Scientific Paper

Pytochemical characterization and total antioxidant activity of different extracts from *Tithonia diversifolia* (Hemsl) A. Gray▲

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Abstract

In order to characterize, qualitatively and quantitatively, the main metabolites present in leaf, stem and root extracts from *Tithonia diversifolia* (Hemsl) A. Gray and determine the total antioxidant capacity of the different plant parts, the plant material was randomly collected in a farm belonging to a farmer of Matanzas province, Cuba. The leaf, stem and root samples were dried, pulverized and extracted with commercial ethanol by maceration. Afterwards, the solvent was removed through a vacuum rotary evaporator. The phytochemical sieving of the different extracts was carried out for the qualitative determination of total phenols, flavonoids, saponins, coumarin, tannins, quinones and terpenoids. The concentrations of total phenols, flavonoids and saponins of the three extracts were determined using a gallic acid pattern, quercetin and 10 % panax ginseng, respectively. The total antioxidant activity was measured through the phosphomolybdate method. The quantification was carried out in triplicate in 96-well microplates. The data were analyzed through descriptive statistics with the package SPSS®, Version 15. The results showed presence of phenols, flavonoids, saponins, coumarins, quinones and terpenoids in ethanol extracts of *T. diversifolia* roots, stems and leaves. The antioxidant activity showed that the root was the organ with higher antioxidant capacity, with 1,10 mg of ascorbic acid/mg of extract. It was followed by the leaves (1,08) and finally, the stem (0,50). The qualitative and quantitative determination of secondary metabolites showed the presence of phenols, flavonoids, coumarins, quinones and terpenoids in ethanol extracts of *T. diversifolia* roots, stems and leaves.

Keywords: leaves, metabolites, roots, stem.

Introduction

Tithonia diversifolia (Hemsl) A. Gray is a non-leguminous, protein, forage plant, belonging to the family Asteraceae. With its utilization in animal feeding, many benefits are obtained due to its nutritional value and diversity in its chemical composition (Mabou-Tagne *et al.*, 2018). According to reports by Galindo *et al.* (2017), its use in the animal diet allows the reduction of methanogens and has beneficial effects on the rumen microbial ecology. Lezcano-Más *et al.* (2016) stated that it contributes to the decrease of the parasite rate in young cattle.

This plant which originated in Central America has been the object of study in natural and traditional medicine for its multiple properties, derived from its secondary metabolism. Its applicability as antimicrobial and anti-inflammatory (Sousa *et al.*, 2019) to fight malaria (Afolayan *et al.*, 2016), diabetes (Sari *et al.*, 2018) and cancer (Di Giacomo *et al.*, 2015) has been recently described. In addition, it has been used as green manure due to its fast growth, high nitrogen-fixing and phosphorus accumulation capacity, with positive effect on poor soils (Scrase *et al.*, 2019). It also constitutes an alternative for insect control, because it has shown insecticidal activity against leaf-cutter ants (Pantoja-Pulido *et al.*, 2017).

The objective of this research was to characterize, qualitatively and quantitatively, the main metabolites present in the leaf, stem and root extracts of *T. diversifolia*, as well as to determine the total antioxidant capacity of the different plant parts.

Materials and Methods

Obtainment of the plant material. The *T. diversifolia* leaves, fresh stems and roots were

▲Paper presented in the 5th International Convention Agrodesarrollo 2019 celebrated on October 22-26, 2019. Plaza America Convention Center. Varadero, Cuba

collected in a farmer's farm, located at 22°49'50,0"N and 81°01'05,3"W, in Matanzas province, Cuba.

Extract preparation. The samples were washed and dried in stove at 50 ºC, during 24 h for the leaves and for 72 h for the stems and roots (López *et al*., 2006). The dried and ground plant material was subject to passive maceration with ethanol during 24 h (three times). Later, they were vacuum filtered and the solvent was withdrawn through a rotary evaporator (López *et al*., 2006). The essays were conducted with ethanol extracts of *T. diversifolia* leaves, roots and stems, in the Biotechnology laboratory of the Pastures and Forages Research Station Indio Hatuey, in Matanzas, Cuba.

Phytochemical sieving of secondary metabolites through colorimetric methods

Total phenols and/or tannins. It was carried out through the ferric chloride method. The extracts were evaluated at a concentration of 10 mg/mL for the stems and roots; while in the leaves the concentration was 2 mg/mL (Chhabra *et al*., 1984).

Flavonoids. Shinoda's test was used and the extracts were evaluated at a concentration of 10 mg/mL for the stems and roots, and of 2 mg/mL for the leaves (Bonilla-Rios *et al*., 2019).

Saponins. Saponin identification was carried out through the foam test. The extracts were prepared at a rate of 10 mg/mL (Robles-García *et al*., 2016).

Coumarins. For the qualitative coumarin determination, the sodium hydroxide test was used. The extracts were arranged at 10 mg/mL (Vázquez and García-Vieyra, 2017).

Quinones. The colorimetric determination of quinones was carried out from 500 µL of the extracts, at a concentration of 10 mg/mL. They were added 500 µL of H_2SO_4 (Vázquez and García-Vieyra, 2017).

Terpenoids. The identification of terpenoids was performed from 500 µL of the extracts at a concentration of 10 mg/mL, according to the methodology proposed by Vázquez and García-Vieyra (2017).

Quantitative determination of the main groups of secondary metabolites

Total phenols. The quantitative determination of total phenols was conducted through the Folin-Ciocalteu method, according to Ocampo *et al*. (2014) with some modifications. The results were expressed in mg of ascorbic acid/mg of extract.

Flavonoids. The quantitative study of flavonoids was performed by the aluminum chloride method, according to explanations cited by Chekol and Desta (2018), some of them modified. The results were expressed as mg of quercetin/mg of extract.

Saponins. The quantification of saponins was done according to the method described by Guzmán *et al*. (2013), with some changes. The results were expressed as mg of 10 % Panax Ginseng/mg of extract.

All the extracts were worked at a concentration of 1 mg/mL.

Total antioxidant activity. The method of phosphomolybdate radical capture was applied. According to the methodology proposed by Umamaheswari and Chatterjee (2008), the essay based on the reduction of Mo (VI)-Mo (V) by the extracts was carried out. Each sample was evaluated in triplicate in three repetitions in time. The results were expressed as mg of ascorbic acid/mg of extract. All the extracts were worked at 10 mg/mL.

Statistical analysis. The variables content of phenols, flavonoids, saponins and total antioxidant activity were analyzed through descriptive statistics with the statistical package SPSS®, Version 15.

Results and Discussion

Table 1 shows the results of the qualitative sieving of the ethanol extracts of *T. diversifolia* leaves, stems and roots, from colorimetric tests for phenols and/or tannins, flavonoids, saponins, coumarin, quinones and terpenoids.

Table 1. Qualitative phytochemical sieving of the *T. diversifolia* ethanol extracts from colorimetric tests.

Metabolite	Extract		
	Leaves	Stems	Roots
Phenols	+	+	+
Flavonoids	+	+	+
Saponins	+	-	+
Tannins	+	+	+
Coumarins	+	+	+
Quinones	+	+	+
Terpenoids	-	+	+

(+) presence, (-) absence

The colorimetric essays allowed to identify the secondary metabolites present in the *T. diversifolia*

extracts (table 1). The root extract showed the presence of all the metabolites. Nevertheless, in the leaf extract there was absence of terpenoids, and for the stem extract, of saponins. Although these metabolites were not found, it does not mean they are not present. Maybe they could be detected with higher concentrations of the extract or through other type of quantitative analytical method, such as high performance liquid chromatography (HPLC) or ELISA.

Each solvent has different extraction capacities and solubility spectrum, for which the quantity of phytocompounds present in one or another extract will depend on their affinity with the extraction solvent used (Soto-García and Rosales-Castro, 2016). The color intensity in the reaction allowed to observe that the roots are the organ with the highest quantity of phytocompounds, followed by the leaves and stems. Due to the color interference, as it is a qualitative method, and not with the same concentrations, only the presence or absence of the metabolite in question was determined.

In other species of the family, such as *Tithonia tubaeformis* (Jacq.) Cass, phytochemical sieving has been performed in methanol-water extracts and the presence of alkaloids, steroids, tannins and coumarins is reported (Hinojosa-Dávalos *et al*., 2013), with absence of saponins. However, Olayinka *et al*. (2015) refer the presence of all the metabolites in *T. diversifolia*, which have also been mentioned in this study.

The quantification of the main secondary metabolites is shown in table 2. The extracts had high phenol and flavonoid contents; while saponins appeared in lower amount. In correspondence with the colorimetric essays, the root extracts showed the highest metabolite concentrations. They were followed by the leaves and, finally, the stems.

Umar *et al*. (2015) conducted a phytochemical study of the leaves, stems and roots in ethanol and aqueous *T. diversifolia* extracts. These authors stated that the secondary metabolites were significantly high in the leaves, followed by the root and stem, with the exception of the phenol concentration whose highest values corresponded to the root. This is in agreement with the results of this study, because the roots showed higher metabolite content, followed by the leaves and, finally, the stem. According to Arguayo (2002), there is high variation regarding the concentration of secondary metabolites in the different plant parts, because they do not show a pattern of maximum production or special storage organs. In addition, the synthesis of these metabolism compounds depends, to a large extent, on the phenological status of the plant, season, soil characteristics, region of the country where it is established and environmental conditions, among other aspects (Sampaio and Da Costa, 2018).

T. diversifolia has shown variability regarding the presence of secondary metabolites, according to reports by several authors (Rivera *et al*., 2018). Hence the differences in the results and versatility of the plant for its adaptation to different environments.

The study of secondary metabolism in the plants influences different biological activities which can bring benefits for the animal and human health. For such reason, once the phytochemical sieving was performed the percentage of antioxidant capacity was measured, which is determined by many of these above-mentioned metabolites.

Figure 1 shows the total antioxidant activity of the ethanol extracts of *T. diversifolia* leaves, fresh stems and roots. As can be observed, the root extract showed the highest antioxidant capacity. It was followed by the leaf extract; while the fresh stem extract showed lower activity. These results are in correspondence with the content of secondary metabolites of the extracts, proving that the antioxidant activity shown in this study can be associated to the phenol and flavonoid content, which is explained by the redox properties of phenolic compounds.

The antioxidant activity of phenols and flavonoids, in general, is given by their free

Table 2. Quantification of secondary metabolites in *T. diversifolia* extracts.

Ethanol extracts of *T. diversifoli*a	Total phenols, mg of gallic acid/mg of extract	Flavonoids, mg of quercetin/mg of extract	Saponins, mg of 10 % Panax Ginseng/mg of extract
Leaves	50,4 ± 0,73	25,6 ± 0,50	1,8 ± 0,03
Fresh stems	33,2 ± 0,55	11,6 ± 0,53	0,6 ± 0,03
Roots	50,9 ± 0,86	38,2 ± 0,31	2,3 ± 0,10

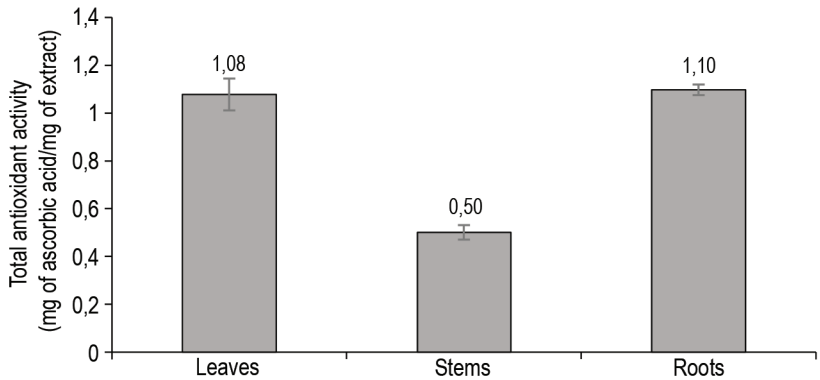

Figure 1. Total antioxidant activity of the ethanol extracts of *T. diversifolia* leaves, fresh stems and roots.

radical sequestration capacity, iron chelant as well as the inhibition of oxidase enzymes. These metabolites are capable of preventing or attenuating oxidative stress, due to the reactive oxygen species (ROS), which prevents the oxidation of important biomolecules (proteins, nucleic acids, lipids and sugars). The above-explained facts are associated to the appearance of certain diseases (cancer, Alzheimer's, ageing, cataracts, diabetes, hypertension, cardiovascular disorders, among others), which have increasing impact and reach in society (Działo *et al*., 2016).

The antioxidant capacity of a plant extract is specified with higher accuracy through the utilization of several analytical methods, because there is high variation in the mechanisms that an antioxidant compound or mixture can exert *in vivo*. All this will depend, to a large extent, on the bioavailability of the mixture of present compounds and on their synergic interactions to produce an antioxidant response at cell level (López-Alarcón and Denicola, 2013).

According to reports by Betancur and Mosquera (2017), the species of the family Asteraceae stood out for presenting high values of antioxidant activity in the presence of the radical DPPH. Also Pantoja-Pulido *et al*. (2017) stated that *T. diversifolia* showed good antioxidant activity, when it was tested on DPPH and in ferric reduction essays.

Conclusions

The qualitative and quantitative determination of secondary metabolites showed the presence of phenols, flavonoids, coumarins, quinones and terpenoids in ethanol extracts of the T. diversifolia roots, stems and leaves.

The root and leaf extracts of *T. diversifolia* showed the highest concentrations of phenols and flavonoids.

The roots constitute the organ with higher antioxidant activity, followed by leaves and, finally, the stem.

T. diversifolia is an important source of diversity, due to the quantity of bioactive compounds it is capable of synthesizing.

Acknowledgements

The authors thank the Financial Fund of Science and Innovation (FONCI, for its initials in Spanish) for the funding to develop this research through the project *Sustainable production for cattle milk production in animal husbandry farms*.

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Received: July 07, 2019
Accepted: August 01, 2019