Anti-oxidant activity from Brazilian Botanical Extracts

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Summary

Antioxidants have been used in cosmetic industry for treatment of aged skin and recently have been also introduced as additives in photoprotection products. In order to determine among the Brazilian botanical species presenting interesting antioxidant activities we have screened several extracts from plants from Rain and Amazonical Tropical Forests, as well as some endemic species, using both TBARs and DPPH˙ methods. Extracts with antioxidant activities were found with *Jacaranda caroba,* Veloso DL , *Spilanthes oleracea* (*Spilanthes acmella* var. *oleracea*), *Orbignya phalerata*, *Pothomorphe umbellata, Chiococca brachiata* and *Polypodium lepidopteris.* Other extracts such as *Camelia sinensis, Sambucus australis, Rosmarinus officinalis L.* were also studied, and showed some antioxidant activity.

Introduction

Free radicals may be generated by products of cellular metabolism and promote the oxidation of proteins, lipids and DNA, turning of into non functional molecules[6]. Other important source of the free radical generation is the UV-light, largely responsible for the skin photoaging[3,4].
It is well-know and very well spread the use of the natural antioxidants in both cosmetic and pharmaceutical products. Those can be from several sources such as tea, wine, fruits, vegetables and spices[1]. However, in the quest of new antioxidants, many other plant species have been investigated due to this increasing interest in finding new molecules and/or extracts with more efficient antioxidant activities. In cosmetics, antioxidants have been largely used for treatment of aged skin and recently have been also introduced as additives in photoprotection products.
Antioxidants are compounds which can neutralize free radicals by two different mechanisms, namely, primary (chain breaking, free radical scavengers) and secondary or preventive. Secondary antioxidant mechanisms may include deactivation of metals, inhibition of breakdown of lipid hydroperoxides to unwanted volatile products, regeneration of primary antioxidants, singlet oxygen quenching and others[1].
A large number of methods have been developed in order to evaluate antioxidant activity. This renders a direct comparison of antioxidants tested by different methods and in different substrates somewhat difficult. For the assay-guided screening of large numbers of complex samples, rapid, simple and reliable tests are required.
In the present study it was compared two widely used methods for the assessment of antioxidant activity, namely, the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH˙) method and the Thiobarbituric acid reactive substances (TBARs) method, and determined their usefulness in assay-guided screening of plant extracts. On the other hand our study focused in the antioxidant activity of plants extracts from Brazilian biodiversity (*Jacaranda caroba, Spilanthes oleracea, Orbignya phalerata, Photomorphe umbellata, Chiococca brachiata* and *Polypodium lepidopteris*) and another exotic species (*Camelia sinensis, Sambucus australis* and *Rosmarinus officinalis L.*).

Material and Methods:

Reagents: All chemicals used were of the highest purity available from Merck Chemical.

Extracts: The extracts of *Camelia sinensis, Sambucus australis, Rosmarinus officinalis L.* and *Jacaranda caroba* were solubled in glycol prolylene. The *Spilanthes oleracea* and *Orbignya phalerata* were dry. And the *Polypodium lepidopteris, Chiococca brachiata* and *Pothomorphe umbellata* were prepared in EtOH.

Table 1: Values of concentration of extracts tested.

Extract	Concentration (%)
Polypodium lepidopteris	20,0
Chiococca brachiata	20,0
Jacaranda caroba	11,73
Spilanthes oleracea	100
Rosmarinus officinalis L.	12,23
Camelia sinensis	11,8
Sambucus australis	10,67
Pothomorphe umbellata	100
Orbignya phalerata	100

Spectrophotometric assay on the reduction of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·)
Reaction mixtures containing 1% of test samples (dissolved in EtOH) and 0,004% DPPH· ethanolic solutions were incubated at 25° C for 30 minutes. Absorbances of the resulting solutions were measured using a Spectra Max Plus (Molecular Devices) at 517 nm and the percent inhibition was determined by comparison with a EtOH treated control group[5].

TBARs assay
Reaction mixtures contained, in a final volume of 1.2 mL, the following reagents at the final concentrations stated: deoxyribose (33,65 mMol/L), KH_2PO_4 buffer, pH 7,4 (0,5 Mol/L), $FeCl_3$ (1,2 mMol/L), NTA (1,2 mMol/L) and H_2O_2 (40 mMol/L). Solutions of $FeCl_3$ were made up immediately before use in deaerated water. Reaction mixtures were incubated at 37°C for 30 minutes, and color developed as describe in discussion.

Results and Discussion

The antioxidant activity has been found in all extracts in different values by the DPPH· method (Table 2 and Figure 1) and by the TBARs methodology (Table 3).
Comparing the results obtained by the two methods (Table 2 and 3), it seems that DPPH· is more sensible than the TBARs method. Probably, this higher sensibility was due to the vehicle used, ethanol.

The DPPH· method is representative of the methods employing model radicals in the evaluation of radical scavengers. The reaction is a homolytic substitution of a hydrogen atom in a *para*-position of one of the phenyl rings[2] thus nitrogen dioxide affords 2-(4-nitrophenyl)-2-phenyl-1-picrylhydrazine and/or the corresponding free radical. Such method has gained high popularity over the last decade because of their rapidity and sensitivity.
The interaction of iron ions with hydrogen peroxide in biological systems can lead to formation of a highly-reactive tissue-damaging species that is thought to be the hydroxyl radical, ·OH. Various reactive iron-oxygen complexes may also exist, such as ferryl, perferryl and $Fe^{2+}/Fe^{3+}/O_2$ species. There has thus been considerable interest in the development of methods for assaying ·OH and related species in biological systems.
The pentose sugar 2-deoxyribose is attacked by ·OH radicals to yield a mixture of products. On heating with thiobarbituric acid at low pH, some or all of these products react to form a pink chromogen that can be measured by its absorbance at 532 nm; this chromogen is indistinguishable from a thiobarbituric acid-malondialdehyde (TBA-MDA) adduct[2].

Figure1: Antioxidants activity of extracts by DPPH· assay.

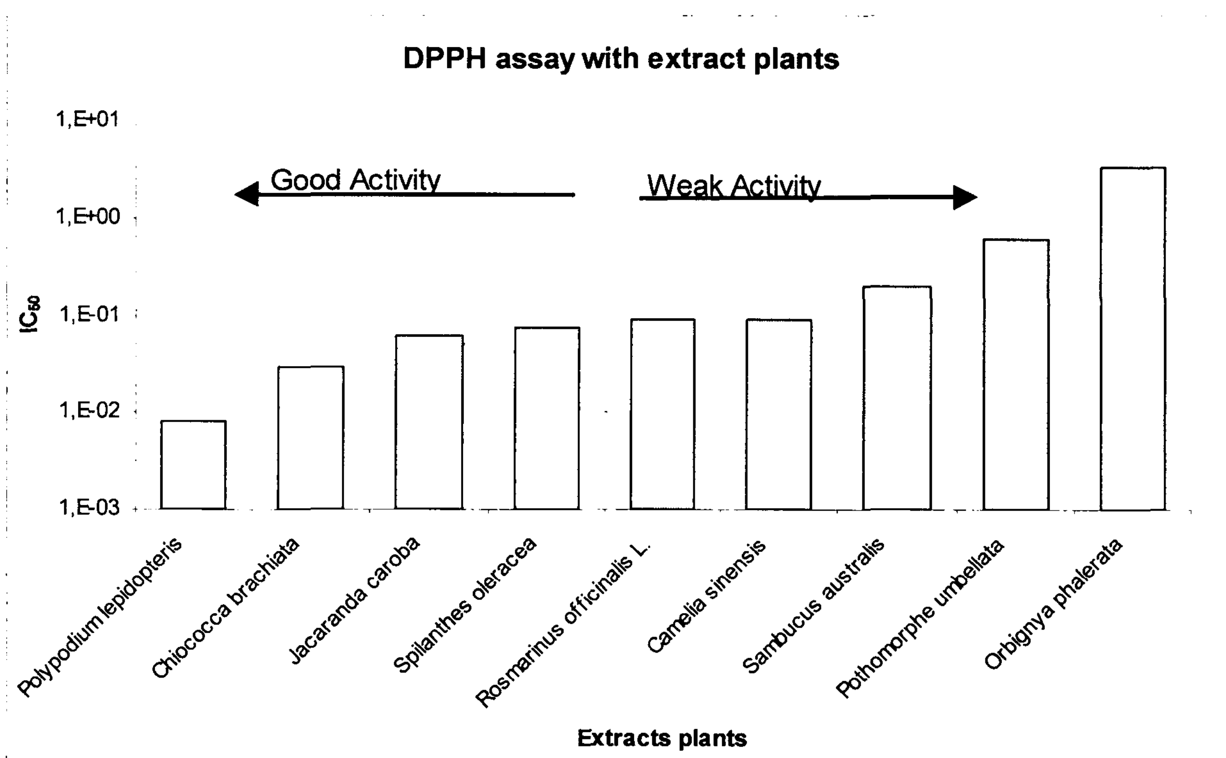

Table 2: Values of IC_{50} of extracts tested on DPPH· assay in mg/mL

Extracts plants	IC50 mg/mL
Polypodium lepidopteris	0,008
Chiococca brachiata	0,029
Jacaranda caroba	0,062
Spilanthes oleracea	0,073
Rosmarinus officinalis L.	0,088
Camelia sinensis	0,089
Sambucus australis	0,193
Pothomorphe umbellata	0,588
Orbignya phalerata	3,303

Table 3: Extracts with active concentration and antioxidant activity used to TBARs assay.

Extract	Concentration (%)	Antioxidant Activity (%)
Polypodium lepidopteris	0,00060	47,95
Chiococca brachiata	0,00020	39,83
Jacaranda caroba	0,00059	53,4
Spilanthes oleracea	0,01000	34,25
Rosmarinus officinalis L.	0,00122	44,3
Camelia sinensis	0,00590	40,5
Sambucus australis	0,00267	47,6
Pothomorphe umbellata	0,00155	41,75
Orbignya phalerata	0,03000	57,9

The TBARs method has vehicle interference with high values arresting on the extracts results.
The DPPH· method is very rapid, simple, sensitive, reproducible, and does not require special instrumentation. These methodology seems to be very useful for the screening of large numbers of samples. Besides practical aspects (time, cost, etc) DPPH· offers other very important possibilities, particularly when it is suitable or necessary to know which molecules are responsible for the antioxidant activity in a crude botanical extract. In this case, one could detect the antioxidant compounds by HPLC in complex mixtures[7,8]. This simple chromatographic methodology could be also useful in the quantification of antioxidant activity of each compound in a mixture. Preliminary results obtained in our lab for the development of a chromatographic method for antioxidant compounds detection in plant extracts, clearly showed its viability. Both lycopene, employed as a standard antioxidant compound, and the *Sambucus australis* glycolic extract showed reduction of the HPLC analytical signals after reaction with DPPH·. This reduction occurred in different levels for compounds presented in the plant extract, once different reaction extension is expected depending on the antioxidant activity of each compound present (data not shown).

Conclusion

All the plant extracts exhibit antioxidant activity in different values, wherein *Chiococca brachiata* and *Polypodium lepidopteris* extracts from the Brazilian Biodiversity, and *Rosmarinus officinalis* L. and *Camelia sinensis* extracts from the exotic plants, showed the best results.
Based on the results obtained in our study, we concluded that DPPH· method is more sensible, rapid, economical than the TBARS. Besides, it could be used to screen a great number of samples simultaneously, producing reproducible results.

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