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Phytochemical screening and antioxidant activity of ethanol extract of *Tithonia diversifolia* (Hemsl) A. Gray dry flowers

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ABSTRACT

Objective: To evaluate the antioxidant activity of extracts of dried flowers of *Tithonia diversifolia* (Hemsl) A. Gray (*T. diversifolia*) dry flower-a shrubby plant belonging to the Asteraceae family and very common in Brazil, providing data to help prevent premature aging skin.
Methods: The tests of phytochemical screening included total phenols, tannins, flavonoids, alkaloids and saponins. The active antioxidant was determined by 2,2-diphenyl-1-picryl-hydrazyl method.
Results: The phytochemical screening of *T. diversifolia* dry flowers revealed the presence of phenolic compounds (tannins, flavonoids and total phenols), while alkaloids and saponins were not detected. The IC_{50} values showed a strong antioxidant activity of the plant extracts.
Conclusions: Therefore, this study suggests the possibility of using dry flowers extracts of *T. diversifolia* for the prevention of cell aging, as was shown to have significant antioxidant activity.

1. Introduction

Skin aging is commonly influenced by several factors, such as genetic and environmental factors (UV light), xenobiotics, and hormonal changes. All these factors can trigger the onset of reactive oxygen species (ROS) that are chemically reactive molecules containing oxygen[1].

They are formed as natural products of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress, ROS levels can dramatically increase that, resulting in significant damage to cell structure[2].

Natural antioxidants present in plant origin protect against these radicals and are therefore important tools in obtaining and preserving good health[3].

Potentially active components from fruits, herbs, roots and leaves have been studied extensively in order to avoid oxidative cellular events. The results suggest that polyphenols, especially the flavonoids possess a high antioxidant power which can protect cells against the adverse effects of ROS[4].

Among many medicinal plant families, Asteraceae family comprises species with arboreous, shrub, herbaceous and liana habits and is widely distributed at tropical, subtropical and tempered regions, particularly in South America, with expression in number of species, composed by some 1 535 genera, 23 000 species and 17 tribes[5].

Tithonia diversifolia (Hemsl) A. Gray (*T. diversifolia*) is a herb family (tribe Heliantheae) occurring from Central America to the West Indies, having been naturalized in the tropics and also has been used as a medicinal plant showing their anti-inflamatory[6], antimalarial[7] and many other biological activities.

The aims of the present study were to evaluate phytochemical screening and also measure the antioxidant activity of ethanol extract of *T. diversifolia* dry flowers using a 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay.

2. Materials and methods

2.1. Botanic material

Plants of *T. diversifolia* were grown in the Garden of

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Medicinal Plants, Faculty of Medicine of ABC, Santo André, São Paulo, Brazil, and flowers were collected in August 2012 (flowering season of this species). After manual collect, the material was dried at 50 °C (hot air chamber) for 1 week and stored under controlled conditions (dry air, dark and at 20 °C).

2.2. Extract preparation

For extract preparation, the flowers were reduced in a mill and kept in ethanol (100%) under stirring at room temperature for 24 h. After filtering, the extract was concentrated on the rotary evaporator attached to a vacuum pump and then used for phytochemical screening.

2.3. Phytochemical screening

All the tests of phytochemical screening (total phenols, tannins, flavonoids, alkaloids and saponins) followed the methodologies described at Brazilian Pharmacopea[8].

2.4. Antioxidant activity

The DPPH method is based on captures of DPPH by antioxidants, producing a decrease in absorbance at 517 nm.

Plant sample solutions (0.1 g/mL) were diluted to final concentrations of 250, 100, 50 and 10 mg/mL, in ethanol. Ascorbic acid solutions (1.8 mg/mL) were diluted to final concentrations of 120, 90, 60 and 30 μg/mL, in ethanol.

About 3 mL of a 0.04 mg/mL DPPH (Sigma Aldrich™) ethanol solution was added to 30 μL of sample solutions of different concentrations, and allowed to react at room temperature. After 30 min, the absorbance values were measured at 517 nm and converted into percentage antioxidant (AA) using the following equation 1 (Eq. 1):

Eq. 1: $AA(\%)=100-\{[(Abs_{sample}-Abs_{blank})\times 100]/Abscontrol\}$

Ethanol (3.0 mL) plus plant extract solution (30 μL) was used as a blank. DPPH solution (3.0 mL; 0.04 mg/mL) plus ethanol (3.0 mL) was used as a negative control. The positive control was those using the standard ascorbic acid (Fluka™) solutions.

The calculation equations of the analytical curves were made by linear regression using the least squares method of plots where the abscissa represented the concentration of test plant extracts or ascorbic acid and the ordinate represented the average percent of antioxidant activity and calculating the linear correlation coefficient. The equation 2 (Eq. 2) and equation 3 (Eq. 3) used to calculate the results of percent of antioxidant activity of ethanol extract of *T. diversifolia* dry flowers and ascorbic acid reference standard, respectively.

Eq. 2: $y=0.236x+1.421\,5$ $R^2=0.997\,8$

Eq. 3: $y=0.621\,2x+3.015$ $R^2=0.995\,9$

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting antioxidant activity. The IC_{50} values of ethanol extract of *T. diversifolia* dry flowers and ascorbic acid reference standard were calculated by Eq. 2 and Eq. 3, respectively[9].

3. Results

3.1. Phytochemical screening

The phytochemical screening test showed that the flowers of *T. diversifolia* contained several active compounds (Table 1). The IC_{50} of dried flower of ethanol extracts of this species and ascorbic acid are presented in Figure 1.

Table 1

Phytochemical composition of ethanol extract of *T. diversifolia* dry flowers.

Phytochemical	Interference
Total phenols	+
Tannins	+
Flavonoids	+
Alkaloids	−
Saponins	−

(+) presence; (−) absence.

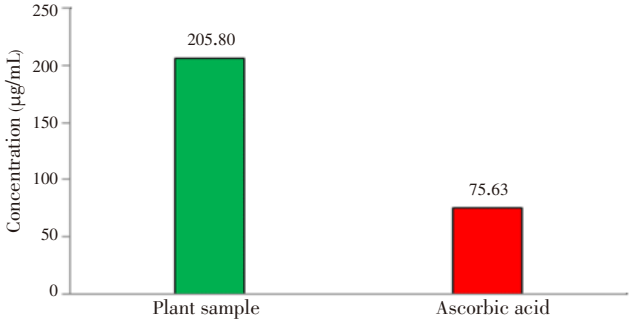

Figure 1. The IC_{50} values (the half maximal inhibitory concentration) of ethanol extract of *T. diversifolia* dry flowers and ascorbic acid.

4. Discussion

In this study, the phytochemical screening of flower extract of *T. diversifolia* revealed that among the substances investigated, presence of phenolic compounds was detected (total phenols, tannins and flavonoids), while alkaloids and saponins were not detected. The presence of some of these secondary metabolites suggests that the plant might be of medicinal importance.

The presence of phenolic compounds (total phenols, tannins and flavonoids) provides pharmacological activities like anti-cancer[10,11], anti-oxidant[11,12], antimicrobial[13,14], wound-healing[15] and anti-inflammatory[6,16], that may suggest an association to the species here investigated.

Similar results of phytochemical screening of flower extract of this species were obtained by Essiett and Akpan[17], differing only in the saponin presence and the result may be related to the parts of the flower used to obtain the extract in the study by Essiett and Akpan that were used only the petals of flowers[17], while in present study were obtained by the whole flower.

The theory of aging skin by the action of free radicals is based on the failure mechanism of natural antioxidant *in vivo*

and *in vitro* since studies suggest a correlation between the aging process and reducing enzymatic and non-enzymatic agents, with a consequent increasing level of ROS[18].

One of the most widely used natural antioxidants studied is ascorbic acid that eliminates most ROS due to the oxidation of ascorbate to monodehydroascorbate and then to dehydroascorbate and has other functions to maintain the normal physiologic state in humans. In the skin, ascorbic acid is a cofactor required for the enzymatic activity of prolyl hydroxylase, which hydroxylates prolyl resulting in procollagen and elastin[19].

Bogdan Allemann and Baumann revised the use of other antioxidants in skin care formulations and found that a 3-month daily regimen of topical using of ascorbic acid provided objective and subjective improvement in photodamaged facial skin[20].

It is common consensus that the cellular aging process can be prevented by plants' phenolic substances, which has motivated the investigation of these plant metabolites and their possible action in the prevention of cellular aging[21].

The result shows the values of IC_{50} of ethanol extract of *T. diversifolia* dry flowers and ascorbic acid as a pattern, and points to a higher antioxidant activity of this plant extract when compared to the standard used ascorbic acid, showing the effectiveness of antioxidant activity.

Therefore, we suggest the possibility that flower extracts of *T. diversifolia* can control the action of free radical activities and thus preventing cellular aging, becoming an alternative in the fight against skin aging, since these plants are easy to grow and produce a lot of flowers during their flowering.

Finally, considering the results obtained, as future perspectives, we intend to evaluate some biological activities, such as wound-healing, anti-inflammatory, antimicrobial and anti-cancer activity, as well as quantify the main phytochemicals present in extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Cadet J, Douki T, Ravanat JL, Di Mascio P. Sensitized formation of oxidatively generated damage to cellular DNA by UVA radiation. *Photochem Photobiol Sci* 2009; **8**: 903-911.

[2] Brieger K, Schiavone S, Miller FJ Jr, Krause KH. Reactive oxygen species: from health to disease. *Swiss Med Wkly* 2012; **142**: w13659.

[3] Khan RA, Khan MR, Sahreen S, Ahmed M. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill. *Chem Cent J* 2012; **6**: 12.

[4] Thielecke F, Boschmann M. The potential role of green tea catechins in the prevention of the metabolic syndrome-a review. *Phytochemistry* 2009; **70**: 11-24.

[5] Játem-Lásser A, Ricardi MS, Adamo G. Herbal traditional medicine of Venezuelan Andes: an ethnopharmacological study. *Phytother Res* 1998; **12**: S53-S59.

[6] Capelari-Oliveira P, Paula CA, Rezende SA, Campos FT, Grabe-Guimarães A, Lombardi JA, et al. Anti-inflammatory activity of *Lychnophora passeria*, Asteracea (Brazilian "Arnica"). *J Ethnopharmacol* 2011; **135**(2): 393-398.

[7] Lacerda AM, Modolo AK, Matias RC, Pistori H, Yano M, Roel AR, et al. Screening of plants with potential phototoxic. *Rev Bras Farm* 2011; **92**(4): 352-355.

[8] Agência Nacional de Vigilância Sanitária. Brazilian Pharmacopea. 5th ed. Brasília: Agência Nacional de Vigilância Sanitária; 2010. [Online] Available from: http://www.anvisa.gov.br/hotsite/cd_farmacopeia/pdf/Volume%201.pdf [Accessed on 12th March, 2014] Portuguese.

[9] Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, et al. Screening of Brazilian plant extracts for antioxidant activity by use of DPPH free radical method. *Phytother Res* 2001; **15**(2): 127-130.

[10] Lin Y, Shi R, Wang X, Shen HM. Luteolin, a flavonoid with potential for cancer prevention and therapy. *Curr Cancer Drug Targets* 2008; **8**(7): 634-646.

[11] Khacha-ananda S, Tragoolpua K, Chantawannakul P, Tragoolpua Y. Antioxidant and anti-cancer cell proliferation activity of propolis extracts form two extraction methods. *Asian Pac J Cancer Prev* 2013; **14**: 6991-6995.

[12] Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr J Biotechnol* 2006; **5**(11): 1142-1145.

[13] Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pak J Pharm Sci* 2009; **22**(1): 102-106.

[14] Hendra R, Ahmad S, Sukari A, Shukor MY, Oskoueian E. Flavonoid analyses and antimicrobial activity of various parts of *Phaleria macrocarpa* (Scheff.) Boerl fruit. *Int J Mol Sci* 2011; **12**: 3422-3431.

[15] Nayak BS, Pinto Pereira LM. *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. *BMC Complement Altern Med* 2006; **6**: 41.

[16] Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K. Mechanism of action of flavonoids as anti-inflammatory agents: a review. *Inflamm Allergy Drug Targets* 2009; **8**(3): 229-235.

[17] Essiett UA, Akpan EM. Proximate composition and phytochemical constituents of *Aspilia africana* (Pers) C. D. Adams and *Tithonia diversifolia* (Hemsl) A. Gray stems (Asteraceae). *Bull Environ Pharmacol Life Sci* 2013; **2**(4): 33-37.

[18] Chiang NY, Verbov J. *Dermatology: a handbook for medical students & junior doctors*. Liverpool: British Association of Dematologists; 2009.

[19] Masaki H. Role of antioxidants in the skin: anti-aging effects. *J Dermatol Sci* 2010; **58**(2): 85-90.

[20] Bogdan Allemann I, Baumann L. Antioxidants used in skin care formulations. *Skin Therapy Lett* 2008; **13**(7): 5-9.

[21] Adil MD, Kaiser P, Satti NK, Zargar AM, Vishwakarma RA, Tasduq SA. Effect of *Emblica officinalis* (fruit) against UVB-induced photo-aging in human skin fibroblasts. *J Ethnopharmacol* 2010; **132**(1): 109-114.