UNIVERSIDAD DE SANTIAGO DE CHILE

BOLETÍN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
17 (1): 1 - 16 (2018)
© / ISSN 0717 7917 / www.blacpma.usach.cl

Blacpma

Revisión / Review

Cuban flora species as a potential source of DNA protective compounds

[Especies de la flora cubana cómo fuente potencial de compuestos protectores del DNA]

Ivette M. Menéndez-Perdomo[1,2], Fabiana Fuentes-León[1] & Ángel Sánchez-Lamar[1]

[1]*Department of Plant Biology, Faculty of Biology, University of Havana, La Habana, Cuba*
[2]*Current address: Department of Biological Sciences, University of Calgary, Calgary, Canada*
Contactos | Contacts: **Ivette M. MENÉNDEZ-PERDOMO** - ***E-mail address:*** **ivette.menendez1@ucalgary.ca**
Contactos | Contacts: **Ángel SÁNCHEZ-LAMAR** - ***E-mail address:*** **alamar@fbio.uh.cu**

Abstract: Environmental exposure to genotoxic agents represents a major health concern for modern society. DNA damage could lead to mutations, which accumulative effect is closely related to degenerative and lethal diseases, such as cancer. Because of their structural and chemical diversity natural products play a fundamental role in pharmaceutical sciences for novel drug discovery. The present review article focuses on pre-clinical studies done with some species from Cuban flora that have been tested with positive antigenotoxic properties against different genotoxins. Special emphasis regarding molecular mechanisms suggested, from antioxidant activity to DNA repair modulation, a critical discussion of the state of art and the perspectives in the use of these plants as a new and promising strategy for genoprotection in the 21st Century are included.

Keywords: genoprotection, *Cymbopogon citratus*; *Mangifera indica*, *Phyllanthus* spp, *Pinus caribaea*, *Punica granatum.*

Resumen: La exposición ambiental a agentes genotóxicos representa un problema de salud significativo en la sociedad actual. El daño al ADN puede generar mutaciones, cuyo efecto acumulativo se encuentra estrechamente relacionado con enfermedades degenerativas y letales como el cáncer. Debido a su diversidad estructural y química los productos naturales juegan un papel fundamental en las ciencias farmacéuticas en el descubrimiento de nuevas drogas. El presente artículo de revisión puntualiza estudios pre-clínicos realizados con determinadas especies de la flora cubana que han sido estudiadas con una respuesta antioxidante positiva frente a diferentes genotoxinas. Se enfatizan especialmente los mecanismos moleculares sugeridos, desde actividad antioxidante hasta modulación de la reparación del ADN, así como una discusión crítica del estado del arte y las perspectivas en el empleo de estas plantas como una estrategia nueva y prometedora para la genoprotección en el siglo 21.

Palabras clave: genoprotección, *Cymbopogon citratus*; *Mangifera indica*, *Phyllanthus* spp, *Pinus caribaea*, *Punica granatum.*

Recibido | Received: April 2, 2017
Aceptado | Accepted: September 9, 2017
Aceptado en versión corregida | Accepted in revised form: December 9, 2017
Publicado en línea | Published online: January 30, 2018
Este artículo puede ser citado como / This article must be cited as: IM Menéndez-Perdomo, F Fuentes-León, Á Sánchez-Lamar1. 2018. Cuban flora species as a potential source of DNA protective compounds. **Bol Latinoam Caribe Plant Med Aromat** 17 (1): 1 – 16.

INTRODUCTION

Genotoxins constitute physical, chemical or biological agents that are capable to damage DNA. They could also be considered as a mutagen, carcinogen, or teratogen if produce mutations in DNA, trigger cancer, or cause a birth defect, respectively. Environmental genotoxic agents such as γ and Ultraviolet (UV) radiation, aromatic amines, hydrogen peroxide (H_2O_2), among many others, represent a major health concern for modern society. Hence, the search for compounds with antimutagenic capacity is an area of increasing research.

Chemoprevention of mutation-related diseases such as cancer is supported by the use of chemical drugs from natural or synthetic sources to reverse, delay, and/or prevent the development or recurrence of cancer (Landis-Piwowar & Iyer, 2014; Kotecha *et al.*, 2016). To achieve this purpose, the search for chemopreventive agents, the assessment of their efficacy and safety, and the knowledge of the mechanisms involved are highly relevant (Seebode *et al.*, 2016).

Many naturally occurring compounds such as flavonoids, coumarins, carotenoids, anthraquinones, and tannins have shown antimutagenic properties. Depending on their mode of action, antimutagens can be classified as desmutagens or bioantimutagens (Bhattacharya, 2011). The former are able to inactivate the mutagens by interacting with it (physical, chemical or biochemically) before it reaches the DNA. Meanwhile the latter suppress the process of mutation after the damage has occur, by modulating DNA repair and replication processes, and hence are able to decrease the frequency of induced, but also spontaneous mutations in the cell (De Flora & Ferguson, 2005). Bioantimutagens could act increasing the DNA polymerase fidelity; inhibiting the error-prone and enhancing the error-free DNA repair mechanisms; inhibiting the expression of mutated genes by epigenetic mechanisms; or by inducing apoptosis.

The therapeutic use of plants is part of the universal humankind culture. Since the isolation of morphine from opium poppy at the beginning of 19 Century (Krishnamurti & Rao, 2016), a revolution in the discovery of new active ingredients from natural sources, especially plant secondary metabolites, has taken place. Currently, medicinal plants and dietary components are considered as excellent sources of chemopreventive agents (Saewan & Jimtaisong, 2015; Kotecha *et al.*, 2016; Montes de Oca *et al.*, 2017) and phytotherapy is considered a key alternative to mitigate the side effects due to indiscriminate use of synthetic drugs.

Cuban flora is extremely rich. Insularity and geologic diversity had created a scenario for a high endemism and extensive habitat variety. In addition, cultural mix of aboriginal settings from pre-columbines times, Spanish conquers, African slaves, as well as Chinese culture, made traditional medicine based in plant remedies a current and fundamental health tool. More than the 50% of the total medicinal plants reported in Cuba (1241 species, 97 of them endemic) are known or employed, showing a broad use of traditional medicine by Cuban population (Fuentes, 2008). Moreover, government efforts to include complementary and alternative medicine into medical school curricula, so that graduates will be able to advise their patients in their correct use, have been acknowledge by several Latin-American and U.S. medical practitioners as an example to follow and incorporate in their educational systems (Appelbaum *et al.*, 2008). Several species commonly used in Cuba folk medicine are approved by Cuban Ministry of Public Health as complementary medicine or raw material for the production of phytopharmaceuticals. However, for quite a few, there is a lack of pharmaceutical studies to sustain the properties attributed by the population. On the other hand, sometimes lab research has shown biological properties beyond their current use in folk medicine, enhancing their bioactive use.

Among the herbal medicines used the most by Cuban population in recent years are syrup and cream based in *Cymbopogon citratus* diuretic, antihypertensive, and anti-inflammatory properties, and a fluid extract from *Pinus caribaea* with antifungal activity (González-Ramírez *et al.*, 2007). Also, it have been proposed to incorporate the species *Mangifera indica* with proved anti-inflammatory and antioxidant properties to the list of medicinal plants in Cuban Pharmacopeia (González-Ramírez *et al.*, 2007). Other plants such as *Punica granatum* and several endemic species of *Phyllanthus* have been employed by its antiviral and antioxidant properties (del Barrio *et al.*, 1995; Sanchez-Lamar *et al.*, 2005; Del Barrio *et al.*, 2014; Sanchez-Lamar *et al.*, 2015). Although there are a few species from Cuban flora that have been studied for its antimutagenic properties (Ramos *et al.*, 2003), we consider that the mentioned plants have a more complete genoprotective profile as have been the target for decades of research on their potentialities as a source of antigenotoxic compounds.

In this review, an up-dated discussion of the genoprotective properties of selected species is presented, essentially focused in the antigenotoxic studies conducted in the country. International databases such as Pubmed Central, as well as national journals, were exhaustively searched for significant data gathering. Possible molecular mechanisms responsible for such activity are discussed and future perspectives outlined.

Toxicological evaluation of some Cuban plants with genoprotective effects

In order to assess the potential genoprotective properties of plant extracts, it is mandatory to first investigate their cyto- and genotoxicity. Every plant extract with antigenotoxic activity that is reviewed in this article has been extensively study by a serial of toxicological tests with different endpoints, from primary structural DNA damage to more complexes levels such as cell or organism viability. This approach allows conducting the genoprotective studies at concentrations that have proven not to be toxic in the particular experimental model, and hence support their safety.

Different formulations and chemical fractions from *C. citratus* have been evaluated in plasmid DNA (González-Pumariega *et al.,* 2016), bacterial cells (Vernhes *et al.*, 2016; Fuentes-León *et al.*, 2017a; Fuentes-León *et al.,* 2017b), and eukaryotic organisms (Cápiro *et al.*, 2001; Fernández-Calienes *et al.*, 2009), showing no toxic effects.

In *M. indica* low toxicity (Fernández-Calienes *et al.*, 2009; Garrido *et al.*, 2009) and lack of DNA primary damage effect, mutagenicity and clastogenicity, have been reported in several *in vitro* and *in vivo* experimental models (Martínez *et al.*, 2000; Cancino-Badías *et al.*, 2001; Rodeiro *et al.*, 2006; Rosario *et al.*, 2009; Rodeiro *et al.,* 2012; Rodeiro *et al.*, 2014).

Acute and sub-chronic toxicity studies proved that *P. orbicularis* aqueous extract did not exert any toxic effect by oral administration at doses recommended for using as antiviral in humans (Rivero & Vidal, 1998), as well as not irritant properties were detected on dermis and ophthalmic tests (Gutiérrez *et al.*, 2002). Likewise, it did not induce either primary DNA damage or mutation when *ex vivo* experiments with plasmid DNA, SOS gene induction, gene reversion and conversion, and SMART assays were performed in different experimental models such as *Escherichia coli*, *Salmonella typhimurium*, *Sacharomyces cerevisiae*, and *Drosophila melanogaster* (Sánchez-Lamar *et al.*, 1999; Sánchez-Lamar *et al.,* 2002; Cuétara *et al.*, 2012; Vernhes *et al.*, 2013a; Vernhes *et al.,* 2013b; Vernhes *et al.,* 2016). *P. williamioides* and *P. chamaecristoides* extracts, showed no toxicity when assessed in human lung cancer cell line A549, neither acute toxicity in *Artemia salina* (Wong, 2013). Recently, we proved that the aqueous extract of *P. williamioides*, *P. chamaecristoides* and *P. microdictyus* are not cytotoxic nor genotoxic (< 2.0 mg/mL) in *Caulobacter crescentus* cells (Menéndez-Perdomo *et al.*, 2016) .

In *ex vivo* experiments, *P. caribaea* aqueous extract did not exert genotoxic effects (Vernhes *et al.*, 2017) and did not induce primary DNA damage (with and without enzymatic activation) in *E. coli* cells (Fuentes *et al.*, 2006b; Cuétara *et al.*, 2012). Additionally, no toxicity was detected in CHO cells exposed to the tannins rich fraction (manuscript in preparation).

Pomegranate whole fruit extract does not affect cells proliferation, neither produce DNA-primary damage in different cells lines measure by Sister Chromatids Exchange (SCE) and DNA fragmentation assays (Sánchez-Lamar *et al.*, 2005; Casadelvalle *et al.*, 2006), and the detected toxic effects of *Punica granatum* fruit extract occurred at higher doses than those used in Cuban folk medicine (Vidal-Novoa *et al.*, 2003).

Selected Cuban flora species with genoprotective properties

Cymbopogon citratus

Cymbopogon citratus (DC) Stapf, known in Cuba as "Caña Santa", is a monocot plant that belongs to the Poaceae Family (Order Poales). The name *Cymbopogon* is derived from the Greek words "kymbe" (boat) and "pogon" (beard), referring to the flower spike arrangement (Shah *et al.*, 2011). The lemongrass is one of the most frequently used plants in Cuban traditional medicine. It is consumed as decoction for treat kidney and respiratory disorders, stomach pains, and also as analgesic, diuretic and anti-inflammatory, although only some of these pharmacological properties have been tested (González-Ramírez *et al.*, 2007; Volpato *et al.*, 2009; Pérez-Machín *et al.*, 2011b). It has been proved that leaves' decoction acts as hypotensive (Carbajal *et al.*, 1989) and antispasmodic (Moron *et al.*, 2000) and the essential oils possesses insecticidal, antimicrobial (Hernández & Rodríguez, 2001) and antifungal activities (Guerra *et al.*, 2004; Pinto *et al.*, 2015).

Antigenotoxic studies using Chromosome Aberrations in CHO cells and Somatic Mutation and Recombination Test (SMART) assay in *Drosophila* showed that *C. citratus* decoction possesses protective properties when tested in co-treatment against alkylating and oxidant agents such as Etilnitrosourea (ENU), Metilmetanesulfonate (MMS), Juglone (JG), 7-12-dimetilbenzoantracene (DMBA) and H_2O_2 (Cápiro *et al.*, 2001; Cápiro *et al.*, 2005). According to the dose-effect curves obtained for each mutagen, authors suggested that *C. citratus* phytocomponents act as desmutagens. In this sense, different mechanisms like chemical or enzymatic inactivation, prevention of formation of active species or antioxidant free radical scavenging, could be involved. Furthermore, aqueous extract showed radioprotective properties in *E. coli* PQ-37 cells, only in the pre-treatment or co-treatment approach (Fuentes *et al.*, 2006a). A possible explanation proposed by these authors was based on free radical scavenging mechanisms in concordance to Cápiro *et al.* (2001) hypothesis. More recently, *Cymbopogon* was tested against the DNA damage induced by UVC light in different experimental models. Aqueous extract from 0.5-4.0 mg/mL reduced CPD formation in co and pre-treatment approaches, using plasmid model (González-Pumariega *et al.*, 2016). Also, when *E. coli* PQ-37 cells were continuously incubated with *C. citratus* extract (before, during and after UVC irradiation), the damage, measured by SOS Chromotest, was significantly reduced. In all concentrations tested 0.1-4.0 mg/mL, a drop of genotoxicity close to 40-50% was found. *E. coli* cells treated after UVC irradiation, decrease the remaining genotoxicity only to 60% (Vernhes *et al.*, 2016). Similar results were found evaluating *Cymbopogon* essential oils in this bacterial model (0.1-2.0 mg/mL). Continuous application reduces UVC genotoxicity, not being that way the incubation after UVC irradiation (Montano-Pérez, 2011). Interestingly, in *C. crescentus* cells same concentrations of essential oils and aqueous fraction didn't reduce primary UVC-induced DNA damage, but showed antimutagenic capacity (Fuentes-León *et al.*, 2017b). Those last results also indicate bioantimutagenic properties in this plant compounds.

C. citratus is widely distributed, extensively used and studied worldwide. There are evidences about its DNA protective capacity in studies conducted around the world. For instance, inhibited chromosomal aberration in human lymphocytes exposed to mitomycin C (Meevatee *et al.*, 1993), micronucleus formation in rats exposed to cyclophosphamide (Pinsaeng, 1993), mutagenesis against several agents in *S. typhimurium* (Vinitketkumnuen *et al.*, 1994), and decreases the DNA adducts induced by azoxymethane (AOM) in rat colon (Suaeyun *et al.*, 1997). Also, the hydroalcoholic and aqueous extracts showed radioprotective potential, decreasing clastogenic damage chinese hamster lung fibroblast cells (V79) using micronucleus assay and plasmid DNA *ex vivo* assay, respectively (Rao *et al.*, 2009; Kanatt *et al.*, 2014). Both authors demonstrated a significant scavenging ability, using several *in vitro* assay systems. These results are in concordance to Balakrishnan *et al.* (2014) who proved that chloroform, methanol and water extracts of *C. citratus* leaves effectively decreased the extent damage caused by H_2O_2 in DNA isolated from human blood serum. Moreover it was reported that lemongrass essential oil exhibited protective action against N-methyl-N-nitrosurea-induced DNA damage (Bidinotto *et al.*, 2011), and particularly Citral (it´s main component) is effective in counteracting the chromosomal aberrations induce by Arsenic in bone marrow mice cells (De *et al.*, 2015). These DNA protective results are in concordance with the findings in the Cuban plants.

Mangifera indica

Known as mango, *Mangifera indica* Linn is a tree from the Family Anacardiaceae (Order Sapindales). Bark and leaves of mango are used in Cuba to treat diabetics, hypertension, digestive, gastrointestinal, and respiratory problems and also as anti-inflammatory, diuretic and antioxidant (González-Ramírez *et al.*, 2007; Volpato *et al.*, 2009; Pérez-Machín *et al.*, 2011a; Machín *et al.*, 2011b). Vimang is a natural product obtained in Cuba from an aqueous extract of this plant bark. This is a complex mix rich in polyphenols, and Mangiferin (**Figure 1**) is the predominant component. This glucosylxanthone is found in large quantities not only in the stem bark but also in leaves and fruit of *M. indica*. (Núñez-Sellés *et al.*, 2002; Núñez-Sellés *et al.*, 2007). Chemical, pharmacological and toxicological studies reinforce Vimang as anti-inflammatory (Garrido *et al.*, 2001; 2004a; Garrido *et al.*, 2004b), analgesic or anti-nociceptive (Garrido *et al.*, 2001; Garrido-Suárez *et al.*, 2010; Garrido-Suarez *et al.*, 2011b; Garrido-Suarez *et al.*, 2011a), immune-modulator (García *et al.*, 2002), antioxidant (Martínez *et al.*, 2000; Pardo-Andreu *et al.*, 2006a;

Pardo-Andreu *et al.*, 2006b; Pardo-Andreu *et al.*, 2008; Garrido *et al.*, 2008) and anti-allergic (García *et al.*, 2006). Furthermore, Vimang inhibited DNA damage caused by copper-phenanthroline and bleomycin systems (Martínez *et al.*, 2000) but not in front of cyclophosphamide in mice pretreated (Cancino-Badías *et al.*, 2001). Also patients with cancer, diabetes mellitus, prostatic hyperplasia, bronchial asthma, psoriasis, lupus erythematosus and dermatitis improved their quality of life with therapeutic use of this extract (Guevara *et al.*, 2004; Guevara *et al.*, 2007).

M. indica bark extract showed antigenotoxic capacity in co-treatment approach, against the damage induced by γ radiation, measure by SOS Chromotest in *E. coli* (250, 500 y 1000 μg/mL) (Rosario *et al.*, 2009) and comet assay in primary human lymphocytes and lymphoblastoid cells (25 and 50 μg/mL) (Rodeiro *et al.*, 2014). Also, concentrations between 50 and 500 μg/plate significantly reduced the mutagenicity mediated by chemical agents like 1-nitropyrene (1-NP), 2-acetylaminofluorene (2-AAF), benzo[a]pyrene (BP), bleomycin, cisplatin, cyclophosphamide, dimethylnitrosamine (DMNA), mitomycin C, and picrolonic acid, and a inhibition of CYP1A1 microsomal activity was observed at 10–20 μg/mL (Morffi *et al.*, 2012). Mangiferin have been proved to be responsible for these antimutagenic properties against chemical agents in *S. typhimurium* assay (Rodeiro *et al.*, 2012) and for the desmutagenic effects of Vimang in human cells (5–25 μg/mL) (Rodeiro *et al.*, 2014). All this authors suggest that DNA protective activity could be explain by antioxidant properties, acting as an oxygen free radicals scavenger, and in addition the capacity of mangiferin to inhibit CYP1 enzymes (Martínez *et al.*, 2000; Rosario *et al.*, 2009; Rodeiro *et al.*, 2012).

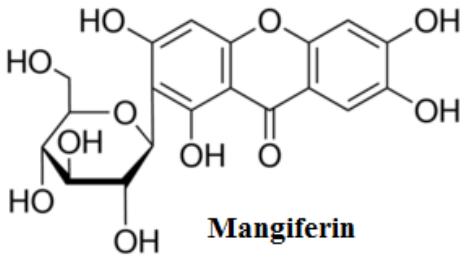

Figure 1
Mangiferin, principal bioactive compound detected in *Mangifera indica*.

Mango is widely used as a food supplement in the form of a pickle, and the fruit is eaten as such or is drunk in the form of juice worldwide. In this sense it has been demonstrated that mango wine and juice are able to protect plasmid DNA against UV, H_2O_2 and γ-irradiation induced DNA damage (Naresh *et al.*, 2014, *Naresh et al.*, 2015). Furthermore treatment of HepG2 cells with mango peel extract prior to oxidative stress was found to inhibit DNA damage in Comet Assay (Kim *et al.*, 2010). It has been proved that mangiferin (50 μg/mL) reduced the γ radiation-induced DNA damage in a concentration-dependent manner in human peripheral blood lymphocytes (Jagetia & Venkatesha, 2006). These authors as Cuban findings confirm mango´s protective *in vitro* actions and thus could be a valuable source of antioxidants. Vascular, cardiac and intestinal effects of an extract of *Mangifera indica* have been studied (Bustamante *et al.*, 1998).

Phyllanthus spp.

Phyllanthus species were described for the first time in 1737 by Linnaeus and belongs to the largest genus of the Family Phyllanthaceae (Order Malpighiales) comprising approximately 1270 species (Hoffmann *et al.*, 2006). *Phyllanthus* plants possess a remarkable diversity and their major significance relies in their multiple ethnomedicinal properties (Sarin *et al.*, 2014). Preclinical and clinical studies carried out with the extracts and purified compounds from these plants support most of their reported uses in folk medicine for the treatment of a wide variety of pathological conditions, particularly their DNA photoprotective properties (Menéndez-Perdomo & Sánchez-Lamar, 2017).

Over the past couple of decades, our research group in the Laboratory of Toxicological Genetics at the University of Havana has studied the genotoxic

and genoprotective properties of Cuban endemic specie *Phyllanthus orbicularis* Kunth, commonly known as "Alegría". *P. orbicularis* is used in folk medicine and several studies conducted with the aqueous extract have proved its antiviral (del Barrio & Parra, 2000; Fernandez *et al.*, 2003; Álvarez *et al.*, 2009; del Barrio *et al.*, 2014) and antioxidant activities (Sánchez-Lamar *et al.*, 1999; Ferrer *et al.*, 2002; Sanchez-Lamar *et al.*, 2015). Phytochemical characterization had revealed the presence of flavonoids, tannins, antocianidins, coumarins, gallic acid-derivates, catechin, epicatechin, and others (Gutiérrez *et al.*, 2011).

P. orbicularis aqueous extract exhibits genoprotective effects against several chemical mutagens. In CHO cells treated with the extract at concentrations ≤ 0.1 mg/mL, it significantly reduced the H_2O_2-induced chromosome aberrations, probably through its antioxidant activity (Sánchez-Lamar *et al.*, 1999). A similar result was detected using the *Salmonella* assay at a dose of 200 μg/plate, and the better antimutagenic effects (less than 30% of remaining mutagenesis) were shown for the pre-treatment approach, when bacterial cells and the extract were incubated before the addition of H_2O_2 (Ferrer *et al.*, 2002). Moreover, through a bioactive-directed fractionation it has been isolated and identified by Gas Chromatography/Mass Spectrometry three phytochemicals responsible for the aqueous extract antimutagenesis against H_2O_2 using the *Salmonella* retromutation assay (Sánchez-Lamar *et al.*, 2015).

Furthermore, when tested by the Ames assay this plant extract produced a significant decrease of the mutagenesis mediated by several aromatic amines, such as *m*-phenylenediamine, 2-aminofluorene, 1-aminopyrene, 2-aminoanthracene, and 9-aminophenantrene (Ferrer *et al.*, 2001). The authors proposed an inhibition/competition for S9 enzymes, as well as chemical modification into non-promutagenic derivatives, as the possible antimutagenic mechanisms depending on the amine tested. Additionally, the plant extract was effective in reducing the mutagenesis of two procarcinogenic aromatic amines (2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine and 4-aminobiphenyl) when assessed by the *Salmonella* assay (Ferrer *et al.*, 2004). The best results were obtained when both amines were metabolized by human liver microsomal enzymes in the presence of 1.0 mg/plate of the extract, with more than a 75% reduction of the mutagenicity. The results indicated an inhibition/modulation of the CYP1A subfamily, responsible for the activation of these promutagenic amines. We were able to link both last activities to 2,4-di-tert-butylphenol, OO'-diphenol-4,4',6,6'-tetra-tert-butyl and 2,6-di-sec-butylphenol (**Figure 2**), and currently studies are being taken in order to modeling *in silico* their molecular action mechanism.

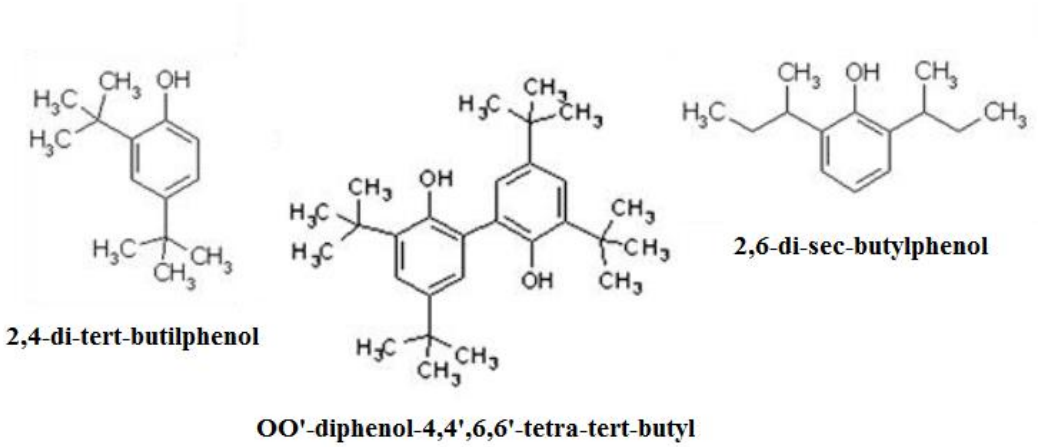

Figure 2
2,4-di-tert-butylphenol, OO'-diphenol-4,4',6,6'-tetra-ter-butyl and 2,6-di-sec-butylphenol principal bioactive compound detected in *Phyllanthus orbicularis*.

P. orbicularis aqueous extract also displays radioprotective effects. The highest antigenotoxic properties of the extract on *E. coli* cells γ-irradiated was reached at a concentration of 2.0 mg/mL (Alonso

et al., 2005). By means of SOS transcriptional-fusion-based assay and using different approaches (pre, co, post-treatment) the authors were able to suggest as the antimutagenic mechanisms implicated in the radioprotection the synergism of a free radicals scavenging mechanism and modulation of DNA repair systems. These results were supported by latter studies using the *Salmonella* reversion assay (Alonso *et al.*, 2010), in which the remaining mutagenesis percentage significantly decrease when cells were irradiated in the presence of the extract at doses ≤ 0.5 mg/mL.

Also, *P. orbicularis* aqueous extract protected DNA against UV-B (≥ 0.1 mg/mL) and UV-C radiation (≥ 0.5 mg/mL) in *ex vivo* experiments using cell-free plasmid DNA (Vernhes *et al.*, 2013b). In the cited study, the transmittance quantification data showed that for 0.1 mg/mL around 50% of UV radiation was blocked by the extract, and 100% of it for higher concentrations tested. These results suggest that there are some phytocomponents in the extract capable of absorbing short- and medium-wave UV radiation and inhibiting bipyrimidine photoproducts formation; although it is also possible that antioxidant components were able to decrease the Reactive Oxygen Species (ROS) induced by UV-B, in a synergic UV absorption/antioxidant photoprotective mechanism. In another study, the photoprotective effect of the extract was also associated to non UV absorption mechanisms. When *P. orbicularis* aqueous extract (1 µg/mL) was tested in DNA repair proficient (MRC5-SV) and deficient (XP4PA, complementation group XPC) human cell lines, it enhanced the removal of cyclobutane pyrimidine dimers (CPD) from genomic DNA in a time-dependent manner, possibly by means of a positive modulation of Nucleotide Excision Repair (NER) system, suggesting the bioantimutagenic capacity of this extract against UV radiation-induced DNA damage (Vernhes *et al.*, 2013a). Additionally, using the SOS Chromotest assay it was demonstrated that *P. orbicularis* aqueous extract (0.1-2.0 mg/mL) applied after irradiation protected DNA from primarily damage induced by UV-C light in *E. coli* cells deficient in NER system, which is indicative that the enhancement of other DNA repair mechanisms, such as Base Excision Repair (BER) and recombination-based repair, could be involved in a bioantimutagenic response (Vernhes *et al.*, 2016).

We also investigated the antimutagenic properties of *P. chamaecristoides*, *P. microdictyus*, and *P. williamioides* aqueous extracts against UV-C artificial light by the Rif^R Assay at concentrations non-toxic in *C. crescentus* cells. All the three aqueous extracts showed DNA photoprotection as they significantly diminish SOS response, meaning that they protect DNA from primary structural damage induced by UV-C radiation, an effect that could be due in part to a possible enhancement of DNA repair systems (Menéndez-Perdomo *et al.*, 2017). *P. chamaecristoides* and *P. microdictyus* extracts significantly decreased the frequency of mutations induced by UV-C radiation, and the relative mutagenicity detected was under 5% at a concentration of 1.0 mg/mL. Moreover, these extracts seemed to act through a bioantimutagenic pathway as 1-1000 µg/mL concentrations permitted the passage of 100% of UV-C radiation indicating low absorption (hence, did not exert the photoprotective action through desmutagenic mechanisms). Nevertheless, for the extracts at 1.0 mg/mL, a 100% (*P. chamaecristoides* and *P. williamioides*) and 50% (*P. microdictyus*) of UV-B absorption was detected, suggesting that some desmutagenic effects may occur for longer UV wavelengths exposure (Menéndez-Perdomo *et al.*, 2017). Currently, some experiments are being carried out in order to unveil the molecular mechanism by which the extracts exert their bioantimutagenic action.

These results are in agreement with worldwide works on *Phyllanthus* species antigenotoxic properties against physical mutagens, such as γ and ultraviolet radiation (Singh *et al.*, 2005; Harikumar & Kuttan, 2007; Londhe *et al.*, 2009; Majeed *et al.*, 2010; Majeed *et al.*, 2011; Thakur *et al.*, 2011; Raja *et al.*, 2011), as well as chemical mutagens like benzopyrene, lead, aluminium, arsenic, cyclophosphamide, aflatoxin B1, dimethylnitrosamine, 2-aminofluorene, 2-aminoanthracene, and 4-nitroquinolone-1-oxide (Dhir *et al.*, 1990; Nandi *et al.*, 1997; Kumar & Kuttan, 2005; Madhavi *et al.*, 2007; Ahmad *et al.*, 2015; Sayed *et al.*, 2015). Moreover, *Phyllanthus spp*. chemopreventive properties against skin carcinogenesis have also been well established in different experimental models, including human skin melanoma MeWo cells (Sancheti *et al.*, 2005; Tang *et al.*, 2010; 2014, 2015).

Pinus caribaea

Known as Caribbean pine (or "Pino macho" in Cuba), *Pinus caribaea* Morelet belongs to the Pinaceae Family (Order Pinales) and is native to

Central America, The Bahamas, the Turks and Caicos Islands and Cuba. In Cuban traditional medicine it is used as a potent antifungal in liquid formulations (González-Ramírez *et al.*, 2007). Scientific evidence refer its antiviral capacity (Rubial *et al.*, 2003) and *in vitro* antioxidant properties through lipid peroxidation inhibition (Santana *et al.*, 2002).

P. caribaea aqueous extract at concentrations ≥ 1.0 mg/mL possesses antigenotoxic properties in *E. coli* cells under irradiation with γ rays (Fuentes *et al.*, 2006a). It have been proven that a tannins fraction obtained from the plant bark aqueous extract is responsible for the radioprotective properties in *E. coli* cells irradiated with γ-rays (Fuentes *et al.*, 2006b). These authors found a significant decrease of genotoxicity (around 70% at 1 mg/mL) when the cells were in contact with the extract before, during, and after the irradiation, but this effect was higher at lower doses when the cells were incubated with the extract only before the irradiation and no significant effect was found for cells treated with the extract only after the irradiation. Together these results suggested a possible antigenotoxic action based on free radical scavenging mechanisms, although more experimental data is needed to understand the underlying molecular interactions.

Recently it was assessed the protective effect of the aqueous extract of *Pinus caribaea* against the damage induced by the UV-B (3000 J/m^2) and UV-C (100 J/m^2) radiation with the cell-free plasmid DNA assay (Vernhes *et al.*, 2017). These experiments are based in the use of the enzyme T4 endonuclease V to detect (and cut) DNA sites where CPDs are formed, generating the open circular (if only cuts one strand) and lineal (if cuts both strands) forms of the original covalent close circular plasmid. At concentrations ≥ 0.1 mg/mL the extract inhibited the photolesions formation due to UV irradiation. Because for concentrations ≥ 0.5 mg/mL transmittance values reflected a complete blocking of UV radiation, the authors suggested that at least in part, the extract exerts its photoprotective effect by a desmutagenic mechanism, by blocking the UV-DNA interaction. The observed protective effect at lower concentrations could be related with the antioxidant properties described for this extract previously, acting by synergism with its desmutagenic potential.

Punica granatum

Called commonly as pomegranate (Granada), *Punica granatum* is a tree native to Asia that belongs to the Lythraceae Family (Order Myrtales). Pomegranate is a fruit consumed fresh or in beverage. It has been widely used in traditional medicine in several parts of the world. In Cuba, is used to treat throat problems and asthma. Some reports have suggested that *Punica* has several important effects on human health, including, antiviral (Casadelvalle *et al.*, 2006; Morffi *et al.*, 2006), antibacterial, anti-malarial (Fernández-Calienes *et al.*, 2010) and antioxidant properties (Sánchez-Lamar *et al.*, 2005). In this sense, whole fruit extract could sequestrate reactive oxygen species caused by H_2O_2, a mechanism that allows it to protect the DNA. In combined treatment (H_2O_2 + punica extract) Sister Chromatid Exchange decrease and Mitotic Index is reestablish to control levels (Sánchez-Lamar *et al.*, 2005).

Investigations conducted in other countries showed that *Punica* extracts or components are effective against several mutagenic agents associated to antioxidant properties. Different extracts have been proved to inhibit DNA damage against sodium azide, cyclophosphamide, pentachlorophenol and hexavalent chromium using *Salmonella* test, MN frequency and chromosomal aberrations in mice, probably due to its free radical scavenging capability (Ghasemian *et al.*, 2006; Valadares *et al.*, 2010; Dassprakash *et al.*, 2012; Agha *et al.*, 2013; Ávila *et al.*, 2013). Also seed oil nanoemulsion entrapping polyphenol-rich ethyl acetate fraction, protected the DNA against UVB-induced damage (Baccarin *et al.*, 2015). Punicalagin and ellagic acid are *Punica granatum* major constituents. They showed antimutagenic potential against sodium azide, methyl methanesulfonate, 2-aminoflourine and benzo[a]pyrene in Ames test and also inhibited benzo[a]pyrene and oxidative-induced DNA adducts (Aqil *et al.*, 2012; Zahin *et al.*, 2014). These results agree with findings in pomegranate Cuban plants.

Table 1
Selected Cuban flora species with genoprotective properties

Species	Positively tested targets	Assay (experimental model)	Molecular mechanism (biactive compound)	References
Cymbopogon citratus (Poaceae)	7-12-dimetilbenzoantracene; H_2O_2; etilnitrosourea; juglone; metilmetanesulfonate	Chromosome Aberrations (CHO cells); SMART (*D. melanogaster)*	Desmutagen	Cápiro *et al.*, 2001; Cápiro *et al.*, 2005
	γ radiation	SOS Chromotest (*E. coli*)		Fuentes *et al.*, 2006a
	UV radiation	*Ex vivo* (plasmid DNA); transmitance quantification		González-Pumariega *et al.*, 2016
		SOS Chromotest (*E. coli; C. crescentus*)	Bioantimutagen	Montano-Pérez, 2011; Vernhes *et al.*, 2016; Fuentes-León *et al.*, 2017
Mangifera indica (Anacardiaceae)	γ radiation	SOS Chromotest (*E. coli*); Comet assay (human lymphocytes, lymphoblastoid)	Free radical scavenging	Rosario *et al.*, 2009; Rodeiro *et al.*, 2014
	1-nitropyrene; bleomycin; cisplatin; mitomycin C; 2-acetylaminofluorene; picrolonic acid; cyclophosphamide; benzo[a]pyrene; dimethylnitrosamine	Ames test (*S. typhimurium*)	Bioantimutagen and desmutagen (mangiferin)	Morffi *et al.*, 2012; Rodeiro *et al.*, 2012; Rodeiro *et al.*, 2014
Phyllanthus orbicularis (Phyllanthaceae)	H_2O_2	Chromosome Aberrations (CHO cells); Ames test (*S. typhimurium*)	Free radical scavenging (2,4-di-tert-butylphenol; OO'-diphenol-4,4',6,6'-tetra-ter-butyl; 2,6-di-sec-butylphenol)	Ferrer *et al.*, 2002; Sánchez-Lamar *et al.*, 1999; Rodeiro *et al.*, 2015
	m-phenylenediamine; 2-aminofluorene; 1-aminopyrene; 2-aminoanthracene; 9-aminophenantrene; 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; 4-aminobiphenyl	Ames test (*S. typhimurium*)	inhibition/competition for S9 enzymes; bioantimutagen	Ferrer *et al.*, 2001; Ferrer *et al.*, 2004
	γ radiation	SOS Chromotest (*E. coli*); Ames test (*S. typhimurium*)	Free radical scavenging and DNA repair systems modulation	Alonso *et al.*, 2005; Alonso *et al.*, 2010
	UV radiation	*Ex vivo* (plasmid DNA); transmitance quantification	UV absorption and free radical scavenging	Vernhes *et al.*, 2013b
		CPD removal (human fibroblasts)	Bioantimutagen, NER modulation	Vernhes *et al.*, 2013a
		SOS Chromotest (*E. coli*)	Bioantimutagen, BER and recombination-based repair modulation	Vernhes *et al.*, 2016
Phyllanthus chamaecristoides P. microdictyus (Phyllanthaceae)	UV radiation	SOS Chromotest; Rif^R Assay (*C. crescentus*); transmitance quantification	Biantimutagen	Menéndez-Perdomo *et al.*, 2017
Phyllanthus williamioides (Phyllanthaceae)		SOS Chromotest (*C. crescentus*)	SOS response modulation	

Pinus caribaea (Pinales)	γ radiation	SOS Chromotest (*E. coli*)	Free radical scavenging (tannins fraction)	*Fuentes et al.*, 2006a; b
	UV radiation	*Ex vivo* (plasmid DNA); transmitance quantification	UV absorption	Vernhes *et al*., 2017
Punica granatum (Lythraceae)	H_2O_2	Sister Chromatid Exchange (CHO cells)	Free radical scavenging	Sánchez-Lamar *et al*., 2005

Concluding Remarks

Cuban flora is vast in species, endemic or not, widely or moderately used in traditional medicine, that constitute a potential source of antigenotoxic compounds (**Table 1**). Even though Cuban folk culture and government policy are positive in the use of plants principles as complementary medicine, few toxicological tests have been performed in order to validate the safety of such practice. Moreover, a fairly limited number of species have been tested for their genoprotective properties, and for those that have been more exhaustive studied such as *Cymbopogon citratus*, *Mangifera indica*, *Phyllanthus spp*., *Pinus caribaea*, and *Punica granatum*, there is still need of more data about the particular molecules and mechanisms underlying their biological activity. In the present review we examined different molecular mechanisms suggested to date, from antioxidant activity to DNA repair modulation, and critically discussed the state of art and perspectives in the use of these plants as a new and promising strategy for genoprotection in the 21st Century. We conclude that there is a long road to drive in order to fulfill this goal, but we consider that important steps have been done in the past couple of decades of research in the nation.

REFERENCES

Agha FE, Hassannane MS, Omara EA, Hasan AM, El-Toumy SA. 2013. Protective Effect of *Punica granatum* Peel Extract Against Pentachlorophenol-Induced Oxidative Stress, Cytogenetic Toxicity and Hepatic Damage in Rats. **Australian J Appl Sci** 7: 853 - 864.

Ahmad MD, Bano S, Anwar S. 2015. Cancer ameliorating potential of *Phyllanthus amarus*: *in vivo* and *in vitro* studies against Aflatoxin B1 toxicity. **EJMHG** 16: 343 - 353.

Alonso A, Fuentes JL, Prieto E, Sánchez-Lamar A, Ferrer M, Barbe J, Llagostera M. 2005. Antigenotoxic effect of the aqueous extract of *Phyllanthus orbicularis* HBK in γ-irradiated *Escherichia coli* cells, vol 10. Recent progress in medicinal plants, vol Phytotherapeutics. Studium Press LLC, Houston-Texas, USA

Alonso A, Fuentes JL, Sánchez-Lamar A, Llagostera M. 2010. Antimutagenic effect of *Phyllanthus orbicularis* against γ-radiation. **Lat Am J Pharm** 29: 1 - 5.

Álvarez AL, del Barrio G, Kourí V, Martínez PA, Suárez B, Parra F. 2009. *In vitro* anti-herpetic activity of an aqueous extract from the plant *Phyllanthus orbicularis.* **Phytomed** 16: 960 - 966.

Appelbaum D, Kligler B, Barrett B, Frenkel M, Guerrera MP, Kondwani KA, Lee BB, Tattelman E. 2008. Natural and traditional medicine in Cuba: lessons for U.S. Medical Education. **MEDICC Rev** 10: 43 - 48.

Aqil F, Munagala R, Vadhanam MV, Kausar H, Jeyabalan J, Schultz DJ, Gupta RC. 2012. Anti-proliferative activity and protection against oxidative DNA damage by punicalagin isolated from pomegranate husk. **Food Res Int** 49: 345 - 353.

Ávila RId, Guerra MT, Borges KdAS, Vieira MdS, Júnior LMdO, Furtado H, Mota MFd, Arruda AF, Valadares MC. 2013. *Punica granatum* L. protects mice against hexavalent chromium-induced genotoxicity. **Braz J Pharm Sci** 49: 689 - 697.

Baccarin T, Mitjan M, Ramos D, Lemos-Senna E, Vinardell MP. 2015. Photoprotection by *Punica granatum* seed oil nanoemulsion entrapping polyphenol-rich ethyl acetate fraction against UVB-induced DNA damage in human keratinocyte (HaCaT) cell line. **J Photochem Photobiol B: Biol** 153: 127 - 136.

Balakrishnan B, Paramasivam S, Arulkumar A. 2014. Evaluation of the lemongrass plant (*Cymbopogon citratus*) extracted in different solvents for antioxidant and antibacterial activity against human pathogens. **Asian Pac J Tropical Dis** 4: S134 - S139.

Bhattacharya S. 2011. Natural Antimutagens: A review. **Res J Medicinal Plants** 5: 116 - 126.

Bidinotto LT, Costa CARA, Salvadori DMF, Costa M, Rodrigues MAM, Barbisan LF. 2011. Protective effects of lemongrass (*Cymbopogon citratus* Stapf) essential oil on DNA damage and carcinogenesis in female Balb/C mice. **J Appl Toxicol** 31: 536 - 544.

Bustamante SE, Garrido G, Martínez JL, Núñez-Selles A, Morales MA. 1998. **Efectos vasculares, cardiacos e intestinales de QF-808. Extracto de *Mangifera indica*.** I Congreso Internacional de la Sociedad Cubana de Farmacología, La Habana, Cuba, 19 al 23 de Octubre de 1998.

Cancino-Badías L, Leiva-González A, Garrido G, Cossío-Ayala M, Prieto-González E. 2001. Vimang: Los efectos antigenotóxico y modulador de las enzimas glutatión peroxidasa y glutatión-S-transferasa. **Rev Cuba Investigaciones Biomed** 20: 48 - 53.

Cápiro N, Sánchez-Lamar Á, Fonseca G, Baluja L, Borges E. 2001. Capacidad protectora de *Cymbopogon citratus* (DC.) Stapf. ante el daño genético inducido por estrés oxidativo. **Rev Cuba Investigaciones Biomed** 20: 33 - 38.

Cápiro N, Sánchez-Lamar A, Baluja L, Sierra LM, Comendador García MA. 2005. Efecto de la concentración de *Cymbopogon citratus* (DC) Stapf sobre la genotoxicidad de mutágenos modelos, en el Ensayo Smart de Ojos W/W$^+$ de *Drosophila melanogaster*. **Rev CENIC Cienc Biol** 36: 1 - 5.

Carbajal D, Casaco A, Arruzazabala L, Gonzalez R, Z T. 1989. Pharmacological study of *Cymbopogon citratus l*eaves. **J Ethnopharmacol** 25: 103 - 107.

Casadelvalle I, Rosario B, Caballero O. 2006. Un extracto de *Punica granatum* L. (BLBu) sobre la apoptosis induida por virus influenxa A y B. **Rev CENIC Cienc Biol** 37: 111 - 117.

Cuétara E, Álvarez A, Alonso A, Vernhes M, Sánchez-Lamar A, Festary T, Rico J. 2012. A microanalytical variant of the SOS Chromotest for genotoxicological evaluation of natural and synthetic products. **Biotecnol Apl** 29: 108 - 112.

Dassprakash MV, Arun R, Abraham SK, Premkumar K. 2012. *In vitro* and *in vivo* evaluation of antioxidant and antigenotoxic potential of *Punica granatum* leaf extract. **Pharm Biol** 50: 1523 - 1530.

De P, Mukhopadhyay MJ, Chakravarty A. 2015. Lemongrass - an anticlastogenic agent against arsenic. **Nucleus** 58: 125 - 129.

De Flora S, Ferguson LR. 2005. Overview of mechanisms of cancer chemopreventive agents. **Mutat Res** 591: 8 - 15.

del Barrio G, Caballero O, Chevalier P. 1995. Inactivación *in vitro* del Ags HB por extractos de plantas del género *Phyllanthus*. **Rev Cubana Med Trop** 47: 127 - 130.

del Barrio G, Parra F. 2000. Evaluation of the antiviral activity of an aqueous extract from *Phyllanthus orbicularis*. **J Ethnopharmacol** 72: 317 - 322.

del Barrio G, Roque A, Kourí V, Morier L. 2014. *Phyllanthus orbicularis* Kunth: una especie endemica cubana con potencial para la obtencion de drogas anivirales. **An Acad Cienc Cuba** 4 (2).

Dhir HA, Kumar A, Sharma P, Talukder G. 1990. Modification of clastogenicity of lead and aluminium in mouse bone marrow cells by dietary ingestion of *Phyllanthus emblica* fruit extract. **Mutat Res** 241: 305 - 312.

Fernandez JA, del Barrio G, Romeu AB, Gutierrez Y, Valdes VS, Parra F. 2003. *In vitro* antiviral activity of *Phyllanthus orbicularis* extracts against herpes simplex virus type 1. **Phytother Res** 17: 980 - 982.

Fernández-Calienes A, Mendiola J, Monzote L, Parra MG, Sariego I, Acuña D, Scull R, Gutiérrez Y. 2009. Evaluación de la toxicidad de extractos de plantas cubanas con posible acción antiparasitaria utilizando larvas de *Artemia salina* L. **Rev Cubana Medicina Tropical** 61: 254 - 258.

Fernández-Calienes A, Mendiola J, Scull R, Gutiérrez Y, Acuña D, Abreu J. 2010. *In vitro* antimalarial activity and cytotoxicity of some selected cuban medicinal plants. **Rev Inst Medicina Tropical Sao Paulo** 52: 197 - 201.

Ferrer M, Sánchez-Lamar A, Fuentes JL, Barbé J, Llagostera M. 2001. Studies on the antimutagenesis of *Phyllanthus orbicularis*: mechanisms involved against aromatic amines. **Mutat Res** 498: 99 - 105.

Ferrer M, Sánchez-Lamar A, Fuentes JL, Barbé J, Llagostera M. 2002. Antimutagenic mechanisms of *Phyllanthus orbicularis* when hydrogen peroxide is tested using *Salmonella* assay. **Mutat Res** 517: 1 - 4.

Ferrer M, Cristófol C, Sánchez-Lamar A, Fuentes JL, Barbé J, Llagostera M. 2004. Modulation of rat and human cytochromes P450 involved in PhIP and 4-ABP activation by an aqueous extract of *Phyllanthus orbicularis*. **J Ethnopharmacol** 90: 273 - 277.

Fuentes-León F, García-Fernádez F, Aguilera-Roque KB, Menck CFM, Galhardo RdS, Sánchez-Lamar Á. 2017a. Fotoprotección al DNA ejercida por fracciones químicas obtenidas de *Cymbopogon citratus* (DC) Stapf. **Rev Cubana Cienc Biol** 5: 1 - 9.

Fuentes-León F, González-Pumariega M, Vernhes M, Menck CFM, Sánchez-Lamar Á. 2017b. Toxic Evaluation of *Cymbopogon citratus* Chemical Fractions in *E. coli.* **Cosmetics** 4: 1 - 5.

Fuentes JL, Alonso A, Cuétara E, Vernhes M, Álvarez N, Sánchez-Lamar Á, Llagostera M. 2006a. Usefulness of the SOS Chromotest in the study of medicinal plants as radioprotectors. **Int J Radiat Biol** 82: 323 - 329.

Fuentes JL, Vernhes M, Cuétara E, Sánchez-Lamar A, Santana JL, Llagostera M. 2006b. Tannins from barks of *Pinus caribaea* protect *Escherichia coli* cells against DNA damage induced by γ-rays. **Fitoterapia** 77: 116 - 120.

Fuentes VR. 2008. Las especies medicinales amenazadas en Cuba. **Rev Jard Bot Nac** 28: 77 - 81.

García D, Delgado R, Ubeira FM, Leiro JM. 2002. Modulation of rat macrophage function by the *Mangifera indica* L. Extracts Vimang and mangiferin. **Int Immunopharmacol** 2: 797 - 806.

García D, Hernández I, Álvarez A, Cancio B, Márquez L, Garrido G, Cuzzocrea S, Delgado R. 2006. Anti-allergic properties of *Mangifera indica* L. extract (Vimang) and contribution of its glucosylxanthone mangiferin. **J Pharm Pharmacol** 58: 385 - 392.

Garrido-Suárez BB, Garrido G, Delgado R, Bosch F, Rabí MdC. 2010. A *Mangifera indica* L. Extract Could Be Used to Treat Neuropathic Pain and Implication of Mangiferin. **Molecules** 15: 9035 - 9045.

Garrido-Suárez B, Garrido G, Delgado R, Bosch F, Rabí MdC. 2011a. Case Series in Patients with Zoster-Associated Pain using *Mangifera indica* L. Extract. **Forsch Komplementmed** 18: 345 - 350.

Garrido-Suárez B, Garrido G, Delgado R, Bosch F, Rabí MdC, Hernández CE. 2011b. Report of cases in patients with acute herpetic neuralgia using a *Mangifera indica* extract. **Braz J Pharmacogn** 21: 1111 - 1117.

Garrido G, González D, Delporte C, Backhouse N, Quintero G, Núñez-Sellés AJ, Morales MA. 2001. Analgesic and antiinflammatory effects of *Mangifera indica* L. extract (VIMANG). **Phytother Res** 15: 18 - 21.

Garrido G, Delgado R, Lemus Y, García D, Beltrán A, Rodríguez J, Quintero G, Delporte C, Backhouse N, Morales MA, Payá M, Muñoz E, Guevara M, Alvarez A, Boza A, Arús L, Cabrera P, Núñez-Sellés AJ. 2004a. Extracto natural de *Mangifera indica* L. (Vimang®): de la etnomedicina a la clínica. **Bol Latinoam Caribe Plant Med Aromat** 3: 107 - 109.

Garrido G, Delgado R, Lemus Y, Rodríguez J, García D, Núñez-Sellés AJ. 2004b. Protection against septic shock and suppression of tumor necrosis factor α and nitric oxide production on macrophages and microglia by a standard aqueous extract of *Mangifera indica* L. (VIMANG). **Pharmacol Res** 50: 165 - 172.

Garrido G, González D, Romay C, Núñez-Sellés AJ, Delgado R. 2008. Scavenger effect of a mango (*Mangifera indica* L.) food supplement's active ingredient on free radicals produced by human polymorphonuclear cells and hypoxanthine–xanthine oxidase chemiluminescence systems. **Food Chem** 107: 1008 - 1014.

Garrido G, Rodeiro I, Hernández I, García G, Pérez G, Merino N, Núñez-Sellés A, Delgado R. 2009. *In vivo* acute toxicological studies of an antioxidant extract from *Mangifera indica* L. (Vimang). **Drug Chem Toxicol** 32: 53 - 58.

Ghasemian A, Mehrabian S, Majd A. 2006. Peel extracts of two Iranian cultivasr of pomegranate (*Punica granatum*) hace antioxidant and antimutagenic activities. **Pak J Biol Sci** 9: 1402 - 1405.

González-Pumariega M, Fuentes-León F, Vernhes M, Schuch AP, Menck CFM, Sánchez-Lamar Á. 2016. El extracto acuoso de *Cymbopogon citratus* protege al ADN plasmídico del daño inducido por radiación UVC. **Ars Pharm** 57: 193 - 199.

González-Ramírez M, Remirez D, Jacobo OL. 2007.

Antecedentes y situación reguladora de la medicina herbaria en Cuba. **Bol Latinoam Caribe Plant Med Aromat** 6: 118 - 124.

Guerra M, Rodríguez M, García G, Llerena C. 2004. Actividad antimicrobiana del aceite esencial y crema de *Cymbopogon citratus* (DC). Stapf. **Rev Cub Plant Med** 9: 1 - 6.

Guevara M, González S, Álvarez A, Riaño A, Garrido G, Núñez-Sellés AJ. 2004. Uso etnomédico de la corteza de *Mangifera indica* L. en Cuba. **Rev Cub Plant Med** 9: 1 - 6.

Guevara M, Garrido G, Rodríguez PC, Riaño A, Álvarez A, Delgado R, Núñez A. 2007. Efectos de la crema antioxidante Vimang® en enfermedades dermatológicas. **Lat Am J Pharm** 26: 237 - 242.

Gutiérrez YI, García G, Miranda M, Valdivieso A, Piloto A. 2002. Evaluación toxicológica del extracto acuoso de *Phyllanthus orbicularis*. **Rev Cub Farm** 36.

Gutiérrez YI, Miranda M, Bello A, Verona S, Montes de Oca R. 2011. Chemical characterization using gas chromatography/mass spectrometry of two extracts from *Phyllanthus orbicularis* HBK. **Rev Cub Farm** 45: 405 - 413.

Harikumar KBN, Kuttan G. 2007. An extract of *Phyllanthus amarus* protects mouse chromosomes and intestine from radiation induced damages. **J Radiat Res** 48: 469 - 476.

Hernández L, Rodríguez M. 2001. Actividad antimicrobiana de plantas que crecen en Cuba. **Rev Cub Plant Med** 2: 44 - 47.

Hoffmann P, Kathriarachchi HS, Wurdack KJ. 2006. A phylogenetic classification of *Phyllanthaceae* (*Malpighiales*; *Euphorbiaceae sensu lato*). **Kew Bulletin** 61: 37 - 53.

Jagetia GC, Venkatesha VA. 2006. Mangiferin protects human peripheral blood lymphocytes against g-radiation–induced DNA strand breaks: a fluorescence analysis of DNA unwinding assay. **Nutrition Res** 26: 303 - 311.

Kanatt SR, Chawla SP, Sharma A. 2014. Antioxidant and radio-protective activities of lemon grass and star anise extracts. **Food Biosci** 6: 24 - 30.

Kim H, Moon JY, Kim H, Lee D-S, Cho M, Choi H-K, Kim YS, Mosaddik A, Cho SK. 2010. Antioxidant and antiproliferative activities of mango (*Mangifera indica* L.) flesh and peel. **Food Chem** 121: 429 - 436.

Kotecha R, Takami A, Espinoza JL. 2016. Dietary phytochemicals and cancer chemoprevention: a review of the clinical evidence. **Oncotarget** 7: 52517 - 52529.

Krishnamurti C, Rao SC. 2016. The isolation of morphine by Serturner. **Indian J Anaesth** 60: 861 - 862.

Kumar KB, Kuttan R. 2005. Chemoprotective activity of an extract of *Phyllanthus amarus* against cyclophosphamide induced toxicity in mice. **Phytomed** 12: 494 - 500.

Landis-Piwowar KR, Iyer NR. 2014. Cancer chemoprevention: current state of the art. **Cancer Growth Metastasis** 7: 19 - 25.

Londhe JS, Devasagayam TPA, Foo LY, Ghaskadbi SS. 2009. Radioprotective properties of polyphenols from *Phyllanthus amarus* Linn. **J Radiat Res** 50: 303 - 309.

Madhavi D, Rudrama-Devi K, Kesava-Rao K, Reddy PP. 2007. Modulating effect of *Phyllanthus* fruit extract against lead genotoxicity in germ cells of mice. **J Env Biol** 28: 115 - 117.

Majeed M, Bhat B, Anand S. 2010. Inhibition of UV induced adversaries by β-glucogallin from Amla (*Emblica officinalis* Gaertn.) fruits. **Indian J Nat Prod Resour** 1: 462 - 465.

Majeed M, Bhat B, Anand S, Sivakumar A, Paliwal P, Geetha KG. 2011. Inhibition of UV-induced ROS and collagen damage by *Phyllanthus emblica* extract in normal human dermal fibroblasts. **J Cosmet Sci** 62: 49 - 56.

Martínez G, Delgado R, Garrido G, Núñez-Sellés AJ, León OS. 2000. Evaluation of the *in vitro* antioxidant activity of *Mangifera indica* L. extract (Vimang). **Phytother Res** 14: 424 - 427.

Meevatee U, Boontim S, Keereeta O, Vinitketkumnuen U, O-ariyakul N. 1993. **Antimutagenic activity of lemon grass.** In: Boot-in S, editor. Man & Environment. Chiang Mai University Press, Chiang Mai, Thailand.

Menéndez-Perdomo IM, Fuentes-León F, Casadelvalle I, Menck CFM, Galhardo RS, Sánchez-Lamar Á. 2016. Assessment of cytotoxic and genotoxic effects of *Phyllanthus* (Phyllanthaceae). **Rev Cub Cienc Biol** 5: 67 - 75.

Menéndez-Perdomo IM, Sánchez-Lamar Á. 2017. *Phyllanthus* plants in photoprotection: a broad spectrum of molecular mechanisms.

Pharmacophore 8: 1 - 10.

Menéndez-Perdomo IM, Wong-Guerra M, Fuentes-León F, Carrazana E, Casadelvalle I, Vidal A, Sánchez-Lamar Á. 2017. Antioxidant, photoprotective and antimutagenic properties of *Phyllanthus* spp. from Cuban flora. **J Pharm Pharmacog Res** 5: 251 - 261.

Montano-Pérez M. 2011. Actividad fotoprotectora de la fracción aceites esenciales de *Cymbopogon citratus* (DC) Stapf, sobre el DNA de *Escherichia coli* frente a la radiación UVC. Tesis de Licenciatura en Biología, Universidad de La Habana, Cuba.

Montes de Oca MK, Pearlman RL, McClees SF, Strickland R, Afaq F. 2017. Phytochemicals for the prevention of photocarcinogenesis. **Photochem Photobiol** 93: 956 - 974.

Morffi J, Peña BdR, Oropesa S. 2006. Actividad antiviral del extracto de *Punica granatum* L. (BLBu) en el modelo experimental de gripe en ratones de la línea Balb/C. **Rev CENIC Cienc Biol** 37: 105 - 109.

Morffi J, Rodeiro I, Hernández SL, González L, Herrera J, Espinosa-Aguirre JJ. 2012. Antimutagenic properties of *Mangifera indica s*tem bark extract and evaluation of its effects on hepatic CYP1A1. **Plants Foods Hum Nut** 67: 223 - 228.

Moron F, Sánchez C, Martínez MC, Morejón Z, Pinedo Z. 2000. **Actividad antiespasmódica *in vitro* de hojas frescas de *Cymbopogon citratus* (DC) Stapf.** Informe TRAMIL Laboratorio Central de Farmacología, Facultad de Ciencias Médicas "Dr Salvador Allende", La Habana, Cuba.

Nandi P, Talukder G, Sharma A. 1997. Dietary chemoprevention of clastogenic effects of 3,4-benzo(a)pyrene by *Emblica officinalis* Gaertn. fruit extract. **Br J Cancer** 76: 1279 - 1283.

Naresh K, Varakumar S, Variyar PS, Sharma A, Reddy OVS. 2014. Impact of irradiation on antioxidant capacity of mango (*Mangifera indic*a L.) wine from eight Indian cultivars and the protection of mango wine against DNA damage caused by irradiation. **Process Biochem** 49: 1819 - 1830.

Naresh K, Varakumar S, Variyar PS, Sharma A, Reddy OVS. 2015. Enhancing antioxidant activity, microbial and sensory quality of mango (*Mangifera indica* L.) juice by γ-irradiation and its *in vitro* radioprotective potential. **J Food Sci Technol** 52: 4054 - 4065.

Núñez-Sellés AJ, Vélez HT, Agüero-Agüero J, González-González J, Naddeo F, Simone FD, Rastrelli L. 2002. Isolation and quantitative analysis of phenolic antioxidants, free sugars, and polyols from Mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. **J Agric Food Chem** 50: 762 - 766.

Núñez-Sellés AJ, Durruthy MD, Balseiro ER, González LN, Nicolais V, Rastrelli L. 2007. Comparison of major and trace element concentrations in 16 varieties of Cuban Mango stem bark (*Mangifera indic*a L.). **J Agric Food Chem** 55: 2176 - 2181.

Pardo-Andreu GL, Delgado R, Núñez-Sellés AJ, Vercesi AE. 2006a. *Mangifera indica* L. extract (Vimang) inhibits 2-deoxyribose damage induced by Fe (III) plus ascorbate. **Phytother Res** 20: 120 - 124.

Pardo-Andreu GL, Riaño-Montalvo A, Avila-González R, Delgado R. 2006b. *Mangifera indica* L. extract (Vimang) protects against LPS induced oxidative damage. **Pharmacologyonline** 3: 712 - 719.

Pardo-Andreu GL, Barrios MF, Curti C, Hernández I, Merino N, Lemus Y, Martínez I, Riaño A, Delgado R. 2008. Protective effects of *Mangifera indica* L extract (Vimang), and its major component mangiferin, on iron-induced oxidative damage to rat serum and liver. **Pharmacol Res** 57: 79 - 86.

Pérez-Machín M, Sueiro ML, Boffill MA, Morón F, Marrero E, Rivas MR, Méndez OR, Mosquera DMG. 2011a. Estudio etnobotánico de las plantas más utilizadas como diuréticas en la Provincia de Villa Clara, Cuba. **Bol Latinoam Caribe Plant Med Aromat** 10: 46 - 55.

Pérez-Machín M, Sueiro ML, Cruz Adl, Boffill MA, Morón F, Méndez OR, Cárdenas J. 2011b. Uso tradicional de plantas medicinales con acción diurética en el Municipio de Quemado de Güines, Cuba. **Int J Tropical Biol** 59: 1859 - 1867.

Pinsaeng K. 1993. **Anti-micronucleus formation of lemon grass extract.** Master's thesis, Chiang Mai University, Thailand.

Pinto ZT, Sánchez FF, Ramos A, Amaral ACF, Ferreira JLP, Escalona-Arranz JC, Queiroz MMDC. 2015. Chemical composition and

insecticidal activity of *Cymbopogon citratus* essential oil from Cuba and Brazil against housefly. **Braz J Veg Parasit** 14: 36 - 44.

Raja W, Hanfi S, Pandey S, Tripathi K, Hanfi S, Agrawal RC. 2011. Effect of *Phyllanthus niruri* leaf extract on antioxidant activity and UV induced chromosomal aberration in swiss albino mice. **Pharmacologyonline** 3: 169 - 174.

Ramos A, Visozo A, Piloto A, García A, Rodríguez CA, Rivero R. 2003. Screening of antimutagenicity via antioxidant activity in Cuban medicinal plants. **J Ethnopharmacol** 87: 241 - 246.

Rao B, Shanbhoge R, Rao B, Adiga S, Upadhya D, Aithal B, Kumar M. 2009. Preventive efficacy of hydroalcoholic extract of *Cymbopogon citratus* against radiation-induced DNA damage on V79 cells and free radical scavenging ability against radicals generated *in vitro*. **Hum Exp Toxicol** 28: 195 - 202.

Rivero F, Vidal A. 1998. Estudio de la toxicidad aguda y subcrónica de un extracto acuoso de *Phyllanthus orbicularis* **Rev Toxicol** 15: 64 - 67.

Rodeiro I, Cancino L, González JE, Morffi J, Garrido G, González RM, Nuñez-Sellés A, Delgado R. 2006. Evaluation of the genotoxic potential of *Mangifera indica* L. extract (Vimang), a new natural product with antioxidant activity. **Food Chem Toxicol** 44: 1707 - 1713.

Rodeiro I, Hernandez S, Morffi J, Herrera JA, Gómez-Lechón MJ, Delgado R, Espinosa-Aguirre JJ. 2012. Evaluation of genotoxicity and DNA protective effects of mangiferin, a glucosylxanthone isolated from *Mangifera indica* L. stem bark extract. **Food Chem Toxicol** 50: 3360 - 3366.

Rodeiro I, Delgado R, Garrido G. 2014. Effects of a *Mangifera indica* L. stem bark extract and mangiferin on radiation-induced DNA damage in human lymphocytes and lymphoblastoid cells. **Cell Prolif** 47: 48 - 55.

Rosario LA, Rodeiro I, Almeida E, Arencibia DF, Leyva O, Alonso A, Rodriguez Y. 2009. Evaluación del efecto radioprotector del extracto acuoso de *Mangifera indica* L (Vimang), en el ensayo de SOS-Chromotest. **RETEL** 19: 53 - 66.

Rubial JL, Dubet M, Luzardo F, Noa E, Vargas LM, Santana JL. 2003. Inhibición de la replicación del virus de la inmunodeficiencia humana por extractos de *Pinus caribaea* Morelet. **Rev Cuba Farm** 37 (2).

Saewan N, Jimtaisong A. 2015. Natural products as photoprotection. **J Cosmet Dermatol** 14: 47 - 63.

Sancheti G, Jindal A, Kumari R, Goyal PK. 2005. Chemopreventive action of *Emblica officinalis* on skin carcinogenesis in mice. **Asian Pac J Cancer Prev** 6: 197 - 201.

Sánchez-Lamar A, Fiore M, Cundari E, Ricordy R, Cozzi R, De Salvia R. 1999. *Phyllanthus orbicularis* aqueous extract: cytotoxic, genotoxic, and antimutagenic effects in the CHO cell line. **Toxicol Appl Phamacol** 239: 231 - 239.

Sánchez-Lamar A, Fuentes JL, Fonseca G, Cápiro N, Ferrer M, Alonso A, Baluja L, Cozzi R, Salvia RD, Fiore M, Llagostera M. 2002. Assessment of the potential genotoxic risk of *Phyllantus orbicularis* HBK aqueous extract using *in vitro* and *in vivo* assays. **Toxicol Letters** 136: 87 - 96.

Sánchez-Lamar A, Cozzi R, Cundari E, Fiore M, Ricordy R, Gensabella G, Degrassi F, De Salvia R. 2005. Extracto de frutos enteros de *Punica granatum* L. como agente protector del daño inducido por el peróxido de hidrógeno. **Rev Cub Med Trop** 10: 1 - 9.

Sánchez-Lamar A, Pérez JA, Ros J, Pons J, Ferrer M, Fuentes JL, Llagostera M. 2015. Fractionation of an aqueous extract of *Phyllanthus orbicularis* Kunth and identification of antioxidant compounds. **Rev Cub Cienc Biol** 4: 56 - 62.

Santana JL, Martinez F, Codorniu E, Vargas LM, Melo P, Garcia M, Isaac K, Estevez P, Roque A, Benitez M. 2002. Radioisotope method for characterization of vegetable tannins, extracted from waste of forestry production in Cuba. **J Radianal Nucl Chem** 253: 101.

Sarin B, Verma N, Martin JP, Mohanty A. 2014. An overview of important ethnomedicinal herbs of *Phyllanthus* Species: present status and future prospects. **Sci World J** 2014: 12.

Sayed S, Ahsan A, Kato M, Ohgami N, Rashid A, Akhand AA. 2015. Protective effects of *Phyllanthus emblica* leaf extract on sodium arsenite-mediated adverse effects in mice. **Nagoya J Med Sci** 77: 145 - 153.

Seebode C, Lehmann J, Emmert S. 2016.

Photocarcinogenesis and skin cancer prevention strategies. **Anticancer Res** 36: 1371 - 1378.

Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann AS. 2011. Scientific basis for the therapeutic use of *Cymbopogon citratus*, Stapf (Lemon grass). **J Adv Pharm Technol Res** 2: 3 - 8.

Singh I, Sharma A, Nunia V, Goyal PK. 2005. Radioprotection of Swiss albino mice by *Emblica officinalis*. **Phytother Res** 19: 444 - 446.

Suaeyun R, Kinouchi T, Arimochi H, Vinitketkumnuen U, Ohnishi Y. 1997. Inhibitory effects of lemon grass (*Cymbopogon citratus* Stapf) on formation of azoxymethane-induced DNA adducts and aberrant crypt foci in the rat colon. **Carcinogenesis** 18: 949 - 955.

Tang YQ, Jaganath IB, Sekaran SD. 2010. *Phyllanthus* spp. induces selective growth inhibition of PC-3 and MeWo human cancer cells through modulation of cell cycle and induction of apoptosis. **PLoS One** 5: e12644.

Tang YQ, Jaganath IB, Manikam R, Sekaran SD. 2014. Inhibition of mapks, myc/max, nfkappab, and hypoxia pathways by *Phyllanthus* prevents proliferation, metastasis and angiogenesis in human melanoma (mewo) cancer cell line. **Int J Med Sci** 11: 564 - 577.

Tang YQ, Jaganath IB, Manikam R, Sekaran SD. 2015. *Phyllanthus* spp. exerts anti-angiogenic and anti-metastatic effects through inhibition on matrix metalloproteinase enzymes. **Nutr Cancer** 67: 783 - 795.

Thakur I, Uma-Devi P, Bigoniya P. 2011. Protection against radiation clastogenecity in mouse bone marrow by *Phyllanthus niruri*. **Indian J Exp Biol** 49: 704 - 710.

Valadares MC, Pereira ERT, Benfica PL, Paula JR. 2010. Assessment of mutagenic and antimutagenic effects of *Punica granatum* in mice. **Braz J Pharm Sci** 46: 121 - 127.

Vernhes M, González-Pumariega M, Andrade L, Schuch AP, Lima-Bess KMD, Menck CFM, Sánchez-Lamar A. 2013a. Protective effect of a *Phyllanthus orbicularis* aqueous extract against UVB light in human cells. **Pharm Biol** 51: 1 - 7.

Vernhes M, González-Pumariega M, Schuch AP, Menck CFM, Sánchez-Lamar A. 2013b. El extracto acuoso de *Phyllanthus orbicularis* K protege al ADN plasmídico del daño inducido por las radiaciones ultravioletas. **Ars Pharm** 54: 16 - 23.

Vernhes M, González-Pumariega M, Fuentes-León F, Baly-Gil L, Menck CFM, Sánchez-Lamar A. 2016. Desmutagenic activity of *Cymbopogon citratus* and *Phyllanthus orbicularis* against UVC damage in *E. coli*. **Adv Pharm J** 1: 80 - 85.

Vernhes M, Schuch AP, Fuentes-León F, Menck CFM, Sánchez-Lamar Á. 2017. Extracto acuoso de *Pinus caribaea* inhibe el daño inducido por radiaciones ultravioletas, en DNA plamídico. **J Pharm Pharmacog Res** 5: 262 - 269.

Vidal-Novoa A, Fallarero A, Peña BR, Medina ME, Gra B, Rivera F, Gutiérrez Y, Vuorela PM. 2003. Studies on the toxicity of *Punica granatum* L. (Punicaceae) whole fruit extracts. **J Ethnopharmacol** 89: 295 - 300.

Vinitketkumnuen U, Puatanachokchai R, Kongtawelert P, Lertprasertsuke N, Matsushima T. 1994. Antimutagenicity of lemon grass (*Cymbopogon citratus*, Stapf) to various known mutagens in *Salmonella* mutation assay. **Mutat Res** 341: 71 - 75.

Volpato G, Godínez D, Beyra A, Barreto A. 2009. Uses of medicinal plants by Haitian immigrants and their descendants in the Province of Camagüey, Cuba. **J Ethnobiol Ethnomedicine** 5: 1 - 9.

Wong M. 2013. **Actividad antioxidante de los extractos acuosos de ocho plantas cubanas del género *Phyllanthus*.** Tesis de Licenciatura en Bioquímica, Universidad de la Habana, Cuba.

Zahin M, Ahmad I, Gupta RC, Aqil F. 2014. Punicalagin and ellagic acid demonstrate antimutagenic activity and inhibition of benzo[a]pyrene induced DNA adducts. **BioMed Res Int** 2014: 1 - 10.