Research Article

Desmutagenic activity of *Cymbopogon citratus* and *Phyllanthus orbicularis* against UVC damage in *E. coli*

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Abstract

Background: Ozone layer hole entails UV radiation increase on the earth surface, raising the risk of skin diseases, including cancer. The use of vegetal compounds is a new strategy to protect people against UV damages. *Cymbopogon citratus* (DC) Stapf and *Phyllanthus orbicularis* Kunt, are Cubans plants with experimentally demonstrated antigenotoxic properties, against several chemical and physical mutagenic agents. There is evidence of its photoprotectives activities acting as desmutagens in *ex vivo* assays but not at cellular level. **Objectives**: In this work we tested, *in vitro,* the genotoxicity and the ability to physically protect against UVC light of aqueous extracts obtained from *Cymbopogon citratus* (CcE) and *Phyllanthus orbicularis* (PoE). **Materials and methods**: The SOS Chromotest in *Escherichia coli* was used to evaluate *in vitro,* the genotoxicity and antigenotoxicity of increasing concentrations of CcE and PoE. To sense this property, two treatment approaches were used: cells were continuously incubated with plant extracts (pre-co-postreatment), and only after UVC irradiation (postreatment). **Results**: Both extracts were not genotoxic and physically protected cells from UVC damage. PoE, also protected bacterial cells after irradiation. Results suggest that CcE exert photoprotection only absorbing radiation, while PoE absolved and also enhance different repair mechanisms. **Conclusion**: Phytocomponentes of *Cymbopogon citratus* and *Phyllanthus orbicularis* protect DNA from primarily damage induced by UVC light in *E. coli* cells. These photoprotective properties are related with their absorptive capacities.

***Keywords*:** plant aqueous extract, photoprotection, SOS Chromotest

Introduction

The ultraviolet (UV) light plays an important role in the generation of DNA damage. The multiplicity of alterations produced in the DNA after exposure are associated with skin carcinogenesis and premature aging (Schuch and Menck, 2010, Brash, 2015). There has been an increase in the number of studies reporting medicinal plants and dietary components as excellent sources of photochemopreventive agents (Saewan and Jimtaisong, 2015, Rojas et al., 2016). The future of sun protection agents will include compounds with the ability of absorbing harmful radiation in combination with substances having the ability to repair damage caused within DNA (Skotarczak et al., 2015).

The Cuban species *Phyllanthus orbicularis* Kunth (Euphorbiaceae) and *Cymbopogon citratus* (DC) Stapf (Poaceae) are widely used by traditional medicine on treatments for different diseases (Alvarez et al., 2009, Quintero et al., 2011, Ekpenyong et al., 2015). Also, both plants have been studied as source of agents that protect

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DNA from UV-induced damage. Phytocompunds of *P. orbicularis* extract (PoE) and *C. citratus* extract (CcE) have shown photochemopreventive potential due to their bioantimutagenic properties in bacteria and human cells, respectively (Vernhes et al., 2013a, Fuentes-León, 2015). For PoE this activity has been related to NER system. Additionally, aqueous extracts of these plants protected a cell-free plasmid DNA against UV damage, because of their absorptive properties (González-Pumariega, 2008, Vernhes et al., 2013b). Nevertheless, there is no scientific evidence of this physical effect at cellular level.

In bacterial cells, the SOS assay is an excellent tool to evaluate the genotoxicity and antigenotoxicity of different compounds, due to their high sensibility and rapidly response against diverse mutagenic agents (Quillardet et al., 1982, Fuentes et al., 2006, Cuétara et al., 2012). This assay has been employed to evaluate the antigenotoxic action of natural compounds against UV damage (Fuentes-León, 2015, Menéndez-Perdomo, 2016).

In order to study agents that protect against UV radiation, it is highly relevant to know the mechanisms involved. In this sense, the employment of diverse design and strains deficient in repair system is an appropriate experimental approach. Particularly, the use of *E. coli* strain NER-genes deficient offers information about compounds whose photochemopreventive action is independent of NER-repair mechanism.

In this work, we evaluate the physical protective properties of aqueous extracts of *P. orbicularis* and *C. citratus*, against UVC light in *E. coli* PQ37 strain. Previously we evaluate the genotoxic activities of extracts. For this, we use a fluorescent version of SOS assay (Cuétara et al., 2012).

Materials and methods

Preparation of *C. citratus* (CcE) and *P. orbicularis* (PoE) aqueous extracts

Leaves of Caña Santa were obtained from adult plants placed in Boyeros, Havana, Cuba. The specimens were verified at the Medicinal Plants Station in Güira de Melena, Artemisa, Cuba (herbarium No. 4593). Fresh leaf pieces were previously oven-dried at 60°C for 3 days. Then they were boiled in 500 mL of water until having a final volume of 275 mL. This solution was filtered and lyophilized as indicated previously by Cápiro et al. (2001).

Specimens of *P. orbicularis* plants were collected at Cajálbana, Pinar del Río, Cuba, and were authenticated by Ph.D. Rosalina Berazain of Cuban Botanical Gardens (No.7/220 HAJB). The lyophilized extract was obtained according to the method described by Barrio and Parra (2000).

In both cases, stock solutions (16 mg/mL) were serially diluted with growth medium to obtain concentrations 0.1, 0.5, 1.0 and 2.0 mg/mL.

Bacterial strains and culture

E. coli PQ37 strain genotype (F thr leu his-4 pyrD thi gal; K o galT lac ΔU 169 sr/300::Tn10 rpoB rpsL uvrA trp::muc+ sfiA::mud(ap,lac) cts) was used in SOS Chromotest. The cells were grown at 37 ºC and 100 rev/min of shaking in Luria-Bertani (LB) media supplemented with Ampicillin 25 μg/mL until an OD_{600nm}=0.4.

UVC irradiation

Irradiation was carried out using a Vilber Loumart Lamp T15M 15 W at a temperature of 25±0.5 ºC and λ=254 nm. The dose used value was 5 J/m^2. A 1.5 mL batch of culture was UV-irradiated in 3 cm diameter Petri dishes.

SOS Chromotest

The SOS assays fluorescent described by Cuétara et al. (2012) was used for the genotoxicity and antigenotoxicity evaluation of extracts. The fluorescence was measured in a fluorometer SUMA PR-531 (TECNOSUMA International, S.A.). The wavelength for substrate excitation was 365 ηm and fluorescence was detected between 420 and 500 nm.

Genotoxicity assay

Exponential phase cultures were 10-fold diluted in a fresh LB media 2-fold concentrated and supplemented with 25 μg/mL of ampicillin, and then dispensed in eppendorf tubes containing different concentrations of CcE or PoE. (v:v) Cells were exposed during 30 min at 4°C and further the culture was grown during 2 h at 37°C. The genotoxicity criterion (Quillardet et al., 1989) was the increase of SOSIF (SOS induction factor), calculated as follows:

$$SOSIF = \frac{\beta\text{galactosidase/alkaline phosphatase(treated cells)}}{\beta\text{galactosidase/alkaline phosphatase(non - treated cells)}}$$

Antigenotoxicity assays

The antigenotoxicity of extracts against UV radiation was evaluated according to approaches described below:

Treatment A: Pre-co-postreatment, *E. coli* cells were preincubated with different concentrations of plant extracts for 30 min at 4ºC, immediately were irradiated, as described above in PBS solution in presence of different concentrations of extracts. Then the samples were incubated in LB medium during 2 h at 37 ºC.

Treatment B: Postreatment, *E. coli* cells were irradiated in absence of extracts and after, the samples were incubated in LB medium with different concentrations of extracts for 2 h at 37ºC.

The antigenotoxicity criterion was the percentages decrease of remaining genotoxicity (% RG) up to 60%. The

% RG was determined for different concentrations of plant extracts as follows:

$$\%\ RG = \frac{(SOSIF)UV\ irradiated\ cells\ with\ extracts - (SOSIF)cells\ with\ extracts}{(SOSIF)UV\ irradiated\ cells - (SOSIF)ells} \times 100$$

All SOSIF and % RG values were calculated from results of a minimal of three independent assays with four replicates for each treatment.

Statistical analysis

Means and standard errors of SOSIF were determined. Controls and treatments were analyzed using the Kolmogorov-Smirnov test. Variance homogeneity (Levene test) and single classification ANOVA were also conducted. Values of SOSIF for different treatment were compared with control using a Dunnett test ($p < 0.05$), according to STATISTICA 6.0.

Results

Before protective effects were assayed, the genotoxicity of CcE and PoE in *E. coli* PQ37 strain cells was investigated. A compound is classified as not genotoxic if the SOSIF remains ≤ 1.5, not conclusive if SOSIF is between 1.5 and 2.0, and genotoxic if SOSIF exceeds 2.0 (Kevekordes et al., 1999). Results showed that extracts not induce genotoxicity at any concentration tested in *E. coli* cells (Figure 1). All SOSIF values for both extracts were minor than 1.5.

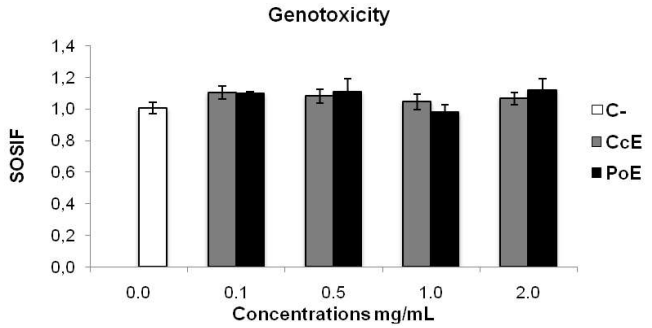

Figure 1. Effects of aqueous extract from *Cymbopogon citratus* (**CcE**) and *Phyllanthus orbicularis* (**PoE**) in the induction of SOS phenomenon in *E. coli* cells. No treatment were significantly different in Dunett Test $p < 0.05$.

Photoprotective properties of *C. citratus* and *P. orbicularis* were evaluated using two approaches. When *E. coli* cells were incubated with plant extracts in pre-co-postreatment, the genotoxicity induced by UVC was significantly reduced for both extracts in all concentrations tested. A drop of genotoxicity close to 40-50% was found in cells continuously treated with CcE or PoE. When the *E. coli* cells were treated after the irradiation, the remaining genotoxicity decrease was not under 60% (Table 1).

Discussion

For evaluating the genotoxic and protective effects from the *C. citratus* and *P. orbicularis* extracts in UVC irradiation damaged cells, the SOS assay in *E. coli* PQ 37 strains was used. Genotoxicity was analyzed using the measures of primary DNA damage in bacteria.

Table 1. Antigenotoxicity effects of aqueous extract from *Cymbopogon citratus* (CcE) and *Phyllanthus orbicularis* (PoE) in the induction of SOS phenomenon in *E. coli* cells.

Concentrations (mg/mL)	Percentage of remaining genotoxicity (%RG)			
	CcE		PoE	
	Pre-co-post treatment	Post treatment	Pre-co-post treatment	Post treatment
0.0	100± 3	100± 2	100± 2	100± 2
0.1	58 ± 6 *	90 ± 5	58± 2 *	69± 2*
0.5	52± 2 *	77 ± 7	49± 4 *	77± 4
1.0	51± 7 *	75 ± 5	50± 2 *	85± 3
2.0	51± 6 *	71 ± 7	62± 3 *	100± 3

(*) significant in Dunett Test $p < 0.05$

Phytocomponents contained in both plants were not genotoxic in *E. coli* cells. In previous studies *C. citratus* decoction have been reported as non toxic or genotoxic (Cápiro et al., 2001). This was reaffirmed by Piloto et al. (2009), fluid extract (70%) was not mutagenic or clastogenic in Ames and MN test, respectively. In plasmid model, aqueous extract didn't induce DNA primary damage neither González-Pumariega (2008). Using *E. coli* PQ-37, Fuentes et al. (2006) and Cuétara et al. (2012) reported genotoxicity in concentrations higher than 2 mg/mL, but the extraction method used, was different.

Similarly, the extract of *P. orbicularis* did not increase the SOSIF values, indicating that this plant extract was not genotoxic to *E. coli* PQ-37 cells, as it was also reported by Cuétara et al. (2012). This result is in concordance with previous reports, where PoE does not induce either primary DNA damage or mutation in CHO cells (Sánchez-Lamar et al., 1999, Sánchez-Lamar et al., 2002). Conversely, it has been found that this extract could be genotoxic in other biological models, although this genotoxicity was associated to its cytotoxicity in vitro and in vivo and times exposure higher than 30 min (Sánchez-Lamar et al., 2002, Vernhes et al., 2013b).

DNA protective agents can act in two ways. The first one is avoiding the interaction between mutagenic agent and DNA molecule, acting as desmutagens. The other way is when damage DNA is repaired or mutation fixing is impeded, acting in these cases as bioantimutagens (Bhattacharya, 2011). Therefore, pre-co-post treatment sense desmutagenic and bioantimutagenic responses. While the postreatment design, only informs about the bioantimutagenic properties.

Photoprotection results shown that CcE and PoE, protected *E. coli* cells from damage induced by UVC light. In both

extracts the effects observed in pre-co-postreament, was higher than the observed in postreatment. These results could be related to the desmutagenic properties of mixtures. Suggesting that, the photodamage diminished in bacterial cells is mainly due to the capacity of extracts to impede the DNA damage formation. This action could be related with the absorptive properties of phytocompounds in the extracts. Previous transmittance studies of these plants revealed that they are capable to absorbed light at λ=254 nm. CcE and PoE shown maximum of 100% of UVC absorption since 0.5 mg/mL, respectively (data no published) (Vernhes et al., 2013b).

The reduction of photons reaching the cells represents a first line of defense. Some blocking phenolic compounds of light UV are phenolic acids, terpenes, monoterpenes, tannins, flavonoids and volatile oils (Rojas et al., 2016). All this phytocomponents acquire structures that could be able to absorb radiations, including UV light. Compounds like limonene, quercetin and kaemferol possess absorption peaks between 250 and 280 nm and are present in lemon grass. Also coumaric, chlorogenic and caffeic acids, geranial and neral possess structures favorable to absorb UV light (Figueirinha et al., 2008, Pinto et al., 2015, Rojas et al., 2016). On the other hand, phytochemistry studies of *P. orbicularis* extract have revealed the presence of flavanols, quercetin glycosides, condensed tannins, gallic acid-derivatives and catechin (Gutiérrez et al., 2000, Alvarez et al., 2009, Gutiérrez et al., 2010). These compounds isolated from different plants extracts protect against UV radiation (Rojas et al., 2016). In this sense, both mixes could potentially act as a barrier for UV radiation and protect DNA from the damage induced.

In case of PoE, the effect found after UV damage (postreatment) could be related with the presence of compounds that modulate the repair mechanism in *E. coli* cells. There is evidence that this mixture modulates the UV-DNA repair system and reduces DNA damage in human cells at low concentrations (Vernhes et al., 2013a). This bioantimutagenic property of PoE can be acting in this biological model, although *E. coli* cells used are NER-deficient. Thus, in this case other DNA repair pathways could also be involved in the protection observed.

There are several examples of natural products that protect DNA, increasing different DNA-repair mechanisms (Bakkali et al., 2006, Das et al., 2013, Rodeiro et al., 2014). Due to the variety of lesions induced by UV light, many repair system are involved in damage removing beside NER system. Base excision (BER) and Recombination repair play an important role in this sense (Rastogi et al., 2010). In biological model NER-deficient, enhanced post-replication repair and recombination mechanisms have been observed after treatment of *E. coli* cells with antimutagenic compounds (Ohta et al. 1988). Phenolic compounds obtained from *Hypericum sps.* increased repair of alkylating DNA damage by base excision repair pathway (Ramos et al. 2013). Also, a lignan isolated from dry seeds of *Piper cubeba* induce recombinational DNA repair (de Rezende et al. 2013). A similar response could explain results found in PoE postreatment.

A strategy for protecting the skin from UV light is topical application of sunscreen products that block the radiation. Several natural compounds with UV absorption property have been used to substitute or to reduce the quantity of synthetic agents in sun-creams (Saewan and Jimtaisong, 2015). Previous studies of the *C. citratus* and *P. orbicularis* extracts had shown the capacity to avoid the UV-DNA damage through the absorptive properties. But, in both cases, this photoprotective mechanism only has been demonstrated in *ex vivo* DNA plasmid assay (Vernhes et al., 2013b, González-Pumariega, 2008). The results obtained in this work confirmed these physical capacities to protect DNA at cellular level. The protective effect against UV light obtained for these extracts in *E. coli* cells are a complement in the photochemopreventive evaluation of *Cymbopogon citratus* and *Phyllanthus orbicularis* plants. These absorptive properties enlarge their potential use in the cosmetic and pharmaceutical industry as protector agents against UV-induced skin damage.

Cymbopogon citratus (DC) Stapf and *Phyllanthus orbicularis* Kunth aqueous extracts do not present genotoxic effects in *E. coli* cells. On the opposite, these extracts reduce the UV-DNA induced damage in bacterial cells and these photoprotective properties can be related with their absorptive properties.

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Declaration of interest

The authors report no declarations of interest.

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