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Identification of Glycosil Flavones and Determination *in vitro* of Antioxidant and Photoprotective Activities of *Alternanthera brasiliana* L. Kuntze

[1]Edja E.S. Silva, [1]José M.T. Alencar-Filho, [1]Ana P. Oliveira, [1]Amanda L. Guimarães, [2]José A. Siqueira-Filho, [1]Jackson R.G.S. Almeida and [1]Edigênia C.C. Araújo

[1]Núcleo de Estudos e Pesquisas de Plantas Medicinais, Universidade Federal do Vale do São Francisco, 56.304-205, Petrolina, Pernambuco, Brazil

[2]Centro de Referência para Recuperação de Áreas Degradadas da Caatinga (CRAD), 56.300-000, Petrolina, Pernambuco, Brazil

Correponding Author: Edigênia C.C. Araújo, Universidade Federal do Vale do São Francisco-UNIVASF, Postal Code: 56.304-20, Petrolina, PE, Brazil Tel/Fax: +55-87-21016863

ABSTRACT

The study *Alternanthera brasiliana* (L.) Kuntze aims to prove its effectiveness in folk medicine. From the study of their chemical constitution were identified by LC-MS technique three glycosylated flavones than were called 2"-O-ramnosylvitexin; 4',5,7-trimethoxy-2"-O-ramnosylvitexin and Ligustroflavone. The tests showed high antioxidant capacity of the specie and in small concentrations showed too high levels of photoprotection. In addition its high capacity for photoprotection also paves its use in cosmetology. *Alternanthera brasiliana* is a species extremely promising with all parameters to become a herbal medicine.

Key words: Antioxidant activity, photoprotection, glycosylated flavones, Amaranthaceae, *Alternanthera brasiliana*

INTRODUCTION

Alternanthera brasiliana (L.) Kuntze belongs to the family Amaranthaceae and is popularly known in Brazil as "Penicillin" and "Terramycin", a herbaceus plant, is used against inflammation, cough and diarrhoea in Brazilian popular medicine (Barua *et al*., 2013).

Some studies were conducted with *Alternanthera brasiliana* (L.) Kuntze, such as its use as a food supplement (Delaporte *et al*., 2005). They also performed *in vitro* studies with a view to pigment production (Silva *et al*., 2005). As for the evaluation of pharmacological activities are few studies as cytotoxic activity by Nihei and Dias (2001) and Lagrota *et al*. (1994) to give an account of showed anti-HSV activity and one fraction showed activity on DNA synthesis of infected cells. In other studies, the plant species showed activity against HIV (Silva *et al*., 2005), antinociceptive (Souza *et al*., 1998), antimicrobial (Brochado *et al*., 2003) and the first phytochemical study with the objective of isolating secondary metabolites was performed in 2003, in which phytochemicals and secondary metabolites were isolated and identified six O-glycosylated flavonoids (Brochado *et al*., 2003).

In recent years, a substantial amount of evidence has shown the key role of free radicals and other oxidants as largely responsible for aging and degenerative diseases associated with aging such as cancer, cardiovascular disease, cataracts, immune system decline and brain dysfunction (Sousa *et al*., 2007).

In search of biologically active molecules and interest in the antioxidant activity of plant extracts, it was decided to conduct this study in order to evaluate the antioxidant and photoprotective *Alternanthera brasiliana* (L.) Kuntze.

MATERIALS AND METHODS

Plant material and preparation of extracts: The aerial parts of *Alternanthera brasiliana* (L.) Kuntze were collected in August 2011 in the city of Petrolina (9.37714°S The coordinates 40.528837°Altitude 391 m), state of Pernambuco, Northeastern Brazil, a region of arid climatic conditions involved for months with strong sunshine and low rainfall. A voucher specimen (19072) is deposited in the Herbarium of the São Francisco (HVASF), Federal University of São Francisco Valley.

The aerial parts of *Alternanthera brasiliana* (L.) Kuntze dried and pulverized (555 g) were subjected to maceration with 95% EtOH for 72 h. The solution was filtered and concentrated on rotaevaporator to 50°C, yielding 101.70 g of ethanol extract. To evaluate the activities, the ethanol extract was suspended in MeOH: H_2O (3:7) and partitioned with hexane, chloroform ($CHCl_3$) and ethyl acetate (AcOEt), in increasing order of polarity to obtain the respective phases which yielded 0.92, 0.95 and 0.35 g, respectively.

HPLC-UV/DAD/ESI+MS profile: The HPLC analyses were conducted for Analytic Central-IQUSP, using a RP-18 column (phenomenex luna, 5 μm, 250/4.6 mm), formic acid 0.1% in deuterated water, such as solvent A and methanol such as solvent B elution gradient mode: 30% of B (15 min), 30-40% of B (15 min), 40-60% of B (10 min), 60-100% of B (5 min) with flow of 1 mL min^{-1}. The UV-DAD detector was set record between 290-320 nm. The extracts and fractions of *A. brasiliana* were diluted in methanol (with standard Merck). In the firgerprinting ESI-MS analysis, the general conditions were: Source temperature of 40°C, capillary voltage of 4.0 kV in the positive ion mode ESI(+)-MS. Structural analysis of single ions in the mass spectra as from *A. brasiliana* was performed by ESI-MS/MS. The compounds were identified by comparison of their ESI-MS/MS fragmentation spectra with the literature data.

Total phenolic determination: Total phenolic contents were assayed using the Folin-Ciocalteu reagent, it is based on the method reported by Slinkard and Singleton (1977) and only the volumes have been reduced (Almeida *et al.*, 2011). Total phenolic contents of the extracts (three replicates per treatment) were expressed as milligram gallic acid equivalents per gram (mg GAE/g) through the calibration curve with gallic acid. The calibration curve range was 50-1000 mg L^{-1} ($R^2 = 0.9938$). All samples were performed in triplicates.

Total flavonoid determination: Total flavonoid contents were determined by using a colorimetric method described previously (Dewanto *et al.*, 2002). The results were expressed as milligram of catechin equivalents per gram of extracts (mg CE/g) through the calibration curve with catechin ($R^2 = 0.9948$). The calibration curve range was 50-1000 mg L^{-1}.

DPPH free-radical scavenging activity: The free radical scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazil (DPPH) assay (De Paola, 2001; Falcon *et al.*, 2006). The absorbance values were measured at 518 nm and converted into the percentage Antioxidant Activity (AA) using the following equation:

$$AA\ (\%) = \frac{\text{Absorbance of the control-Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Ethanol (1.0 mL) plus plant extracts solutions (2.5 mL) were used as a blank. DPPH solution (1.0 mL) plus ethanol (2.5 mL) was used as a negative control. The positive controls (ascorbic acid, BHA and BHT) were those using the standard solutions. Assays were carried out in triplicate. The IC_{50} values were calculated by linear regression using by GraphPad Prism 5.0 program.

β-carotene bleaching test: The β-carotene bleaching method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion (Wannes *et al*., 2010). Ascorbic acid, BHA and BHT were used as positive control. In the negative control, the extracts were substituted with an equal volume of ethanol. The antioxidant activity (%) was evaluated in terms of the bleaching of the β-carotene using the following equation:

$$\text{Antioxidant activity (\%)} = \left[1-\frac{(A_0-A_t)}{(A_0^0-A_t^0)}\right] \times 100$$

where, A_0 is the initial absorbance and A_t is the final absorbance measured for the test sample, A_0^0 is the initial absorbance and A_t^0 is the final absorbance measured for the negative control (blank). The results are expressed as percentage of antioxidant activity (AA %). Tests were carried out in triplicate.

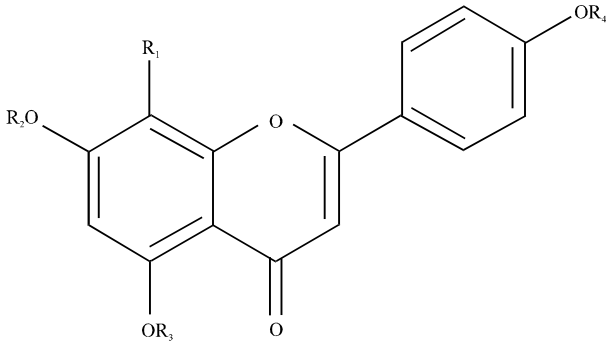
Determination of the maximum absorption wavelength and Sun Protection Factor (SPF) *in vitro*: For determining of the maximum absorption wavelength (λ_{max}), the dried extracts were diluted in absolute ethanol, obtaining concentrations of 5, 25, 50 and 100 mg L^{-1}. Subsequently, was performed spectrophotometric scanning at wavelengths between 260-400 nm, with intervals of 5 nm. The readings were performed using 1 cm quartz cell and ethanol used as blank (Violante *et al*., 2009). Calculation of SPF was obtained according to the equation developed by Mansur *et al*. (1986).

Statistical analysis: The data obtained in the experiments was statistically analyzed using the GraphPad Prism® version 5.0 and expressed as Mean±SD. The differences at $p<0.05$ were considered significative.

RESULTS AND DISCUSSION

This study, identified the presence of flavonoid compounds in the extract and phases of *A. brasiliana*. The ESI-MS technique with direct infusion was used to characterize the presence of compounds with potent free-radical scavenging and photoprotective activities presented in this study.

The analysis by ESI-MS showed that of some constituents which absorb in the UV range in the samples Ab-ETOH and Ab-AcOEt were coincided with the mass of the flavones: (1) 2"-O-ramnosylvitexin, (2) 4',5,7-trimethoxy-2"-O-ramnosylvitexin and (3) Ligustroflavone (Table 1, Fig. 1). Structural analysis of single ions in the mass spectra was made by comparison with the literature data (Zhang *et al*., 2010; Pieroni *et al*., 2000). The peaks were justified according the fragmentations the following and retention factor (R_f): (1) $C_{27}H_{30}O_{14}$, $R_f = 20.0$, 601 $[M+Na]^+$,



R_1 = Glucosly(1→6) ramnoside, $R_2 = R_3 = R_4 = H$
R_1 = Glucosly(1→6) ramnoside, $R_2 = R_3 = R_4 = CH_3$
$R_1 = H$, R_2 = Glucosly(2→1) ramnosyl, (6→1) ramnoside, $R_3 = R_4 = H$

Fig. 1: Glycosilated flavones identified in the extract and phase ethyl acetate of *Althernathera brasiliana*

Table 1: Compouds identified in the ethanolic extract (Ab-EtOH) and phase ethyl acetate (Ab-AcOEt) from *Althernathera brasiliana*

Compouds	Retention time (min)	Protonated ions $[M+H]^+$ (m/z)	MS/MS ions (m/z)
1	20	579	433, 415, 397, 367, 313, 271
2	36	621	475, 457, 439, 379, 325, 313
3	38	725	579, 561, 543, 525, 271

Table 2: Total Phenolics (TP), Total Flavonoids (TF) and antioxidant activity of the dried extracts of *Alternanthera brasiliana*

Extracts	TP (mg GAE q/g)	TF (mg Ce q/g)	DPPH (EC_{50}, μg mL^{-1})	β-carotene (AA %)
Ab-EtOH	81.77±2.08	92.90±35.22	190.30±28.31	41.72±1.570
Ab-Hex	69.43±33.38	---	226.10±17.99	81.82±15.91
Ab-$CHCl_3$	72.43±2.89	---	173.20±8.97	73.70±13.18
Ab-AcOEt	167.40±34.12	87.06±1.25	28.90±1.03	73.70±10.96
Ascorbic acid	---	---	9.72±4.33	5.19±5.380
BHA	---	---	2.31±0.18	102.90±3.870
BHT	---	---	0.27±0.06	56.01±6.480

EC_{50} values were obtained by interpolation from linear regression analysis with 95% of confidence level, EC_{50} is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%, Values are given as Mean±SD (n = 3)

579 $[M+H]^+$, 433 [$M+H^+$-rhamnose], 271 [$M+H^+$-rhamnose-glucose]; (2) $C_{30}H_{36}O_{14}$, R_f = 36.0, 643 $[M+Na]^+$, 621 $[M+H]^+$, 475 [$M+H^+$-rhamnose], 313 [$M+H^+$-rhamnose-glucose]; (3) $C_{38}H_{40}O_{18}$, R_f = 38.0, 747 $[M+Na]^+$, 725 $[M+H]^+$, 578 [M+H -rhamnose], 271 [M+H -rhamnose-glucose-rhamnose] (Fig. 1). Factors such as byosynthesis, analysis of MS/MS, range characteristic UV absorption and comparison with literature data were considered to define the structures. The similarity between the aglycone and the agreement of the data with the classes of secondary metabolites isolated from *Alternanthera brasiliana* and published in the literature (Facundo *et al.*, 2012) assisted in the structural proposition.

Thus, we determined the total phenolic and flavonoid contents of the crude ethanol extract (Ab-EtOH) and phases hexane (Ab-Hex), chloroform (Ab-$CHCl_3$), ethyl acetate (Ab-AcOEt) of *Alternanthera brasiliana*. As show in the Table 2, of the four samples Ab-AcOEt (ethyl acetate phase) showed the highest total phenolic content (167.43 mg g^{-1}) and the highest total flavonoid content (87.06 mg g^{-1}) about a half of the total phenolic content.

DPPH is one of a few stable available organic nitrogen radicals and has a UV-Vis absorption maximum at 515-518 nm. The data showed that the Ab-AcOEt sample exhibited excellent free radical scavenging activity, with a value of IC_{50} of 28.90±1.03 μg mL^{-1} (Table 2). The antioxidant activity of extracts was also evaluated too by the β-carotene-linoleic acid bleaching method. The rate of the β-carotene bleaching can be slowed down in the presence of antioxidants (Kulisic *et al.*, 2004). In this model, the samples showed high antioxidant activity (41-81%) and the most active extract was the Ab-Hex with percentage of antioxidant activity of 81.82±15.91 (Table 2).

This specie to possess, in the chemical constituents, phenolics and flavonoids compounds that are important antioxidant substances that are obtained from most natural plants. According to these results, it was concluded that dried samples from *Alternanthera brasiliana* have potent antioxidant activity, mainly by scavenging abilities observed against DPPH radical. Previous studies have identified flavonoids and other phenolic compounds isolated from *A. brasiliana* (Facundo *et al.*, 2012). The existing data give new information for the antioxidant potential and polyphenolic content of plant species that have been traditionally used as medicinal plant.

Table 3 shows the spectrophotometric absorption profile of the dried extracts of *A. brasiliana*. Analyzing the data can be observed that phase Ab-AcOEt showed characteristic absorption bands in regions UVC, UVB and UVA in a concentration dependent manner, suggesting a possible photoprotective potential. The SPF *in vitro* was determined by the spectrophotometric method developed by Mansur *et al.* (1986) using the UVB region, considered to be the region of greatest incidence during the day in which people are exposed for longer period (Dutra *et al.*, 2004). In Table 4, it can be observed that the phase Ab-AcOEt showed higher SPF at concentration 100 mg L^{-1} (11.320±3.47).

The results obtained from photoprotective potential can be justified by the high content of flavonoids present mainly in Ab-AcOEt. The maximum absorption wavelength (λmax = 400 nm), with absorption bands in regions UVA, was observed for all other samples Ab-EEB, Ab-Hex and Ab-$CHCl_3$ (Table 3). According to the literature, the content of flavonoids produced by a plant is considered an important factor for protecting plants against ultraviolet radiation (Souza *et al.*, 2005). The ultraviolet absorption spectrum of the flavonoids shows in general two peaks of

Table 3: Maximum absorption wavelength (λmax) and absorption type of dried extracts of *Alternanthera brasiliana*

Extracts	λ_{max} (nm)	Absorption type
Ab-EEB	275, 330, 400	UVC, UVA, UVA
Ab-Hex	275, 400	UVC, UVA
Ab-$CHCl_3$	280, 325, 400	UVC, UVA, UVA
Ab-AcOEt	270, 320, 335	UVC, UVB, UVA

UVC: 200-290 nm, UVB: 290-320 nm, UVA: 320-400 nm

Table 4: Sun Protection Factor (SPF) *in vitro* of the dried extracts of *Alternanthera brasiliana*

	FPS-UVB dried extracts (mg L^{-1})			
Extracts	5	25	50	100
Ab-EEB	0.175±0.016	0.871±0.009	1.526±0.418	3.677±0.413
Ab-Hex	0.083±0.080	0.631±0.150	1.290±0.257	2.675±0.642
Ab-$CHCl_3$	0.215±0.023	0.969±0.285	1.749±0.556	3.662±0.617
Ab-AcOEt	0.676±0.431	3.055±1.729	5.831±2.031	11.320±3.467

Values are given as Mean±SD (n = 3)

maximum absorption (240-280 and 300-550 nm) (Bobin *et al*., 1995). The results about SPF also showed standard concentration-dependent (Table 4). Although, the test has been carried out *in vitro*, it was demonstrated that this method correlates well with *in vivo* tests, because relates the absorbance of the substance in question with the erythematogenic effect of radiation and intensity of light at specific wavelengths between 290 and 320 nm (UVB region) (Violante *et al*., 2009). As the population already uses the species as a healing skin protection factor adds value to herbal treatments.

CONCLUSION

The excellent results of photoprotective and antioxidant activity obtained for *Alternanthera brasiliana* suggest that this species, which is already widely used in folk medicine, may become an herbal.

REFERENCES

Almeida, J.R.G.S., M.R. Oliveira, A.L. Guimaraes, A.P. Oliveira, L.A.A. Ribeiro, A.S.S.C. Lucio and L.J.Q. Junior, 2011. Phenolic quantification and antioxidant activity of *Anaxagorea dolichocarpa* and *Duguetia chrysocarpa* (Annonaceae). Int. J. Pharm. Biol. Sci., 2: 367-374.

Barua, C.C., S.A. Begum, D.C. Pathak and R.S. Bora, 2013. Wound healing activity of *Alternanthera brasiliana* kuntze and its anti oxidant profiles in experimentally induced diabetic rats. J. Applied Pharm. Sci., 3: 161-166.

Bobin, M.F., M. Raymond and MC. Martini, 1995. [Absorption properties UVA/UVB natural products]. Cosmet. Toil., 7: 44-50, (In Portuguese).

Brochado, C.O., A.P. Almeida, B.P. Barreto, L.P. Costa and L.S. Ribeiro *et al*., 2003. Flavonol robinobiosides and rutinosides from *Alternanthera brasiliana* (Amaranthaceae) and their effects on lymphocyte proliferation *in vitro*. J. Braz. Chem. Soc., 14: 449-451.

De Paola, M.V., 2001. [Formulating principles of sunscreens]. Cosmet. Toiletries, 13: 74-81, (In Portuguese).

Delaporte, R.H., K.P. Guzen, O.S. Takemura and J.C.P. Mello, 2005. [Evaluation of the mineral species *Alternanthera brasiliana* (L.) kuntze and *Bouchea fluminensis* (vell) mold]. Braz. J. Pharma., 15: 133-136, (In Portuguese).

Dewanto, V., W. Xianzhong, K.A. Kafui and and H.L. Rui, 2002. Organic foods contain higher levels of *Chemical* dangerous to infants. J. Agric. Food Chem., 50: 3010-3014.

Dutra, E.A., D.A.G.C. Oliveira, E.R.M. Kedor-Hackmann and M.I.R.M. Santoro, 2004. Determination of Sun Protection Factor (SPF) of sunscreens by ultraviolet spectrophotometry. Rev. Bras. Cienc. Farm., 40: 381-385.

Facundo, V.A., M.S. Azevedo, R.V. Rodrigues, L.F. Nascimento, J.S.L.T. Militao, G.V.J. Silva and R. Braz-Filho, 2012. Chemical constituents from three medicinal plants: *Piper renitens*, *Siparuna guianensis* and *Alternanthera brasiliana*. Braz. J. Pharm., 22: 1134-1139.

Falcon, D.Q., E.R. Costa, D.S. Alviano, C.S. Alviano, R.M. Kuster and F.S. Menezes, 2006. [Antioxidant and anti-microbial activity from calceolaria chelidonioides Humb. Bonpl. and Kunth.]. Braz. J. Pharm., 16: 73-76, (In Portuguese).

Kulisic, T., A. Radonic, V. Katalinic and M. Milos, 2004. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem., 85: 633-640.

Lagrota, M.H.C., M.D. Wigg, M.M.G. Santos, M.M.F.S. Miranda, F.P. Camara, J.N.S.S. Couceiro and S.S. Costa, 1994. Inhibitory activity of extracts of *Alternanthera brasiliana* (amaranthaceae) against the herpes simplex virus. Phytother. Res., 8: 358-361.

Mansur, J.S., M.N.R. Breder, M.C.A. Mansur and R.D. Azulay, 1986. [Determination of the sun protection factor by spectrophotometry]. Anais Bras. Dermatol., 61: 121-124, (In Portuguese).

Nihei, J.S.D.A. and P.S.P. Dias, 2001. [Evaluation of anti-tumor activity *in vitro* of plant extracts of the plant family Amaranthaceae]. Completion of Course Work, US., Federal University of Sao Paulo, Sao Paulo, (In Portuguese).

Pieroni, A., P. Pachaly, Y. Huang, B. van Poel and A.J. Vlietinck, 2000. Studies on anti-complementary activity of extracts and isolated flavones from *Ligustrum vulgare* and *Phillyrea latifolia* leaves (Oleaceae). J. Ethnopharmacol., 70: 213-217.

Silva, N.C.B., A.F. Macedo, C.L.S. Lage, M.A. Esquibel and A. Sato, 2005. Developmental effects of additional ultraviolet a radiation, growth regulators and tyrosine in *Alternanthera brasiliana* (L.) kuntze cultured *in vitro*. Braz. Arch. Biol. Technol., 4: 779-786.

Slinkard, K. and V.L. Singleton, 1977. Total phenol analysis: Automation and comparison with manual methods. Am. J. Enol. Vitic, 28: 49-55.

Sousa, C.M.M., H.R. Silva, G.M. Vieira Jr., M.C.C. Ayres and C.L.S. Costa *et al*., 2007. [Total phenolics and antioxidant activity of five medicinal plants]. Quimica Nova, 30: 351-355, (In Portuguese).

Souza, M.M., P. Kern, A.E.O. Floriani and V. Cechinel-Filho, 1998. Analgesic properties of a hydroalcoholic extract obtained from *Alternanthera brasiliana*. Phytoter. Res., 12: 279-281.

Souza, T.M., L.E. Santos, R.R.D. Moreira and V.L.B.I. Rangel, 2005. [Sunscreen activity evaluation of *Achillea millefolium* L. (Asteraceae)]. Braz. J. Pharm., 15: 36-38, (In Portuguese).

Violante, I.M.P., I.M. Souza, C.L. Venturini, A.F.S. Ramalho, R.A.N. Santos and M. Ferrari, 2009. [*In vitro* sunscreen activity evaluation of plants extracts from Mato Grosso cerrado]. Braz. J. Pharm., 19: 452-457, (In Portuguese).

Wannes, W.A., B. Mhamdi, J. Sriti, M.B. Jemia and O. Ouchikh *et al*., 2010. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. italica L.) leaf, stem and flower. Food Chem. Toxicol., 48: 1362-1370.

Zhang, W., M. Xu, C. Yu, G. Zhang and X. Tang, 2010. Simultaneous determination of vitexin-4-O-glucoside, vitexin-2-O-rhamnoside, rutin and vitexin from hawthorn leaves flavonoids in rat plasma by UPLC-ESI-MS/MS. J. Chromatogr. B, 878: 1837-1844.