease of absorbance at 517 nm induced by the extracts (Aazza *et al.*, 2011).

The capacity for scavenging DPPH free radicals by samples obtained from young plants were higher in leaf extracts either in those samples dried at 70 or 40 ºC. In adult plants, leaf and flower extracts had also the best capacity for scavenging those free radicals (Table 2).

Young leaves dried at 70 ºC had higher capacity for scavenging DPPH free radicals (5.49 ± 1.56 µg mL^{-1}) than adult leaves (10.00 ± 3.85 µg mL^{-1}) dried at the same temperature ($p<0.05$, t-Student). Adult leaves dried at 40 ºC (1.13 ± 0.62 µg mL^{-1}) presented significant higher capacity ($p<0.01$, t-Student) for scavenging those radicals than young leaves (3.23 ± 0.90 µg mL^{-1}). The leaf extracts obtained from young plants dried either 70 or 40 ºC did not reveal significant differences in the capacity for scavenging free radicals in contrast to those observed from adult plants, in which drying temperature of 70 ºC was worse than the remaining drying temperatures (Table 2). Stalk and leaf extracts obtained from young and adult plants dried at 70 ºC were the best for scavenging superoxide anion radicals. At 70 ºC of drying temperature, in both young and adult plants, the capacity for scavenging superoxide anion radicals did not differ significantly ($p<0.05$, t-Student) (Table 2). When drying temperature of plant material was 40 ºC, stalk extracts of young plants had better capacity for scavenging these radicals (42.53 ± 1.00 µg mL^{-1}) than the respective adult plants (59.22 ± 7.87 µg mL^{-1}) ($p<0.01$, t-Student). The principle of the method used in the present work, that measures the capacity for scavenging hydroxyl radicals, is based on the formation of

malondialdehyde from the decay of the desoxyribose which reacts with thiobarbituric acid giving rise to a pink pigment, such as reported for the TBARS method (Boulanouar *et al.,* 2013). Stalk extracts obtained from young plants dried at 40 ºC or at room temperature had the best capacity for scavenging free hydroxyl radicals. In adult plants and at 70 ºC of drying temperature, flower extracts had the best capacity for scavenging these free radicals (Table 2). When comparing all samples, stalks of young plants dried at room temperature had the best activity (1.65 µg mL^{-1}) (Table 2). Overall, extracts obtained from young plants had better capacity for scavenging hydroxyl radicals than extracts obtained from adult plants, independent on the drying temperature. Two homogenous polysaccharides isolated from flue-cured tobacco leaves and flowers presented capacity for scavenging DPPH and hydroxyl free radicals, according to the results reported by some authors (Xu *et al.,* 2013, 2014). These authors associated such activities with the polysaccharides present in the fractions. According to the same authors, the activities might be attributed to their hydroxyl groups and other functional groups (COOH, C=O, -O-) which could donate electrons, reducing the radicals to a more stable from or reacting with free radicals to terminate the radical chain reaction (Leung *et al.,* 2009). In our case, we did not determine the polysaccharide content of extracts, only phenol content was evaluated. The content of phenols were generally superior in young plants than in adult ones (Table 1) and therefore responsible for the best antioxidant activity found, nevertheless we cannot rule out the hypothesis that the polysaccharides also have an important role in the ability to scavenge free radicals. Fe(III) reduction can be used as an indicator of electron-donating activity and therefore reflects an important mechanism of phenolic antioxidant action. In this study, the reducing power was evaluated by monitoring the ferric-ferrous transformation at 700 nm (Bentes *et al.,* 2009). Figures 1-4 present the reducing power of different extracts of plant parts of tobacco dried at 70 or 40 ºC. In all cases, such activity was dose-dependent. The reducing power was always higher in extracts obtained from leaves, independent on the developmental stage or drying temperature (Fig. 1-4). In the opposite site, the extracts obtained from roots had the worst reducing power. Leaf extracts obtained from young plants and dried at 70 ºC had higher reducing power than those dried at 40 ºC. In contrast, leaf extracts obtained from adult plants had higher activity when dried at 40 ºC. The effect of temperature on antioxidant activity was already reported previously by Larrauri *et al.* (1997) but in other plant material. These authors reported that higher drying temperatures decreased the antioxidant activity of samples. In our case and only considering those extracts with the best activities (leaves), the drying temperature was also important on the reducing power of extracts but this property was also highly dependent on the developmental stage of plants, because in young samples, the best drying temperature was 70 ºC, whereas 40 ºC was the best one for obtaining extracts with the best reducing power.

Table. 1: Phenol and flavonoid content (mg g^{-1}, dry weight) found in plant extracts (Mean ± standard error).

Part of plant	Phenols (mg GAE g^{-1})					
	Young plant			Adult plant		
	70 ºC	40 ºC	Room temperature	70 ºC	40 ºC	Room temperature
Roots	$4.63\pm1.59^{b,A,**}$	$3.25\pm0.44^{c,C++}$	$4.00\pm1.68^{b,B}$	$1.56\pm1.05^{c**,oo}$	$4.63\pm1.90^{c++,oo}$	nd
Petioles	$6.32\pm1.59^{b**}$	nd	nd	$4.65\pm1.05^{b**,NS}$	$4.02\pm1.90^{c,NS}$	nd
Stalk	$5.33\pm1.59^{b;A;ns}$	$5.03\pm0.44^{b;B,ns}$	$6.36\pm1.68^{b,A}$	$5.70\pm1.05^{b;ns;NS}$	$4.98\pm1.90^{c;ns,NS}$	nd
Leaves	$23.05\pm1.59^{a,A,*}$	$4.54\pm0.44^{b,B,++}$	$18.65\pm1.68^{a,A}$	$14.46\pm1.05^{a*,A}$	$20.34\pm1.90^{a++,A}$	18.36 ± 5.16^{A}
Flowers	nd	nd	nd	$12.11\pm1.05^{a;NS}$	$13.33\pm1.90^{b,NS}$	nd
Plantlets	$7.13\pm1.59^{b;NS}$	$8.64\pm0.44^{a;NS}$	nd	nd	nd	nd
			Flavonoids (mg QE g-1)			
	70ºC	40ºC	Room temperature	70ºC	40ºC	Room temperature
Roots	0.05 ± 0.43^{d}	nd	nd	Ndet	Ndet	nd
Petioles	$1.36\pm0.43^{bc,ns}$	nd	nd	$1.37\pm0.28^{bc,ns,oo}$	$0.40\pm0.15^{c,oo}$	nd
Stalk	$0.50\pm0.43^{cd,**}$	nd	nd	$0.83\pm0.28^{c,**,NS}$	$0.76\pm0.15^{bc,NS}$	nd
Leaves	$3.26\pm0.43^{a,ns,NS}$	nd	3.08 ± 1.02^{NS}	$3.16\pm0.28^{a,ns,B}$	$4.77\pm0.15^{a,A}$	3.73 ± 0.789^{B}
Flowers	nd	nd	nd	$2.12\pm0.28^{b,NS}$	$1.17\pm0.15^{b,NS}$	nd
Plantlets	2.44 ± 0.43^{ab}	nd	nd	nd	nd	nd

Nd: not determined, Ndet: not detected.

In phenols, values followed by the same lower case (in the columns) are not significantly different ($p<0.05$, Tukey test for multiple comparisons).
In flavonoids, values followed by the same lower case (in the columns) are not significantly different ($p<0.05$, Tukey test for multiple comparisons).
**: statistically significant differences between content of phenols or flavonoids in young and adult plants comparing the same part of plant and dried at 70 ºC ($p\leq0.01$; Student t-test), *: statistically significant differences between content of phenols or flavonoids in young and adult plants comparing the same part of plant and dried at 70ºC ($p\leq0.05$; Student t-test), $^{++}$: statistically significant differences between content of phenols or flavonoids in young and adult plants comparing the same part of plant and dried at 40ºC ($p\leq0.01$; Student t-test), $^{+}$: statistically significant differences between content of phenols in young and adult plants comparing the same part of plant and dried at 40ºC ($p\leq0.05$; Student t-test), ^{oo}: statistically significant differences between content of phenols or flavonoids in adult plants or young plants comparing the same part of plant and dried at 70ºC and 40ºC ($p\leq0.01$; Student t-test)., ns: not significant when comparing content of phenols or flavonoids in young and adult plants comparing the same part of plant and dried at 70ºC ($p\leq0.05$; Student t-test), *ns*: not significant when comparing content of phenols or flavonoids in young and adult plants comparing the same part of plant and dried at 40ºC ($p\leq0.05$; Student t-test)., In phenols, values followed by the same upper case (in the rows) are not significantly different ($p<0.05$, Tukey test for multiple comparisons)., In flavonoids, values followed by the same upper case (in the rows) are not significantly different ($p<0.05$, Tukey test for multiple comparisons)., NS: not significant when comparing content of phenols or flavonoids in adult plants comparing the same part of plant and dried at 70ºC and 40ºC ($p\leq0.05$; Student t-test).

Table. 2: Antioxidant activity of hydro-alcoholic extracts obtained from different parts of tobacco plants dried at different temperatures, expressed in IC_{50} (µg mL^{-1}) (Mean ± standard error).

Part of plant	Young plant			Adult plant		
	TBARS					
	70 ºC	**40 ºC**	**Room temperature**	**70 ºC**	**40 ºC**	**Room temperature**
Roots	96.91±3.85$^{a,\circ\circ}$	58.16±10.45$^{a,++,\circ\circ}$	nd	nd	95.64±2.95$^{a,++}$	
Petioles	50.55±3.85^{b,*}	nd	nd	93.38±4.15^{a,*,NS}	91.33±2.95^{a,NS}	
Stalk	100.18±3.85$^{a,ns;A}$	83.30±10.45^{a,ns,A}	60.03±0.84^{a,A}	87.74±4.15^{a,ns,NS}	74.58±2.95^{b,ns,NS}	
Leaves	57.56±3.85^{b,ns,A}	68.24±10.45^{a,ns,A}	24.89±0.84$^{c,B,\underline{NS}}$	44.21±4.15^{b,ns,B}	69.23±2.95^{b,ns,A}	32.24±1.52$^{\underline{NS},B}$
Flowers	nd	nd	nd	nd	nd	
Plantlets	nd	nd	35.85±0.84^{b}	nd	nd	
	TEAC					
	70 ºC	**40 ºC**		**70 ºC**	**40 ºC**	
Roots	21.06±2.03^{b,*}	21.94±1.76$^{\underline{ns},+}$	14.77±6.08^{a}	68.90±5.01^{a,*,NS}	64.62±4.03$^{a,+,NS}$	
Petioles	47.37±2.03^{a,ns}	nd	nd	46.38±5.01^{b,ns,NS}	29.39±4.03^{b,NS}	
Stalk	24.99±2.03^{b,ns}	24.34±2.12$^{\underline{ns},ns}$	15.44±6.08^{a}	19.78±5.01^{c,ns,NS}	19.28±4.03^{bc,ns,NS}	
Leaves	4.76±2.03^{c,ns}	nd	5.17±6.08$^{b,\underline{NS}}$	4.24±5.01^{c,ns,A,}	7.94±4.03^{c,A}	6.34±0.57$^{\underline{NS},A}$
Flowers	nd	nd	nd	8.45±5.01$^{c,\circ}$	19.05±4.03$^{c,\circ}$	
Plantlets	10.24±2.03$^{c,\circ}$	nd	6.08±6.08$^{b,\circ}$	nd	nd	
	DPPH					
	70 ºC	**40 ºC**		**70 ºC**	**40 ºC**	**Room temperature**
Roots	48.70±1.56$^{b,**,\circ\circ}$	10.63±0.90$^{b,++,\circ\circ}$	nd	97.18±3.85^{a,**,NS}	96.51±0.62$^{a,++,NS}$	nd
Petioles	91.27±1.56^{a,**}	nd	nd	45.44±3.85^{b,**}	94.00±0.62^{b}	nd
Stalk	44.34±1.56$^{b,ns,\circ}$	23.89±0.90$^{a,+,\circ}$	nd	29.31±3.85$^{c,ns,\circ\circ}$	94.60±0.62$^{ab,+,\circ\circ}$	nd
Leaves	5.49±1.56^{d,*,NS}	3.23±0.90$^{c,++,,NS}$	nd	10.00±3.85^{d,*,A}	1.13±0.62$^{c,++,B}$	3.58±0.42^{,B}
Flowers	nd	nd	nd	20.16±3.85^{d}	2.84±0.62^{c}	nd
Plantlets	34.19±1.56^{c,A}	12.91±0.90^{b,B}	8.98±0.24^{,B}	nd	nd	nd
	Superoxide					
	70 ºC	**40 ºC**		**70 ºC**	**40 ºC**	**Room temperature**
Roots	60.92±2.93^{bc,*}	74.21±1.00^{a}	nd	77.93±2.99^{a,*}	nd	nd
Petioles	86.23±2.93^{a,*}	nd	nd	77.70±2.99^{a,*}	48.36±7.87^{a}	nd
Stalk	56.52±2.93^{c,ns,B}	42.53±1.00$^{c,++,B}$	93.60±0.18^{A}	55.95±2.99^{b,ns,NS}	59.22±7.87$^{a,++,NS}$	nd
Leaves	52.89±2.93^{c,ns}	nd	nd	56.57±2.99^{b,ns,NS}	67.04±7.87^{a,NS}	
Flowers	nd	nd	nd	76.54±2.99^{a}	43.40±7.87^{a}	nd
Plantlets	70.14±2.93^{b}	58.52±1.00^{b}	nd	nd	nd	nd
	Hydroxyl					
	70 ºC	**40 ºC**		**70 ºC**	**40 ºC**	**Room temperature**
Roots	58.27±3.16’’,^{**,NS}	59.80±5.59$^{b,+,NS}$	nd	86.49±2.29^{a,**}	89.02±3.16$^{a,+}$	nd
Petioles	nd	nd	nd	74.34±2.29^{b}	74.34±3.16^{b}	nd
Stalk	nd	23.19±5.59$^{c,+}$	1.65±3.93^{b}	72.15±2.29^{b}	66.48±3.16$^{b,+}$	nd
Leaves	nd	44.59±5.59$^{b,++}$	61.31±3.93^{a}	38.49±2.29^{c}	66.86±3.16$^{b,++}$	14.43±3.84
Flowers	nd	nd	nd	29.19±2.29^{d}	Nd	nd
Plantlets	93.95±1.85’’,^{NS}	91.24±5.59^{a,NS}	73.08±3.93^{a}	nd	Nd	nd

nd: not determined

In every antioxidant activity, values followed by the same lower case (in the columns) are not significantly different ($p<0.05$, Tukey test for multiple comparisons).

**: statistically significant differences between antioxidant activity in young and adult plants comparing the same part of plant and dried at 70ºC ($p\leq0.01$; Student t-test), *: statistically significant differences between antioxidant activity in young and adult plants comparing the same part of plant and dried at 70ºC ($p\leq0.05$; Student t-test), $^{++}$: statistically significant differences between antioxidant activity in young and adult plants comparing the same part of plant and dried at 40ºC ($p\leq0.01$; Student t-test), $^{+}$: statistically significant differences between antioxidant activity in young and adult plants comparing the same part of plant and dried at 40ºC ($p\leq0.05$; Student t-test), $^{\circ\circ}$: statistically significant differences between antioxidant activity in adult plants or young plants comparing the same part of plant and dried at 70ºC and 40ºC ($p\leq0.01$; Student t-test)., $^{\circ}$: statistically significant differences between antioxidant activity in adult plants or young plants comparing the same part of plant and dried at 70ºC and 40ºC ($p\leq0.05$; Student t-test)., ns: not significant when comparing antioxidant activity in young and adult plants comparing the same part of plant and dried at 70ºC ($p\leq0.05$; Student t-test), *ns*: not significant when comparing antioxidant activity in young and adult plants comparing the same part of plant and dried at 40ºC ($p\leq0.05$; Student t-test)., In every antioxidant activity, values followed by the same upper case (in the rows) are not significantly different ($p<0.05$, Tukey test for multiple comparisons)., NS: not significant when comparing antioxidant activity in young and adult plants comparing the same part of plant and dried at 70ºC and 40ºC ($p\leq0.05$; Student t-test), NS: not significant when comparing antioxidant activity in young and adult plants comparing the same part of plant and dried at room temperature ($p\leq0.05$; Student t-test).

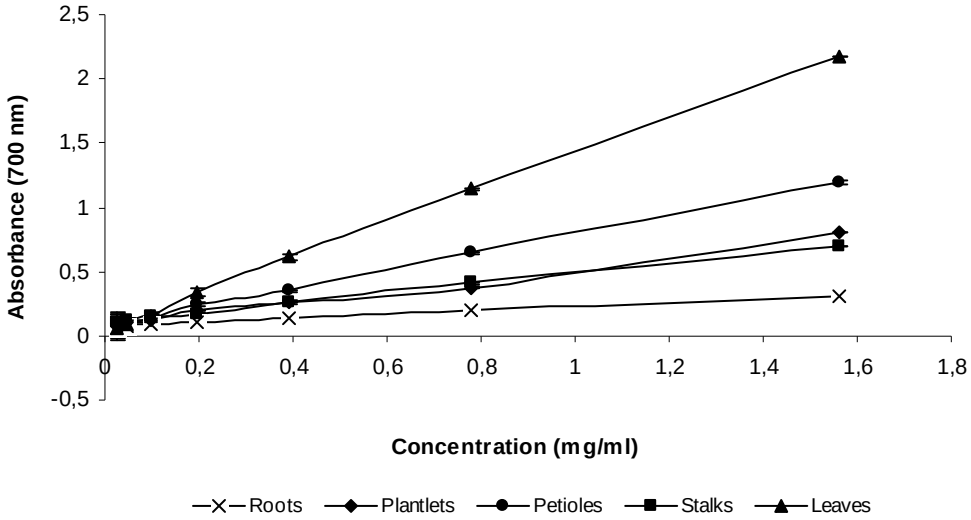

Fig. 1: Reducing power of the extracts obtained from young tobacco samples dried at 70 ºC.

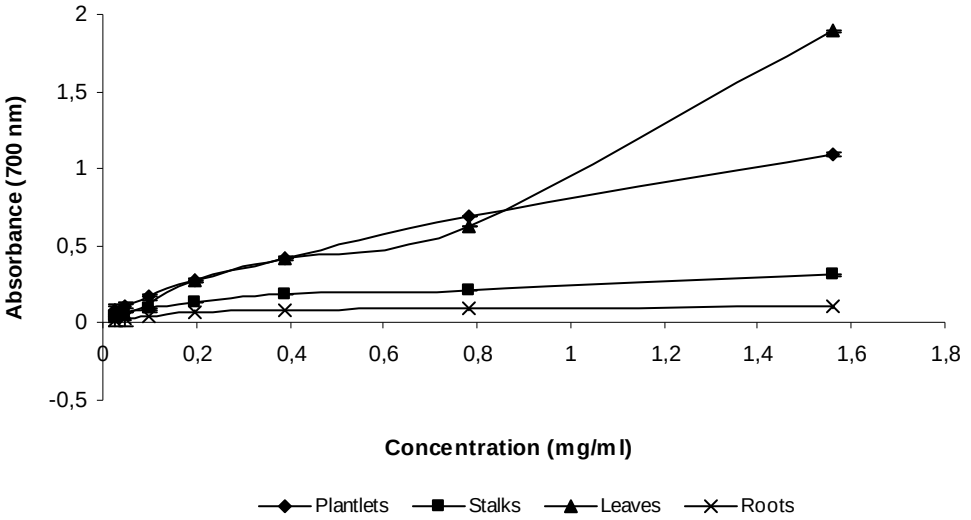

Fig. 2: Reducing power of the extracts obtained from young tobacco samples dried at 40 ºC.

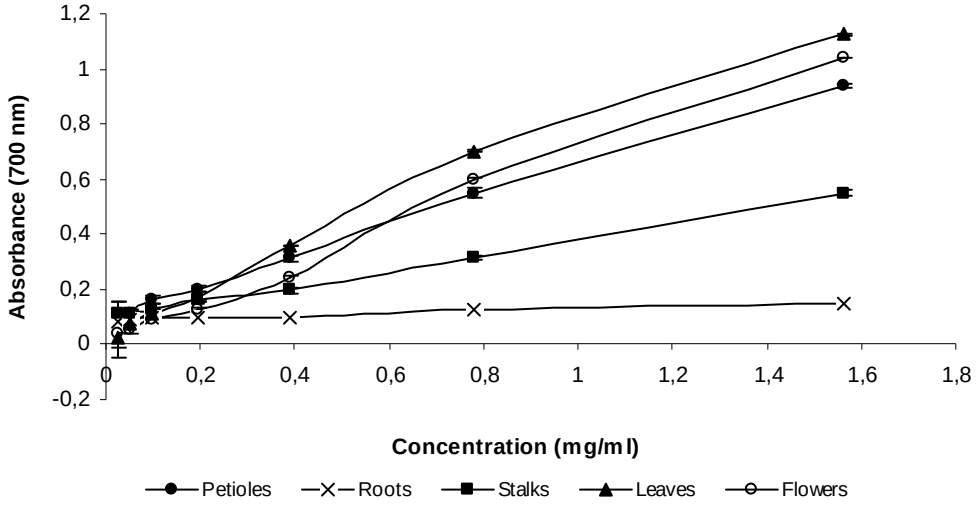

Fig. 3: Reducing power of the extracts obtained from adult tobacco samples dried at 70 ºC.

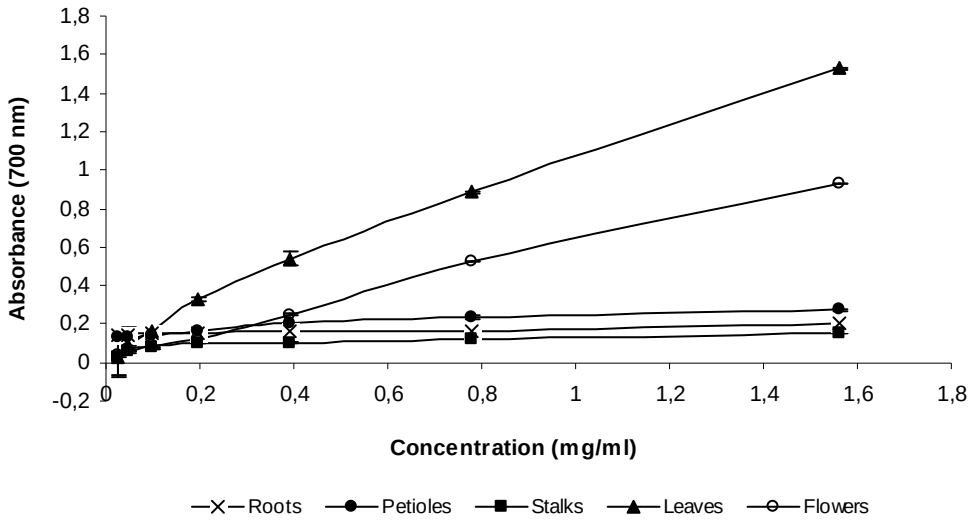

Fig. 4: Reducing power of the extracts obtained from addult tobacco samples dried at 40 ºC.

CONCLUSION

In young and adult plants, leaves generally had higher amounts of phenols than the remaining parts of the plant, independent on the temperature used. Leaves and flower had significantly higher concentrations of flavonoids in contrast to roots which presented the lowest amounts. The antioxidant activity was generally higher in leaf extracts, although stalk extracts had also a good capacity for scavenging hydroxyl radicals. Generally, young plants had the best capacity for scavenging DPPH and hydroxyl free radicals. Concerning drying temperatures, the results were not conclusive, in this way studies that address energy costs in the drying process of the plant material must be carried out in the future.

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