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Study of the mutagenesis and antimutagenesis of *Rheedia acuminata* using *Salmonella typhimurium* retromutation assay

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ABSTRACT

Rheedia acuminata belongs to the Guttiferae (Clusiaceae) family; it was characterized by its xanthones production (natural products with significant biological activities). The mutagenesis of ethanol dichlorometane plant extract (from 2 to 2000µg/plate) was studied in the *Salmonella typhimurium* retromutation assay (TA98, TA100, TA102, TA1535 and TA1537 strains) with and without metabolic activation (S9). Likewise, mutagenesis of three fractions from plant extract (from 0.002 to 200µg/plate) was studied in TA102 strain. In both cases, data found showed that the *R. acuminata* is neither mutagenic nor promutagenic for all strains. Antimutagenesis assays of plant extract and fractions were performed by treating TA102 cells with hydrogen peroxide (H_2O_2) and adding different concentrations of extract and fractions (from 0.002 to 200µg/plate). Under these assay conditions, data found showed that *R. acuminata* extract and fractions possess a strongly antioxidant activity, confirmed for its antimutagenic activity and for its free radical scavenging activity.

1. INTRODUCTION

Cellular damage caused by exposition to reactive oxygen species is associated with aging, cancer, as well as to autoimmune, inflammatory, cardiovascular and neurodegenerative diseases, such as Alzheimer, Parkinson, multiple sclerosis, Down syndrome, etc. (Aruoma, 2003). Therefore, the search for antioxidant substances which can prevent these diseases is of great importance. During the last two decades, studies on protective effects against human carcinogenesis processes, on edible plants and on their isolated compounds have been increased (Ferguson, 2001).

Guttiferae (Clusiaceae) family is characterized for its high production of xanthones (metabolites with proven biological activity). Inside this family, *Rheedia* was characterized for its production of xanthones with cytotoxic activity, antioxidant activity, antifungal activity, antibacterial activity and activity against malaria (Bennett and Lee, 1989; Torrico *et al*., 2001). In addition, *Rheedia acuminata* has evidenced a significant activity to avoid the lipid peroxidation in the cellular walls and to capturing free radicals such as peroxides (Mollinedo *et al*., 2001).

In the present work, mutagenesis and antimutagenesis of the dichloromethane extract from ethanol of *Rheedia acuminata* were evaluated. Furthermore, mutagenesis and a antimutagenesis of three fractions obtained from the extract were tested.

2. MATERIAL AND METHODS

2.1 Ethanol dichloromethane extract of R. acuminata and its fractions

The ethanol dichloromethane extract of R. acuminata employed in this work was obtained from the bark of the plant, collected from Tumunpasa canton (470 meters over sea level) of San Buena Ventura Municipality of Iturralde County of La Paz - Bolivia in February of 2002. Both, the extract and fractions were provided by the Laboratory of Natural Products of the Institute of Chemical Investigations of the Mayor de San Andrés University.

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2.2 Mutagenesis

Mutagenesis was evaluated by the standard method of plate incorporation described by Maron and Ames (1983), using TA1535, TA1537, TA98, TA 100, and TA 102 strains and three plates per dose. Dimethylsulfoxide (DMSO) was used as negative control and well-known specific mutagens as positive controls for each strain with and without metabolic activation.

Rat liver S9 fraction was provided by Moltox Company, and was obtained from Sprague Dawley male rats pretreated with phenobarbital/*beta*-naphtoflavone.

Results were processed by Analysis of Variance (ANVA, *alpha* = 0.01) in order to estimate the mean of fixed effects. And, to analyze the differences between media concentrations of the extract or fractions with respect to negative control, the Dunnett test of multiple comparisons was used.

2.3 *Antimutagenesis*

Hydrogen peroxide (H_2O_2) was used as free radical generator and TA102 strain was used, it was frequently reported for its high sensibility to diverse oxidative agents (Levin *et al*., 1982).

Once assays were carried out, plates were incubated at 37°C for 72 hours, followed by counting the number of revertant colonies.

Antimutagenesis was determined as the percentage of remaining mutagenesis (%RM). This value is calculated from results of a minimum of two independent experiments as follows: %RM = 100 x (number of induced revertants per plate in the treatment with both mutagen and extract or fraction/number of induced revertants per plate with mutagen without extract or fraction). Number of induced revertants was calculated subtracting the number of spontaneous revertants obtained in the negative control (Ferrer *et al*., 2002).

3. RESULTS AND DISCUSSION

3.1 Mutagenesis.

The number of revertants obtained for all strains and concentrations are shown in Table 1. Data don't even duplicate the value of the negative control (spontaneous revertants). Furthermore, there is no significant difference regarding negative control (F = 1.150; P = 0.417), indicating that the extract is not mutagenic for any strain even at high concentrations (2,000 microg/plate). Moreover, the extract demonstrates not to be pro-mutagenic, as shown by revertant values with enzymatic S9 fraction, that is to say that even exposed to a metabolic activation there is no mutagenic activity (F = 3.877; P = 0.085). In the case of complex mixtures, *e.g.*, an extract, all their components and type of their biological action are not known exactly, thus being possible to find some compound that can exhibit a noxious effect when metabolized.

Table 1. Number of revertants of *R. acuminata* Extract with and without metabolic activation (S9).

Treatment[a]	S9	TA1535	TA1537	TA98	TA100	TA102
control +[b]	-	1184.7 ±69.4 (SA)	2493.7 ±453.9 (9AA)	1067.3 ±46.9 (2NF)	2454.0 ±302.2 (SA)	1293.0 ±1.4 (MC)
	+	1313.3 ±69.3 (2NA)	89.0±4.4 (1AP)	2352.7±45.1 (2AF)	1214.7 ±311.6 (2AF)	680.0±22.6 (Dt)
control -	-	32.3 ±2.5	23.0 ±4.6	20.3±4.0	115.0 ±11.5	364.7 ±5.7
	+	32.3 ±3.5	27.7 ±8.1	30.0±2.6	126.3±10.5	344.0±28.3
2000 µg/plate	-	34.0 ±4.6	20.3 ±7.1	18.7±0.6	102.7±22.9	320±5.7
	+	33.3 ±4.2	43.3±2.1	28.3±8.4	116.3 ±3.8	367.0±15.6
200 µg/plate	-	27.7 ±3.8	19.7± 3.8	21.3 ±3.1	110.3 ±16.3	374.0 ±5.7
	+	32.3 ±3.1	21.3±5.5	29.3±6.7	119.0±1.0	303.0±1.4
20 µg/plate	-	30.3 ±1.5	27.0±4.6	20.3±3.8	137.0±51.4	380.0 ± 36.8
	+	29.3±3.2	19.0 ±2.0	24.0 ±2.0	112.3 ±6.4	339.0 ±4.2
2 µg/plate	-	28.7 ±3,2	20.7 ±5.0	20.0 ±2.6	132.0 ±7.1	407.0±7.1
	+	29.3 ±3.5	24.7 ±2.3	27.3 ±7.4	115.3 ±2.1	257.0 ±125.4

[a]Standard deviations of a minimum of two independent plate-incorporation experiments are shown.
[b]Positive controls are : sodium azide (AS), 1-aminopyrene (1AP), 2-naphtylamine (2NA), 9-aminoacridine (9AA), 2-nitrofluorene (2NF), 2-aminofluorene (2AF), mytomicin C (MC), 1,8-dihydroxyantrachinone (Dt).

3.2 Antimutagenicity.

Table 2. Optimal antimutagenic effect of the different fractions from *Rheedia acuminata* against hydrogen peroxide (H_2O_2)

Evaluated Fraction	Fraction (µg/plate)	Number of revertants per plate: Fraction	Number of revertants per plate: Fraction + H_2O_2 (100 µg/plate)	%RM
Extract	0	-	1293.0 ± 1.4	100
	0.02	360.0 ± 22.6	499.0 ± 7.1	14.5
B1	0	-	1293.0 ± 1.4	100
	0.02	341.0 ± 7.5	475.0 ± 7.1	11.9
X1	0	-	1293.0 ± 1.4	100
	20	314.0± 2.8	683.0 ± 5.7	34.3
X2	0	-	1293.0 ± 1.4	100
	0.02	316.0 ± 14.1	426.0 ± 19.8	6.6

[a]Standard deviations of a minimum of two independent plate-incorporation experiments are shown. The value of negative control (TA102 strain with solvent) was 364.7 ± 7 revertants/ plate

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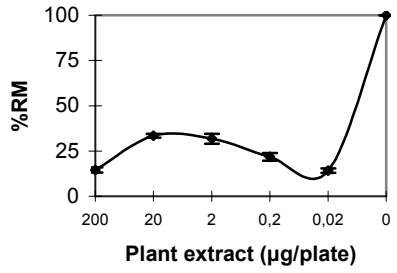
According to Table 2, the percentage of remaining mutagenesis indicates that both extract and fractions are potent inhibitors of the mutagenesis caused by hydrogen peroxide. Also, it showed that the highest antimutagenic activity was found with 0.02µg/plate of fraction X2. This fraction was preliminarily identified as a xanthone (not published data); for this reason, its chemical structure could condition this activity.

On the other hand, *R. acuminata* extract showed two concentrations with high antimutagenic activity: the highest and the lowest. Because of the lowest concentration has shown the highest antimutagenic effect, it was considered as the optimal concentration (see Fig. 1).

Figure 1. Antimutagenic effect of different concentrations of ***R. acuminata*** extract against H_2O_2



Additionally, it has been demonstrated that the extract and its fractions used for this work possess a free radical scavenging activity (not published data). These results evidence the potent antioxidant activity of the extract and its fractions.

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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