In vitro ANTIOXIDANT ACTIONS OF *Musa paradisiaca* L. EXTRACT (ACITAN®)

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Summary

Musa paradisiaca L is a plant of the family Musaceae. Differents parts of this plant (fruit, pulp, leaves) has been used as a folk medicine for treatment of peptic ulcers. Chemical constituents of the Acitan®a natural product obtained from *Musa paradisiaca* stem have been investigated, and some of them identified (tannins and phenols). In the present study, in order to characterize pharmacological actions of Acitan®, we investigated effects of the extract on antioxidant activity, using commonly accepted assays. Acitan® scavenged both superoxide anions and hydroxyl radicals, and inhibited the degradation of deoxyribose mediated by hydroxyl radicals. Also inhibited the lipid peroxidation induced by iron, in rat brain homogenates. Additionally, Acitan® inhibited DNA damage by bleomycin or copper-phenanthroline system. The antioxidant activity increased with an increasing amount of Acitan®. According to these results, it may be hypothesized that antioxidant effect of Acitan® could be related to presence of polyphenolic compounds.

Key words. *Musa paradisiacal* L, antioxidant, DNA damage, lipid peroxidation.

Introduction

Musa paradisiaca L is a plant of family Musaceae (1). The parts different of this plant (fruit, pulp, leaves) has been used as a folk medicine for treatment of peptic ulcers, analgesic, anti-asthmatic (2,3,4). Chemical constituents of the Acitan® have been investigated, and some of them identified (tannins and phenols). However, there is not information available on the pharmacological actions of stem of this plant, in order to characterize pharmacological actions of Acitan®, we investigated effects of the extract on antioxidant activity, using commonly accepted assays.

The role of free radicals has been shown in various pathological conditions, such as atherosclerosis, inflammation, arthritis, Parkinson´s disease, ischaemia-reperfusion injury, lung and heart disease, diabetes, cancer and AIDS (5,6,7,8,9,10). Reactive oxygen species (ROS) have aroused significant interest among scientist in the past decade. ROS are continuously produced during normal physiologic events, and are remove by antioxidant defence mechanism (11). There is a balance between generation of ROS and antioxidant system in organism. In pathological condition, ROS are overproduced and result in lipid peroxidation and oxidative stress. The imbalance between ROS and antioxidant defence mechanisms leads to oxidative modification in the cellular membrane or intracellular molecules (12).

There is also a consensus that the micronutrients and nonnutrient components of fruits and vegetables play a preventive role in the development of chronic diseases. Epidemiological studies have found that diets high in fruits, vegetables, herbs and spices correlate with low incidence of cancer and heart disease (13). These foods posses high levels of vitamins (vitamins E and C) and other constituents (such as fiber, carotenoides, flavonoids and other phenolic compounds), which could protect against disease (14).

Material and Methods

Plant material

The stem of *Musa paradisiacal L*. were collected in March 2002, and botanical authentication was carried out. It's was washed, cut into small pieces and dryness. The stem was pulverized. The powder was extracted with water; then the extract was lyophilized and then diluted in water to obtain the assay sample.

Chemicals

All chemicals were of the highest purity available and were obtained either from Sigma Chemical Company (Poole, Dorset, UK) or from BDH Chemicals Company (Gillingham Dorset, UK).

*Phospholipid peroxidation***.** The ability of Acitan® to inhibit peroxidation of membrane lipids at pH 7.4 was tested using rat-brain phospholipid as described by Aruoma et al (15). Male Sprague-Dawley rats (180-200 g of weight) from CENPALAB (Bejucal, La Habana, Cuba) were used to obtain the brain.

*Deoxyribose assay***.** The deoxyribose assay (16) was used to detect hydroxyl radical ($OH^{\cdot}$) scavenger activity and the iron-binding ability of Acitan®. Experiments were performed in triplicate.

*Bleomycin-iron dependent and copper-phenanthroline dependent DNA damage***.** The bleomycin and the copper-phenanthroline assays were conducted to test the pro-oxidant activity of Acitan®. Salmon testes DNA was used has a target in these assays (17). Experiments were performed in triplicate.

*Pirogallol assay***.** The pirogallol assay was used to detect anion superoxide radical ($O_2^{\cdot -}$) scavenger activity of Acitan®. Experiments were performed in triplicate.(18).

Thiobarbituric acid-reactive substance (TBARS) assay. TBARS were determined as an indicator of phospholipid peroxidation and as measure of the extent of DNA and deoxyribose damage. The assay was conducted as described by Buege and Aust (19).

Statistical analysis. The results obtained are expressed as the means ± standard deviation (s.d.) of triplicate experiments. Percent inhibition was calculated by comparing control with test experiments.

Results and Discussion

Both ferrous and ferric ions induce lipid peroxidation trough various mechanism involving reactive species. Most of the antioxidants inhibit iron-induced lipid peroxidation (15). Hence, Acitan® was tested for its effect on lipidic peroxidation induced by ferric ions (Table 1) in rat brain homogenates, it showed concentration-dependent inhibition of peroxidation, but less active than trolox, a water-soluble derivate of vitamin E, used as a standard drug for comparison (17).

Table 1. Effect of *Musa paradisiaca* Extract (Acitan®) on the lipid peroxidation induced in rat brain homogenates.

Sample	FC (%)	Extent of peroxidation (A 532nm)	Inhibition (%).
PBS(control)	-	0.198 ± 0.015	-
Trolox C	100μM	0.108 ± 0.012	45
Acitan®	0.010	0.134 ± 0.039	32
Acitan®	0.016	0.096 ± 0.033	51
Acitan®	0.050	0.074 ± 0.013	62*

Trolox C (100 μM); established antioxidant, added as positive control. Values are the means from triplicate experiments, varying by not more than 10 %. Acitan® concentration is indicated as % w/v, FC final concentration. * $p<0.05$ significantly different compared with control

The effect of Acitan® on scavenging hydroxyl radicals was measured by studying the competition between Acitan® and deoxyribose for the hydroxyl radical generated from the ferric-ascorbate-EDTA-H_2O_2 system and can inhibit deoxyribose degradation depending on its concentration and rate constant for reaction with $OH^{\cdot}$ The hydroxyl radical attacks deoxyribose and sets off a series of reactions that eventually result in TBARS formation. When a molecule scavenges a hydroxyl radical, it decreases TBARS formation. Figure 1 shows inhibition of TBARS formed in presence and absence of EDTA. In the absence of Acitan® a higher rate of deoxyribose damage was obtained, especially in presence of EDTA, desferroxamine, a know hydroxyl radical scavenger was included as control for comparison. The formation of hydroxyl radical was not accelerated when Acitan® was added to the reaction, it shows inhibition concentration-dependent ($r= 0.99$); decreased damage by over 50%. In the absence of EDTA, deoxyribose undergoes site-specific degradation, when the hydroxyl radical is generated from de ferric-ascorbate-H_2O_2 system (16). Iron binds directly to deoxyribose in the absence of chelator (EDTA) to cause site-specific degradation. Hence, the compounds

that can inhibit deoxyribose degradation in the absence of EDTA are those that are capable of chelating iron, thus rendering it inactive poorly active in the Fenton reaction, (IC_{50}=0.159 w/v). The effect of Acitan® was tested in such a system (Figure 1); Acitan® was active in such a system indicating that it is a complex agent. In presence of EDTA IC_{50}=0.0.087 w/v

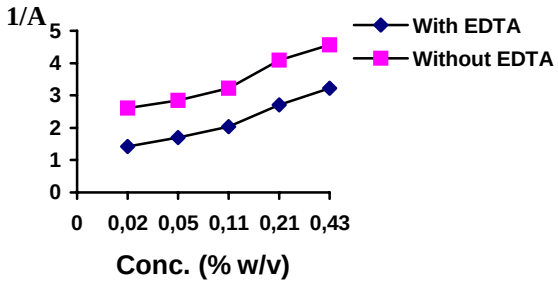

Figure 1. Action of Acitan® on hydroxyl radical-dependent degradation of deoxyribose in presence and absence of EDTA. A (absorbance at 535nm)

Bleomycin-iron III (Fe^{3+}) and phenanthroline cupric (Cu^{2+}) ions mixtures, in the presence of a reductor agent, mediated damage to DNA which could be detected using a TBA assay (16). Data in Table 2 and 3 shows that Acitan® did not promote DNA damage cupric II phenantroline or in bleomycin- iron III systems. In both cases, the extent of DNA damage was similar or lower than basal value, which pointed out that Acitan®, did not show pro-oxidant activity.

Table 2. Effects of upon copper phenantroline-mediated DNA damage

Extent of DNA damage Cu-phenantroline A $_{532\ nm}$		
Sample	**FC(%)**	**OD ± s.d**
Control (+)	-	0.175 ± 0.005
Acitan®	0.002	0.125 ± 0.001
Acitan®	0.125	0.116 ± 0.002
Acitan®	0.250	0.070 ± 0.002
Acitan®	0.500	0.038 ± 0.000

Values are the means from triplicate experiments. Acitan® concentration are indicated as % w/v). FC, final concentration (%); OD, optical density.

These differences are probably related in the way that each method caused damage to DNA. In the bleomycin-iron III system, the sugar moiety of DNA is predominantly damaged, while in the copper phenanthroline system the damage seems to be predominantly directed at the bases (17). On the other hand, in the assay that ascorbic acid was added, Acitan® showed a concentration-dependent protection to DNA damage. The square correlation coefficient between logarithm of concentration and percentage of DNA protection was 0.80 and 0.88 for bleomycin-iron III/ascorbic acid and phenantroline-cupric II ions ascorbic acid, respectively. The half protective concentration was estimated in 0.016 and 0.0084 % w/v in each case.

Table 3. Effects of upon bleomycin-iron mediated DNA damage

Extent of DNA damage Bleomycin-iron A_{532} nm		
Sample	**FC(%)**	**OD ± s.d**
Control (-)	-	0.002 ± 0.000
Control (+)	0.00035	0.079 ± 0.000
Acitan®	0.00320	0.033 ± 0.011
Acitan®	0.00650	0.022 ± 0.000
Acitan®	0.01600	0.004 ± 0.001
Acitan®	0.03100	-0.031 ± 0.008

Values are the means from triplicate experiments with values agreeing to within 5 %. FC, final concentration (%); OD, optical density.

Data in Table 4 shows that Acitan® scavenger superoxide radical.

Table 4. Effect of *Musa paradisiaca* L. extract on the O2.- scavenging activities.

Drug	**FC(%)**	**OD ± s.d**	**%**
PBS(control)	-	0.020 ± 0.001	-
SOD(control +)	200 U/L	-	92**
Acitan®	0.053	0.014 ± 0.001	30
Acitan®	0.107	0.013 ± 0.001	35
Acitan®	0.200	0.007 ± 0.001	61*

SOD (200 U/L); established antioxidant, added as positive control. Values are the means from triplicate experiments which varied by not more than 10 %. Acitan® concentration are indicated as % w/v. FC, final concentration (%); OD, optical density. *P<0.05, **P<0.001

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