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Purifying activity of free radicals and mutagenic controls of Loranthaceae extracts

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INTRODUCTION

Free radicals are highly reactive species, responsible for diverse health dysfunctions, *e.g.*, heart problems, cell aging processes and carcinogenesis. Mutagenic activity also involves the action of free radicals at genomic level, with hereditary alterations. Punctual DNA mutations and chromosomal reorganizations activate prooncogens or inactivate tumor suppressive genes, which are processes involved in carcinogenesis. The demand of antioxidants (free radical purifying substances) of natural origin has been increased, partly owing to the doubts raised regarding the safety in the use of synthetic antioxidants, such as butylated hydroxytoluene (BHT) or butylated hydroxyanisol (BHA). This work studies comparatively the antioxidant and antimutagenic activities of plant extracts from two species of the Loranthaceae family. The study of the mutagenic activity was also included.

METHODOLOGY

The selected plant species were *Psittacanthus cuneifolius* (Ruiz & Pav.) Blume and *Tripodanthus acutifolius* (Ruiz & Pav.) Van Tieghem. Plant material was collected in northwestern Tucumán province, each species being taxonomically identified and further herborized. Dry and powdered leaves were separately subjected to three types of extractions: infusions, decoctions and tinctures (according to the popular use forms). The extracted material (EM) and the content of phenolic compounds (CP) (Singleton *et al*., 1999) were determined for each extraction. Antioxidant activity was evaluated by different methods:

- Purifying capacity of DPPH (Yamaguchi *et al.,* 1998): The stable free radical DPPH (1,1-diphenyl-2-picryl-hydrazyl) was used. At least 5 dilutions of each plant extract were mixed in ethanol with DPPH (75 μM) up to 2 mL final volume. Quercetin, rutin and BHT were used as reference drugs. The absorbance at 515 nm was measured after 20 min incubation (light protected).

- Purifying capacity of the superoxide anion (O_2^-) (Liu *et al.*, 1997): The superoxide anion was generated in 1 ml Tris HCl buffer (20 mM, pH 8.3), with the addition of NADH (1 mM), NBT (0.1 mM), PMS (10 μM) and 0.1 mL of plant extracts at different concentrations. *L*-Ascorbic acid was used as purifying reference. The colour of the reaction was spectrophotometrically measured at 560 nm for 1 min.

- Purifying capacity of $HO^{\cdot}$ radical (Halliwell *et al*., 1987) The deoxyribose test was used in order to determine the competence between deoxyribose and plant extracts (or reference substance) for hydroxyl radicals which are generated by the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The $HO^{\cdot}$ radicals attack deoxyribose generating malondialdehyde (MDA), able to form aducts with thiobarbituric acid (TBA), which are measured at 532 nm. Slight modifications allowed to determine the specific antioxidant, non-specific antioxidant, and pro-oxidant activity of the plant extracts. Mannitol was used as purifying reference substance.

The values of 50% purifying concentration (IC_{50}, extract or reference substant concentration able to purify 50% of the reactive species of the medium)

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were graphically determined for each assay.

Mutagenic and antimutagenic activities were evaluated using *Escherichia coli* WP2 uvrA pKM 101 Trp- strains, susceptible to mutagen action. Terbutyl hydroperoxide was used (TBH) as mutation inductor agent. When incorporating S9 metabolic activator promutagens were detected. The values of the 50% antimutagenic concentration were graphically determined (AC_{50}, extract dose able to inhibit 50% of the reversion of *E. coli* strains).

RESULTS AND DISCUSSION

The assays with DPPH$^{\cdot}$ showed that the tincture of *T. acutifolius* and the infusion of *P. cuneifolius* possessed the highest purifying activity (IC_{50} = 0.1 and 0.2 µg CP/mL, respectively). Regarding reference drugs, that of higher activity was quercetin (IC_{50} = 1.6 µg/mL). All extracts of *P. cuneifolius* and the tincture of *T. acutifolius* showed higher activity than quercetin, while the tincture and infusion of *T. acutifolius* exhibited higher activity than rutin (IC_{50} = 5.7 µg/mL). BHT presented smaller purifying activity regarding all tested substances (IC_{50} = 60 µg/mL). The superoxide anion scavenging technique demonstrated that the highest activity was that of the infusion of *P. cuneifolius* and the tincture of *T. acutifolius* (IC_{50} 2.0 and 2.8 µg CP/mL, respectively). All tested extracts presented higher purifying activity than ascorbic acid (IC_{50} = 65 µg/mL). The purifying capacity of HO$^{\cdot}$ radical showed the highest non-specific (CD_{50} of 20 µg CP/mL) and specific (CD_{50} of 3.8 µg CP/mL) purifying activity for the infusion of *P. cuneifolius*. The prooxidant activity was absent in most cases. All tested extracts had higher activity than mannitol (IC_{50} = 506 µg/mL). Plant extracts with outstanding mutagenic activities were the tinctures of *T. acutifolius* and *P. cuneifolius*, whose AC_{50} values were 38 and 16 µg CP/mL, respectively. The tincture of *T. acutifolius* exhibited higher antimutagenic power than that of *P. cuneifolius* at high concentrations (100-200 µg CP/mL). Aqueous extracts didn't show antimutagenic action. The tests of mutagenic activity generated similar growth percentages to those of spontaneous reversion controls, what would imply absence of mutagenic or promutagenic activity. Antimutagenicity tests generated results similar to those of free radical scavenging tests, since the extractive forms with better purifying capacities (tinctures) also showed antimutagenic activity. The latter effect can be partially attributed to the capacity that would have tinctures to capture the alkylperoxyl (ROO$^{\cdot}$) radical generated from TBH mutagen.

CONCLUSIONS

Free radicals are species that act at genetic level. The IC_{50} values obtained with extracts were lower than those of some reference drugs, including BHT (used in food conservation). The tested antimutagenic properties complete the study of free radical scavenging. Tests didn't detect mutagenic or promutagenic actions in the extracts. Our results would indicate that the purifying capacity of free radicals depends on the type of extraction, suggesting the potential use of plant extracts as a source of genomic protective agents or carcinogenesis preventive agents.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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