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ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACTS AND ALKALOID FRACTIONS OF FOUR MEXICAN MEDICINAL PLANTS OF SOLANACEAE

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Abstract

Background: Methanol extracts and alkaloid fractions of different parts of four plant species belonging to Solanaceae family and used in Mexican traditional medicine were investigated for their total phenolic contents, anti-inflammatory and antioxidant properties.
Materials and Methods: The total phenolic compounds of each extract was determined according to the Folin-Ciocalteu method, while the *in vitro* radical scavenging activities of the extracts were assessed using the DPPH and ABTS radicals. The *in vivo* anti-inflammatory activity was determined using the TPA-induced mouse ear edema model.
Results: The methanol extracts contained the highest concentrations of phenolic compounds and also exhibited the best reducing power on the DPPH and ABTS radicals, in a concentration-dependent fashion. However, the anti-inflammatory activity did not follow the same trend, as some alkaloid fractions that showed low radical reducing power exhibited the strongest anti-inflammatory activity.
Conclusion: The methanol extract obtained from the flowers of *Nicotiana glauca* presented the best overall performance with the largest amount of phenolic compounds (111 µg garlic acid equivalents/g of extract), the best antioxidant activity (94.80% inhibition of DPPH and 97.57% of ABTS) and the highest anti-inflammatory activity (81.93% inhibition of the inflammation).

Keywords: Solanaceae family, antioxidant activity, anti-inflammatory activity.

Introduction

Nicotiana glauca Gram. (Tabaquillo), *Nicotiana trigonophylla* Dunal (Tabaquillo delgado), *Solanum rostratum* Dunal (Abrojo), and *Solanum nigrescens* Mart. & Gal. (Hierba Mora), all belonging to Solanaceae, are plants used in traditional medicine of the state of Querétaro, Mexico, to treat inflammation. The infusion of the whole plant of *S. rostratum* and the juice obtained from crushed young leaves of *S. nigrescens* are applied on swollen feet. The epidermis of the leaves of *N. glauca* is placed on the swollen cheek for toothache. The whole plant of *N. trigonophylla* is crushed in alcohol and this extract is rubbed on the swollen parts.
It is well documented that injury to cells may provoke the production of re-active oxygen and nitrogen species, which are thought to promote inflammation (Reuter *et al.*, 2010; Kielland *et al.*, 2009; Kao *et al.*, 2005). Antioxidants are known to suppress inflammation in a rat arthritis model and in a mouse model of contact hypersensitivity (Yoshino *et al.*, 2006). Other studies indicate that alkaloids and especially phenolics contained in medicinal plants have antioxidant and/or anti-inflammatory activity (Kao *et al.*, 2005; Barbosa-Filho *et al.*, 2006; Lopes-Souto *et al.*, 2011; Shaheen *et al.*, 2005). Relationship between these two biological activities has also been established (Scalbert *et al.*, 2005; Jensen *et al.*, 2008).

Nicotiana glauca is native to Argentina, but now is widely distributed throughout the United States. It grows well in warm-arid and semiarid climates on marginal unfertile lands (Biblioteca digital de la medicina tradicional Mexicana, 2009). High levels of anabasine, a piperidine alkaloid isomer of nicotine, have been reported in this plant as far as 1935 (Smith, 1935; Galiana and VigueraLoko, 1964), and confirmed in many other chemical studies (Lisko *et al.*, 2013). Ingestion of this alkaloid can result in severe or lethal poisoning in humans, livestock and poultries (Papavisiliu and Heliakis, 1947; Castorena *et al.*, 1987; Sims *et al.*, 1999; Mizrachi *et al.*, 2000; *Schep et al.*, 2009; Botha *et al.*, 2011; Semmler *et al.*, 2012). Also, teratogenic activity has been attributed to this alkaloid (Keeler *et al.*, 1981; Green *et al.*, 2012). However, no intoxication after external application of preparations from its leaves to treat inflammation has been reported. Besides anabasine, other compounds have been identified in this plant species (Morel *et al.*, 1988; Backheet and Sayed, 2002; Zaher *et al.*, 2009).

Nicotiana trigonophylla is native to northern and western America. It lives in warm, semi-dry and mild climates, as wild plant associated with desert scrub, grassland, forests of oak and pine. This small snuff accompanies the highways and roads in arid areas of our country (Biblioteca digital de la medicina tradicional Mexicana, 2009). Phytochemical studies have shown only the presence of nicotine and nor-nicotine in this species (Bowen, 1945; Baldwin and Ohnmeiss, 1993).

Solanum rostratum is native to Mexico, and is present in warm and dry climates. It grows along rivers, roads and crops, associated with tropical forest, desert scrub, grassland, forests of oak and pine (Biblioteca digital de la medicina tradicional Mexicana, 2009). There are many studies on the chemical composition of this species. The alkaloids α-solanine, β-solanine, γ-solanine α-chaconine, β-chaconine, and γ-chaconine and glycosidic alkaloids have been identified (Zhang *et al.*, 2012; Novruzov *et al.*, 1973) as well as methylprotodioscin (Author *et al.*, 2004), and β-sitosteryl glucoside (Alvarez *et al.*, 2009).

The origins of *Solanum nigrescens* have been located in the southeastern United States, Chile and Argentina. Present in warm and dry climates, it is associated with disturbed vegetation of tropical rain forest, desert scrub, forests of oak and pine (Biblioteca digital de la medicina tradicional Mexicana, 2009). Although this plant is toxic to humans and livestock due to the presence of solanine, tender leaves are cited as edible, while an edible jelly is prepared from the ripe fruits (Vibrans, 2009).
As a contribution to the deep knowledge of our local medicinal plants, the present study was aimed at investigating the *in vivo* anti-inflammatory and *in vitro* antioxidant potential of methanolic and alkaloid extracts from each part of these plants, in order to obtain data that might support the ethno-medical use.

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Materials and Methods

Chemicals

Solvents were either analytical or HPLC grade and were obtained from Baker (Mallinckrodt Baker Inc., Phillipsburg, N.J., U.S.A.). DPPH (1,1-diphenyl-2-picrylhydrazyl), Folin-Ciocalteu reagent, Na_2CO_3, gallic acid, TPA, Trolox [6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid], ferrozine, ferric chloride, $FeSO_4$, 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate ($K_2S_2O_8$), aluminum chloride ($AlCl_3.6H_2O$), acetic acid and sodium acetate were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

Experimental Animals

Adult male mice CD-1 with a body weight ranging from 22 to 28 g were used. All animals had free access to food and water and were kept on a 12/12 hrs light-dark cycle. All experiments with the animals were performed in accordance with the Mexican Official Standard NOM-062-ZOO-1999 for the production, care, and use of laboratory animals (Norma Oficial Mexicana, 2001).

Plant Material

The plants analyzed are annual wild species which grow only during the wet season of the year in semiarid and uncultivated lands, from June to September. Whole plants at the mature stage were collected in the rainy season of 2009 in the following locations in the state of Querétaro: Escolásticas (municipality of Pedro Escobedo): *N. glauca* (voucher no. 877), Doviló (Cadereyta): *N. Trigonophylla* (voucher no. 140), Juriquilla (Querétaro): *S. rostratum* (voucher no. 1), and El Gallo (Colón): *S. nigrescens* (voucher no. 1043). The voucher specimens have been deposited in the Ethno-botanical Collection of the Herbarium of Queretaro "Dr Jerzy Rzedowski", located at the School of Natural Sciences, University of Queretaro, Mexico.

Preparations of methanol extracts and alkaloid fractions

The plants were cleaned and each part separated and immediately subjected to drying process at 39 °C. Dry plant material was then milled and stored protected from light at 4 °C for later use. The plant material was extracted by exhaustive maceration in darkness with methanol. The mixture was filtered and the extract was evaporated under vacuum until dry and then stored at 4 °C. Extraction of the alkaloids was carried out according to methods described in the literature (Maiza-Benabdesselam *et al.*, 2007; Jones and Kinghorn, 2006). Briefly, the plant material (10 g), was defatted with *n*-hexane and extracted with methanol. The MeOH extract was evaporated until dryness, and the residue was then taken up in 20 mL of 1% HCl at pH = 4-5. The aqueous acid solution was adjusted to pH = 8 with concentrated ammonium hydroxide and extracted with chloroform. The organic extracts were dried over anhydrous sodium sulphate and the solvent evaporated to obtain the crude alkaloid extract.

Determination of total phenolics

The total phenolic content of the methanolic extract and the antioxidant power of the alkaloid extract were determined according to the Folin-Ciocalteu colorimetric method (Dewanto et al., 2002). The appropriate dilutions of the extracts were oxidized with 250 mL of 1 N Folin-Ciocalteu reagent. After 5 min, 1.25 mL of a 20% aqueous Na_2CO_3 was added to neutralize the mixture, which was then allowed to stand for 2 hrs. After that, the absorbance was measured against a prepared blank at 760 nm using a spectrophotometer (UV/VIS Lambda 40; Perkin Elmer). The results were expressed as µg of garlic acid equivalents (GAE) per g of extract. All the data were reported as the average of three measurements.

DPPH radical scavenging activity

The radical scavenging activity of the extracts was determined using the stable radical DPPH, according to the method reported by Fukumoto and Mazza (2000). All the reactions were conducted in 96 well micro-plates (Nalge Nunc International, NY, USA). An aliquot of 20 µ L of a methanolic solution of the extracts at various concentrations (10, 100, 500, 1000, 2500, 5000 μg/mL), was mixed with 200 µ L of 150 μM of DPPH in 80% methanol. The controls contained all the reaction reagents, except the extract or positive control substances (Trolox). After 30 min of incubation in darkness at ambient temperature, the resultant absorbance was recorded at 520 nm in a spectrophotometer (SpectraMax Tunable Microplate Reader) (Molecular Devices Co., Sunnyvale, Calif., U.S.A.). The experiments were carried out by using a randomized block design (three blocks): inside each block, every treatment was independently applied three times. The percentage of absorbance inhibition was calculated according to the equation: % inhibition = ([absorbance of control – absorbance of samples]/absorbance of control) × 100.

ABTS Assay

For ABTS assay, the procedure followed the method of Nenadis *et al*. (2004) using the 2,2´-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The method was modified to be conducted in 96 well micro-plates (Nalge Nunc Intl., N.Y., U.S.A.). Briefly, 20 μL aliquots of the extracts were mixed with 230 μL of a previously prepared $ABTS^{\bullet+}$ solution. The controls contained all the reaction reagents except the extract or Trolox. The absorbance was recorded at 730 nm at 0 and 6 min in a Spectra Max Tunable Microplate Reader (Molecular Devices Co.). The percentage of absorbance inhibition was calculated using the same equation as previously described.

Anti-inflammatory activity of methanolic and alkaloid extracts on TPA-induced mouse ear edema

This assay was conducted following the protocol previously described (Qadeer *et al.*, 2007), where groups of three male CD-1 mice were used. Edema was induced by a topical application of 2.5 µg per ear of the edemogen TPA (12-O-tetradecanoylphorbol-13-acetate) dissolved in EtOH. Solutions of the extracts (1 mg/ear) and the standard drug indomethacin (1 mg/ear) were applied to both sides of the right ear (10 µL each side) simultaneously with TPA. The ear swelling was measured before the TPA application and 4 h after, and the edema was expressed as an increase in thickness.

Statistical analysis: Experimental values were given as means ± standard deviation (SD). Statistical significance was determined by one-way analysis of variance (ANOVA). Differences at $P < 0.05$ were considered to be significant. A Tukey test for comparison of multiple means was used.

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Results and discussion

Phenolic compounds, to which many health benefits are attributed (Scalbert *et al.,* 2005; Kaur and Kapoor, 2001), are among the bioactive phytochemicals widely distributed in the plant kingdom. These compounds, reputed to be good antioxidant agents, have great value in preventing the onset and progression of many human diseases caused by the free radicals (Bors and Michel, 2002). Many studies have claimed that the antioxidant activity of plant samples was mainly due to the amount of phenolic compounds they contained (Kaur and Kapoor, 2001). Tables 1-4 summarize the results obtained of total phenolic content (TPC), and anti-inflammatory activity (AIA), of the methanol and alkaloid extracts prepared from *N. glauca*, *N. trigonophylla*, *S. rostratum*, and *S. nigrescens*. TPC, expressed as µ g gallic acid equivalents (GAE), per g of extract was determined according to the Folin-Ciocalteu (F-C) method. However, as it has been proven by some studies, this method is likely to overestimate the phenolic content, as many other compound classes besides phenols also react with the F-C reagent (Ikawa et al., 2003; Everette et al., 2010; Prior *et al.,* 2005), making it more suitable for the estimation of total antioxidant capacity rather than phenolic content (Prior *et al.,* 2005). For this reason, the method was also applied on the alkaloid extracts. The highest content of phenolics was found in different plant organs from one species to another. As shown in Tables 1 and 2, the methanolic extracts of the flowers of *N. glauca* and *S. rostratum* had the highest phenolic contents ($P < 0.05$), which reached 111.7 and 109.50 µ g of GAE/g of extract respectively. *N. trigonophylla* accumulated more phenolics (69.53 µg of GAE/g of extract, $P < 0.05$) in the roots (Table 3), and *S. nigrescens*, in the leaves (85.93 µ g of GAE/g of extract, $P < 0.05$) (Table 4).

The DPPH and ABTS assays (Thaipong *et al.,* 2006), are often used to evaluate the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damages caused by oxidative stress. In these *in vitro* assays, both radicals are reduced to their stable or less reactive derivatives by the antioxidant compounds. Such activity has also been recognized for certain alkaloids (Shaheen *et al.,* 2005; Oloyede *et al.,* 2010; Maiza-Benabdesselam *et al.,* 2007), compounds that are the main chemical characteristic of the Solanaceae family. The results obtained for our extracts are summarized in Tables 1-4. The extracts were capable of scavenging DPPH and ABTS radicals ($P < 0.05$) in a concentration-dependent fashion. Maximum scavenging activities, expressed as percentage of inhibition, was found at the concentrations of 3000 μg/mL and 1000 μg/mL in the DPPH and ABTS methods, respectively. In general, the methanolic extracts showed greater antioxidant activity than the respective alkaloid ones in both assays. As shown in Table 1, the methanolic extract from the flowers of *N. glauca* exhibited the highest percentages of inhibition of DPPH and ABTS radicals, with 94.8% and 97.57%, respectively. However, in the DPPH assay, this extract showed 88.70% inhibition at a much lower concentration (1500 µg/mL). Regarding *N. trigonophylla*, the best inhibition of the radical DPPH was obtained with the methanolic extracts prepared from the flowers (57.61 %), while the greatest inhibition (64.7%), of the radical ABTS was obtained with the methanolic extract from the roots (Table 3). The DPPH scavenging activities of the different extracts from *S. rostratum* are shown in Table 2. Four methanolic extracts (roots, leaves, flowers and fruits) from this species exhibited inhibitions of the DPPH radical between 86.76 and 90.24 %, while the methanolic extracts from leaves, fruits and flowers inhibited the ABTS radical up to 95.84%, 98.04% and 92.71%, respectively. In this plant, the alkaloid extract from stems gave a 93.5 % inhibition of DPPH and 98.87% of ABTS (Table 2). Table 4 shows the results obtained for *S. nigrescens*. The highest antioxidant activity was found for methanolic extracts from leaves, reaching 82.00 % inhibition of the DPPH radical. The behaviors of the alkaloid extracts from this plant species were similar to those observed for the other three plants, that is to say, they showed lower antioxidant activities than those of the respective methanolic extracts. The antioxidant activities obtained in this study are lower than some reported for Asteraceae family plants (Khalighi-Sigaroodi et al., 2012; Gandhiappan and Rengasamy, 2012), which were assessed with extracts obtained from the whole plants, instead of plant parts. However, Mosquera and coworkers (Mosquera *et al.,* 2009) even report no antioxidant activity at all for several species of Asteraceae family.

However, the overall antioxidant power of the extracts obtained from these medicinal plants may be considered as significant compared with that reported for pure quercetin (96.6% at a concentration of 25 □g/ml) (Kruzlicova *et al.,* 2012).

Because phenolics are the most abundant antioxidants in plants, the F–C assay used does provide an acceptable estimation of total phenolic content of the methanolic extracts. However, the alkaloid extracts preparation used in the present study does not allow phenolic compounds to be present in these extracts. So, in this case, the F-C results must be seen as the genuine total antioxidant capacity of the alkaloids themselves. It is worth remembering that the most characteristic secondary metabolites of these four plant species are alkaloids such as anabasine and nicotine in *N. glauca*, nicotine and nor-nicotine in *N. trigonophylla*, α-, β- and γ-solanines, as well as α-, β-, and γ-chaconines in *S. rostratum* and solanine in *S. nigrescens.*

There is evidence suggesting that free radicals and excited-state species play a key role in inflammatory processes (Closa and Folch-Puy, 2004). Damaging of the mucous membrane cells may provoke the production of reactive oxygen species (ROS), which promote inflammation (Yoshino *et al.,* 2006). In this regard, the anti-inflammatory activities of the methanolic and alkaloid extracts from the four plant species were evaluated against TPA-induced mouse ear edema and their inhibitory activities compared with that of indomethacin, a commercially available anti-inflammatory drug that has 91.35% inflammation at a dose of 1.0 mg/ear (Qadeer *et al.,* 2007). As evidenced in Table 1, the extracts from *N. glauca* that showed the highest anti-inflammatory activity were those from the stems (methanolic) (85.99%), flowers (methanolic) (81.93%), and roots (alkaloid), (80.5%). It is important to point out that alkaloid extracts from the roots, seeds, and barks showed a higher percentage of inhibition of inflammation than their respective methanolic extracts. In contrast, the leaves that are the plant parts used in traditional medicine did not produce the greatest anti-inflammatory effect (Figure 1).

As shown in Table 3, all alkaloid extracts from *N. trigonophylla* presented higher anti-inflammatory values than the respective methanolic extracts, the most potent (89.25%) being that obtained from the leaves. In contrast, the methanolic extracts from the stems and flowers exhibited negative values (-32.21% and -7.89% respectively), this indicating pro-inflammatory effects. Despite their lower antioxidant activities (less than 50% inhibition), the methanol (80.29%) and alkaloid extracts (89.25%) from the leaves produced the best anti-inflammatory activities (80.29% and 89.25% respectively).

Of all the extracts prepared from *S. rostratum*, only the alkaloid extract from the leaves markedly inhibited the TPA-induced inflammation, by 94.37% at the dose tested in the experiment (Table 2); nonetheless, this plant had an overall detrimental behavior, as it showed not only the lowest inhibition, but also a pro-inflammatory effect. *S. nigrescens*, on the other hand, had its best anti-inflammatory activity with the methanol extracts prepared from the stems (63.03%), and the leaves (53.46%).

Although antioxidant activity of alkaloids is also documented (Shaheen *et al.,* 2005; Oloyede *et al.,* 2010; Maiza-Benabdesselam *et al.,* 2007), these compounds did not have a reputation as good antioxidant agents; even those of pyridine type, like nicotine, are rather credited with pro-oxidant activity (Sudheer *et al.,* 2005). This is in accordance with the results obtained in the present study. Previous reports have established a good correlation between phenolic content and the radical scavenging activity of an extract (Scalbert *et al.,* 2005). Our results suggest that total phenolics were the major contributors to the DPPH and ABTS scavenging activities of the plants. However, in some cases, the extracts showed an antioxidant activity greater than expected, or on the contrary, a low activity that did not match the content of phenolic compounds.

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Table 1: Total phenolic content, and antioxidant and anti-inflammatory activities of *Nicotiana glauca*.

Plant organ	Methanolic extracts				Alkaloid extracts			
	AIA*(1 mg extract/ear)	Antioxidant activity			AIA*(1 mg extract/ear)	Antioxidant activity		
	% inhibition	F-C**(µg of GAE/g of extract)	DPPH % inhibition	ABTS % inhibition	% Inhibition	F-C**(µg of GAE/g of extract)	DPPH % inhibition	ABTS % inhibition
Roots	56.78±4.00	91.14±0.003^{b}	84.28±0.10^{b}	75.44±3.14^{c}	80.50±1.16	46.05±2.65^{a}	49.17±0.67^{b}	23.60±1.38^{a}
Stems	85.99±0.41	80.80±0.003^{c}	85.54±0.28^{b}	73.29±0.80^{c}	44.73±7.95	39.40±0.001^{a,b}	58.65±0.23^{a}	25.66±1.93^{a}
Leaves	54.60±1.97	61.76±1.150^{d}	63.73±1.82^{d}	77.11±1.02^{c}	37.35±1.44	17.37±1.96^{e}	28.13±0.22^{c,d}	23.48±1.38^{a}
Flowers	81.93±1.53	111.7±0.002^{a}	94.80±0.78^{a}	97.57±0.09^{a}	21.54±1.01	20.39±2.17^{e}	19.78±1.87^{c}	20.98±2.98^{a}
Seeds	49.55±3.08	95.97±0.003^{b}	78.52±1.84^{c}	83.86±0.96^{b}	69.58±5.04	27.66±3.71^{d}	10.09±1.74^{e}	25.16±4.05^{a}
Bark	28.29±1.14	78.19±2.420^{c}	83.50±1.05^{b}	84.80±0.50^{b}	46.49±2.00	35.31±1.61^{b,c}	23.50±2.16^{d}	25.54±3.77^{a}
Trunk	70.41±1.17	61.71±2.300^{d}	80.09±0.98^{c}	77.53±2.05^{b}	22.98±1.41	31.15±0.001^{c,d}	49.51±0.67^{b}	21.49±2.72^{a}

*AIA: anti-inflammatory activity; **FC: Folin-Ciocalteu method for determination of phenolic content in methanolic extract and total Anti-oxidant capacity of the alkaloid extracts. Values are expressed as mean ± SD (n=3). Different letters mean a statistical difference (Tukey test, $\alpha = 0.05$).

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Table 2: Total phenolic content, and antioxidant and anti-inflammatory activities of *Solanum rostratum*.

Plant organ	Methanolic extracts				Alkaloid extracts			
	AIA* (1 mg extract/ear)	Antioxidant activity			AIA* (1 mg extract/ear)	Antioxidant activity		
	% inhibition	F-C** (µg of GAE/g ofextract)	DPPH % inhibition	ABTS % inhibition	% Inhibition	F-C** (µg of GAE/g of extract)	DPPH % inhibition	ABTS % inhibition
Roots	39.23±0.38	23.61±3.05^{c}	90.24±0.99^{a}	52.48±0.68^{c}	-13.95±1.33***	26.89±0.004^{b}	19.14±2.83^{b}	46.69±0.002^{c}
Stems	-17.79±3.0***	74.84±0.001^{b}	62.10±1.92^{b}	44.10±2.35d	-31.76±1.91***	48.12±0.004^{a}	93.50±0.21^{a}	98.87±0.049^{a}
Leaves	4.33±0.83	98.62±1.21^{a}	86.76±0.06^{a}	95.84±1.77^{a,b}	94.37±1.17	33.71±4.24^{b}	13.10±0.52^{b}	50.08±2.44^{b}
Flowers	8.17±0.83	109.5±0.003^{a}	87.15±1.98^{a}	92.71±1.98^{b}	14.28±1.33	34.56±1.26^{b}	16.72±0.72^{b,c}	18.30±1.34^{d}
Fruits	14.19±0.63	106.90±6.18^{a}	89.98±0.79^{a}	98.04±0.04^{a}	ND	ND	ND	ND

*AIA: anti-inflammatory activity; **FC: Folin-Ciocalteu method as described in Table 1; ***Values with negative sign represent pro-inflammatory activity; Values are expressed as mean ± SD (n=3). Different letters mean a statistical difference (Tukey test, $\alpha = 0.05$).

Table 3: Total phenolic content, and antioxidant and anti-inflammatory activities of *Nicotiana trigonophylla*.

Plant organ	Methanolic extracts				Alkaloid extracts			
	AIA* (1 mg extract/ear	Antioxidant activity			AIA* (1 mg extract/ear)	Antioxidant activity		
	% inhibition	F-C**µg of GAE/g of extract	DPPH % inhibition	ABTS % inhibition	% inhibition	F-C** µg of GAE/g of extract	DPPH % inhibition	ABTS % inhibition
Roots	22.25±3.13	69.53±4.06^{a}	39.81±1.86^{b}	64.70±2.33^{a}	47.83±4.65	37.11±2.33^{a}	21.15±0.76^{a}	26.28±3.61^{b,c}
Stems	-32.21±9.8***	56.27±0.008^{a,b}	38.62±0.85^{b}	51.63±1.31^{b}	66.75±1.17	24.73±3.21^{c}	20.48±1.68^{a}	23.03±0.50^{b,c}
Leaves	80.29±1.66	30.48±0.33^{c}	19.70±1.30^{c}	47.39±0.21^{c}	89.25±2.65	9.32±0.57^{d}	10.99±1.53^{b}	39.43±7.63^{a}
Flowers	-7.89±0.87***	22.33±0.37^{c}	57.61±2.12^{a}	31.97±2.01^{d}	55.24±2.46	42.82±0.37^{a}	12.99±1.74^{b}	30.84±6.53^{a,b}

*AIA: anti-inflammatory activity; **FC: Folin-Ciocalteu method as described in Table 1; ***Values with negative sign represent pro-inflammatory activity. Values are expressed as mean ± SD (n=3). Different letters mean a statistical difference (Tukey test, $\alpha = 0.05$).

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Table 4: Total phenolic contents, and antioxidant and anti-inflammatory activities of *Solanum nigrescens*.

Plant organ	Methanolic extracts				Alkaloid extracts			
	AIA* (1 mg extract/ear)	Antioxidant activity			AIA* (1 mg extract/ear)	Antioxidant activity		
	% inhibition	F-C** (µg of GAE/g of extract)	DPPH % inhibition	ABTS % inhibition	% Inhibition	F-C** (µg of GAE/g of extract)	DPPH % inhibition	ABTS% inhibition
Roots	10.46±0.50	37.12±0.003^{c}	43.57±1.66^{b}	53.98±1.60^{b}	7.39±1.77	32.15±5.51^{b}	35.97±1.46^{b}	23.35±6.72^{a}
Stems	63.03±4.10	57.12±0.002^{b}	53.47±1.98^{c}	45.79±1.69^{c}	2.35±1.04	42.80±5.40^{a}	39.32±1.18^{a}	24.40±2.08^{a}
Leaves	53.46±1.27	85.93±0.104^{a}	82.00±0.98^{a}	62.03±0.53^{a}	42.35±2.04	49.69±1.51^{a}	20.79±1.16^{c}	20.14±1.17^{a}
Fruits	36.37±1.78	72.85±0.001^{a}	75.56±0.86^{b}	63.37±0.71^{a}	21.51±2.10	7.49±1.91^{c}	40.58±1.28^{a}	27.92±5.88^{a}

*AIA: anti-inflammatory activity; **FC: Folin-Ciocalteu method as described in Table 1; Values are expressed as mean ± SD (n=3).
Different letters mean a statistical difference (Tukey test, $\alpha = 0.05$).

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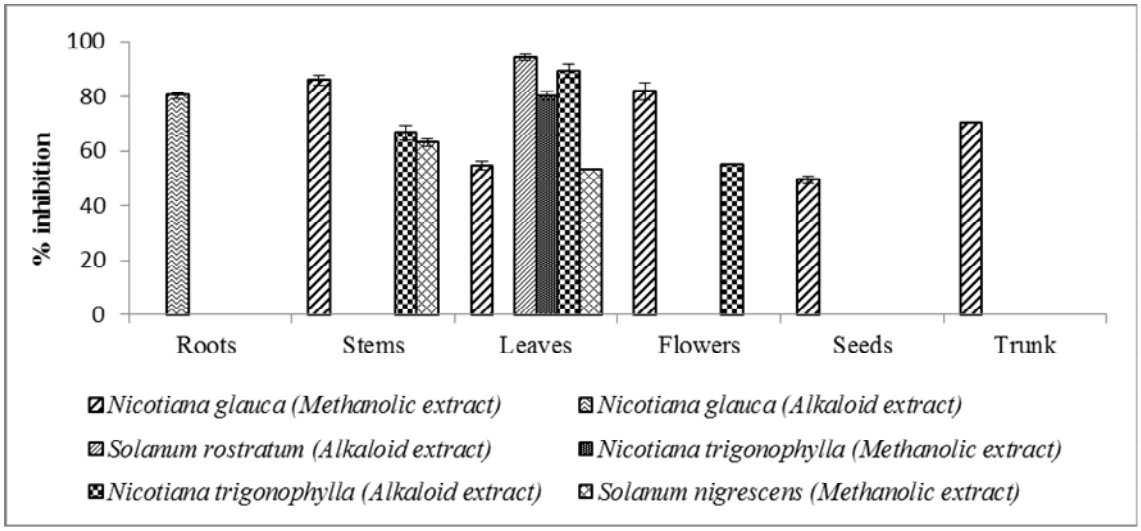

Figure 1: Plants'parts with anti-inflammatory activity.

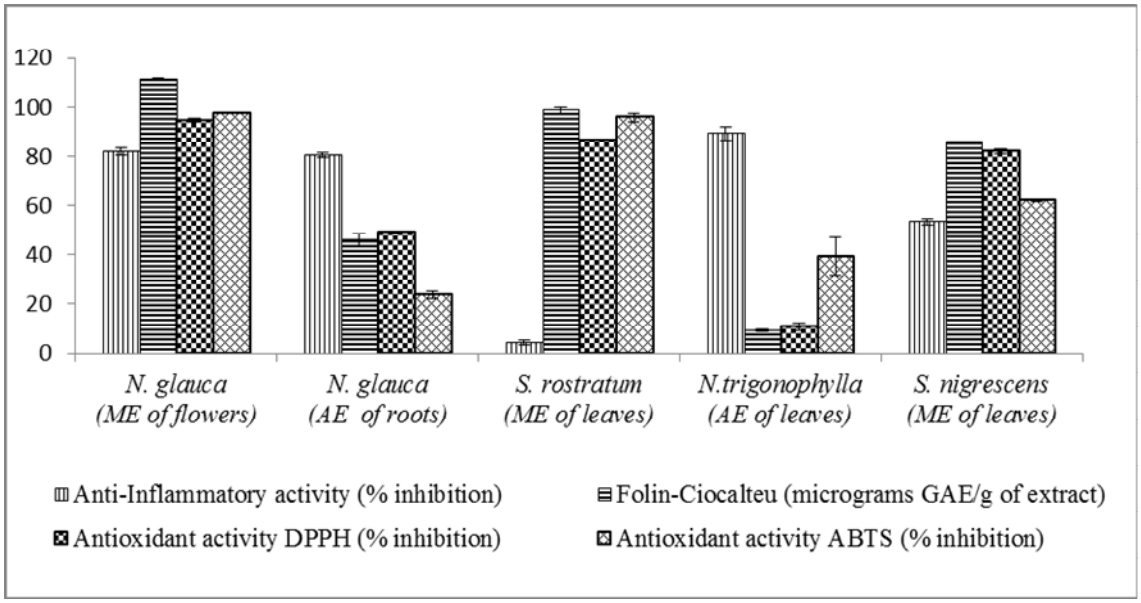

Figure 2: Anti-inflammatory *versus* antioxidant activities of the extracts (ME = methanolic extract, AE = alkaloid extract).

This indicates that the antioxidant capacity of a plant is due to the combined effect of various factors such as the presence of other types of antioxidant metabolites, or a pro-oxidative activity that may reduce the total antioxidant strength (Fukumoto and Mazza, 2000; Molyneux, 2004). This may be the case with the methanol (roots), and alkaloid (stems) extracts from *S. rostratum*.
Another important remark is that the extracts with the highest antioxidant activity were not the same ones that showed the best anti-inflammatory activity (Figure 2).

Of all the alkaloid extracts, those from the leaves of *Nicotiana trigonophylla* and *Solamun rostratum* showed the best anti-inflammatory activity. The anti-inflammatory activity of *S. rostratum* already reported was attributed to β-sitosteryl 3-O-ß glucopyranoside (Maiza-Benabdesselam, et al., 2007), not to any alkaloid found in the species. However, there are many studies reporting anti-inflammatory activity of steroid (solasonine) and pyridine (nicotine) alkaloids (Barbosa-Filho *et al.*, 2006; Lakhan and Kirchgessner, 2011; Kalra *et al.*, 2004).

Conclusions

The results obtained in the present investigation support the traditional use of three of the plant species as anti-inflammatory agents and as expected, extracts from the leaves of *N. glauca* and *S. nigrescens* that are the parts traditionally used do have a good anti-inflammatory effect (more than 50% inhibition). Furthermore, the methanolic extract from flowers of *N. glauca* with the largest amount of phenolic compound presented the best overall performance, *i.e*. the best antioxidant and the highest anti-inflammatory activities. However, in this work, no correlation was found between the antioxidant and anti-inflammatory activities, since alkaloid fractions of some plant parts that had low antioxidant activity

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produced better anti-inflammatory activity than the methanolic extracts. Finally, regardless of the extract, the stems of *S. rostratum* exhibited pro-inflammatory activity.

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