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Vol. 8(35), pp. 1101-1109, 17 September, 2014
DOI: 10.5897/JMPR2014.5471
Article Number: 4BAE6C147589
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Effect of extracts from field and *in vitro* plants of *Petiveria alliacea* L. on plasmidial DNA

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Received 9 May, 2014; Accepted 11 September, 2014

***Petiveria alliacea* L. is a native herbaceous species from the Amazon region traditionally used in folk medicine for its various pharmacological activities. This study aimed to perform a comparative assessment of genotoxic and antigenotoxic potential of field- and *in vitro*-grown plants. Specimens from different populations from Rio de Janeiro State were used as donor plants for *in vitro* culture on Murashige and Skoog (MS) medium. Aqueous extracts from field and *in vitro* plants were evaluated by analyzing their effect on the integrity of pUC 9.1 plasmid. DNA genotoxic potential was assessed after treatment of plasmid DNA with increasing concentrations of aqueous extracts (1.0, 5.0 to 10.0 mg/ml) from the populations studied and the *in vitro* plants originated from them. The electrophoretic analysis showed changes in the structural conformation of plasmid DNA, indicating the occurrence of single- and double-strand breaks caused by the extracts, demonstrating the genotoxic potential at higher concentrations. The antigenotoxic potential was assessed based on plasmid protection induced by the extracts after DNA treatment with stannous chloride. Thus, data also pointed to a antigenotoxic effect of extracts against oxidative damage. In addition, the intensity of the effect of extracts on plasmid DNA varied according to origin of plants.**

Key words: Antigenotoxicity, bioactivity, DNA topology, genotoxicity, micropropagation.

INTRODUCTION

Petiveria alliacea L. (Phytolaccaceae) is a plant endemic to the Amazon Forest. In Brazil, it is popularly known as “guiné” or “tipi.” The leaves are widely used in folk medicine and African-Brazilian cults (Ponte et al., 1996; Azevedo and Silva, 2006; Gomes et al., 2008). Research on the biological effects of this species has aroused great interest regarding its therapeutic and prophylactic use. Various pharmacological uses have been demonstrated, such as analgesics (Di Stasi, 1988), insect repellent (Johnson et al., 1997),

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(Ponte et al., 1996), acaricide (Johnson et al., 1997), antibacterial (Szczepan et al., 1972; Williams et al., 2003), antifungal (Benevides et al., 2001), and treatment for bovine viral diarrhea virus (BVDV) (Ruffa et al., 2002). Many of these effects may result from a cytotoxic mechanism also described for this species as the inhibition of antioxidant status *in vitro* and *in vivo*, featuring pro-oxidant effects in a concentration-dependent manner (Andrade et al., 2012). On the other hand, components of the extracts have shown a well-defined antioxidant activity (Delle-Monache et al., 1996; Okada et al., 2008), as well as immunomodulatory (Quadros et al., 1999) and neuropharmacological activities (Cifuentes et al., 2001). Research in the last two decades focused on polysulfides with antineoplastic action produced in this species (Mata-Greenwood et al., 2001; Rosner et al., 2001; Ruffa et al., 2002; Williams et al., 2003; Williams et al., 2007; Webster et al., 2008).

Paradoxically, both beneficial and harmful effects are common to many plant extracts by the large amount of substances produced from secondary metabolism. Previous work on *P. alliacea* revealed the presence of triterpenoids, saponins, polyphenols, coumarins, benzaldehyde, benzoic acid, flavonoids, fredelinol, pinitol and allantoin, varying their concentrations in the root, stems and leaves (De Sousa, 1990; Kubec et al., 2002, 2003; Delle-Monache and Suarez, 1992; Delle-Monache et al., 1996). Either alone or together, these substances can cause different biological effects in humans.

Despite the importance of these plants and their pharmacological activities, few studies have reported on the specific action of crude extracts on DNA coupled with an assessment of genotoxic potential. Such data are crucial since toxicity is a key determinant in the use of phytochemicals for medicinal purposes. Despite the risks, demand is increasing for plants with medicinal properties, as described earlier, causing a simultaneous increase in extraction and erosion in natural plant populations possibly containing the genotypes of greatest interest. However, the use of tissue culture methods can produce bioactive substances by providing for the multiplication of specific genotypes, but without the influence of environmental factors. Among these techniques, micropropagation allows for the multiplication (Borgaud et al., 2001; Tripathi and Tripathi, 2003; Lima et al., 2010) of genetically uniform and healthy plants in a short time. In addition, the controlled culture environment allows the manipulation of environmental factors to minimize or modify their effects (Vanisree et al., 2004). This technique develops plant stocks for commercial and industrial use without depleting natural resources and also facilitates genetic improvement, management, germplasm exchange and conservation (Rout and Das, 2000). Baseline genotoxicity can be used to monitor the quality of plants produced in culture for pharmacological purposes and can be quantified through the assessment of conformational changes in supercoiled plasmid DNA structure (De Mattos et al., 2000). Therefore, this study aimed to analyze both the genotoxic and antigenotoxic potential of extracts from different populations of field and *in vitro* plants of *P. alliacea* based on the effect of the extracts on the DNA molecule.

MATERIALS AND METHODS

Plant

Samples were obtained from spontaneously growing populations in different regions of Rio de Janeiro State. These plants were potted and kept in a greenhouse with 50% shade for identification and preparation of extracts. The species were identified by Dr. Alexandre G. Christo (Rio de Janeiro Pontifical Catholic University), and voucher specimens were kept at the Herbarium of Rio de Janeiro State University (HRJ 11.131 - 11.711 - 11.710 - 11.618, respectively). Samples were tagged according to area codes: (i) MG (22° 64' 32.18"S and 43° 12' 22.26"W, elev. 13 m); (ii) MH (22° 51' 24.46"S and 43° 22'13.75"W, elev. 17 m); (iii) NT (22° 53' 55.95"S and 43° 05' 09.37"W, elev. 54 m); and (iv) VI (22° 54' 57.57"S and 43° 14' 18.54"W, elev. 22 m). Botanical characterization of these samples was previously reported (Soares et al., 2013) and the plants were classified as *P. alliacea* L. based on the morphological characteristics, as well as the herbaceous habit and garlic odor. However, plants collected from MH, VI, and NT were identified as belonging to the variety *P. alliacea alliacea*. Plants collected from MG were identified as var. *P. alliacea tetrandra* (B.A. Gomes) Hauman (Marchioretto, 2010). Harvested plants were maintained in the greenhouse of the Plant Biotechnology Center at Rio de Janeiro State University. The seeds produced by these samples were used to initiate *in vitro* cultures.

Culture growth conditions

The *in vitro* cultures were incubated in growth chambers at 30 ± 2°C and 16 h photoperiod under an irradiance of 46 µmol $m^{-2}s^{-1}$ supplied by cool white fluorescent lamps.

In vitro germination

Seeds from different populations were used to establish primary cultures *in vitro*. Aiming to decontaminate the seeds were washed three times with detergent in tap water, immersed in 70% ethanol for 5 minutes and then in 1% NaOCl solution for 5 minutes. In addition, incubation was performed with 1% Benlate® and 1% Agrimicina® for 15 min. Afterwards, the seeds were inoculated on MS medium (Murashige and Skoog, 1962) without growth regulators (MS0) supplemented with 0.04 g/L Benlate® and 300 mg/L Agrimicina®, and incubated in flasks for one week under culture conditions, as described earlier. After this period, all flasks containing microorganismal contaminates were discarded, and the microorganism-free seeds were transferred to MS medium and maintained under the same growth conditions.

Micropropagation

Shoot apices and nodal segments to the third position from the top down (0.5 cm long) were excised from *in vitro*-grown plants derived from seed germination and cultured on MS medium

solidified with 0.7% agar plus 30 g/L sucrose. The pH was adjusted to 5.8 before autoclaving for 15 min at 121°C. The shoots were transferred to MS medium supplemented with Indole-3-acetic acid (IAA) 0.6 µM for rooting. These cultures were incubated in growth chambers at the described conditions. Primary regenerants in the form of whole plants were subcultured six to eight weeks by inoculation of shoot apices on growth regulator-free MS medium. The *in vitro* plants were used as donors of leaf explants for preparation of the extracts.

Extract preparation

After leaves were dried at 45°C for 48 h, the aqueous extract was prepared by boiling 10% wt/wt of the dried powdered plant leaves in sterile distilled water for 10 min. The aqueous extract was then left to cool at room temperature overnight.

Extraction of plasmid DNA

Cultures of *Escherichia coli* DH5αF'IQ were used as host for plasmid pUC 9.1. Plasmid DNA was extracted using the Invisorb® Spin Plasmid Mini Two plasmid extraction kit. The quantification of DNA was performed by absorbance at 260 nm.

DNA topology analysis

In order to evaluate possible genotoxic activity, aliquots of plasmid DNA (100 ng) were incubated with aqueous extracts (1.0, 5.0 to 10.0 mg/ml) at room temperature for 60 min. In order to assay the possible antigenotoxic potential of plant extracts, 1.11 mM stannous chloride ($SnCl_2$) solution was mixed with the extracts, added to the plasmid suspension and incubated at the same conditions described earlier. Stannous chloride was used as a positive control because it is a reactive oxygen species generator and can induce DNA strand breaks. After incubation, 10 µl of each sample were subjected to electrophoresis in a 0.8% normal melting point agarose gel (100 V for 30 min in 1× TAE buffer, pH 8.0). The gel was stained with ethidium bromide (0.5 mg/ml), visualized under a transilluminator system and digitalized with a Cannon XYZ system. Each assay was repeated at least three times, and the DNA bands from the best result were quantified (%) through Image J software, ver. 1.46 U.

Statistical analysis of DNA strand breaks

The data collected from light densitometer scanning gave us the null events percentage [no breaks = p (0; µ)] for each of the extracts tested. In this way, using the Poisson distribution, it is possible to obtain the mean value of breaks for each of the concentrations from the percentage of DNA supercoiled forms, as follows: $\mu = -\ln p(0; \mu)$ (Remington and Schor, 1985).

RESULTS

Micropropagation

The samples of *P. alliacea* collected in the field (Figure 1A, C, E and G) were introduced into the culture through *in vitro* germination of their seed, constituting different lineages *in vitro* (MG, NT, MH and VI) which were maintained by monthly subcultures (Figure 1B, D, F and H). From these primary cultures, more subcultures were performed monthly using shoot apices and nodal segments as explants which showed regeneration frequencies of 90 to 100% independent of position. Growth initiation occurred from apical or axillary buds after 6 to 7 days.

Genotoxic potential

The plasmid treated with *P. alliacea* aqueous extracts from field samples (Figure 2A, C, E and G; lanes 3, 4 and 5) and *in vitro* plants (Figure 2B, D, F and H; lanes 3, 4 and 5) caused single- (Figure 2A, C, E and G; lane 3) and double-strand breaks (Figure 2B and F; lanes 3, 4 and 5). In the *in vitro* samples from MG and VI, double-strand breaks were observed in lane 5. This treatment led to the conversion from Form I (supercoiled intact DNA) to Form III (open circle). This effect was dependent on both the concentration of the extract and the origin of the plants. Thus, different profiles were observed between NT field and *in vitro* samples, where apparently *in vitro* extracts of all plants were able to cause DNA breaks at different concentrations (Figure 2A and B; lanes 3, 4 and 5), as shown by quantitative analysis of corresponding bands on the gel and the decrease in Form I (supercoiled plasmid DNA), along with the presence of broken DNA strands, including Form II (circular) and/or Form III (open circle), as shown graphically in Figure 2A and B. MG field and *in vitro* samples showed a similar electrophoretic profile, as demonstrated through quantitative analysis showing that field extracts produced both single- (Form II) (Figure 2C, lanes 3 and 4) and double-strand breaks (Form III) (Figure 2C, lane 5), while low concentrations of *in vitro* extracts produced only DNA changes as indicated by Form III (Figure 2D, lanes 3 and 4). DNA changes as indicated by Form II appear just at the highest concentration of the extract (Figure 2D, lane 5). MH field samples only showed significant formation of double- and single-strand breaks at the highest tested concentration (Figure 2E, lane 5). On the other hand, all concentrations of *in vitro* samples exhibited double- and single-strand break formations, starting from the lowest concentration (Figure 2F, lanes 3, 4 and 5). VI field samples showed single-strand breaks at all tested concentrations, while no double-strand breaks could be detected (Figure 2E, lanes 3, 4 and 5). VI *in vitro* samples also showed single-strand breaks at the lowest concentration (Figure H, lane 3), while concentrations shown in lanes 4 and 5 showed both kinds of lesions (Figure H, lanes 4 and 5).

Antigenotoxic potential

The antigenotoxic potential of the extracts was evaluated based on their capacity to block or reduce the occurrence

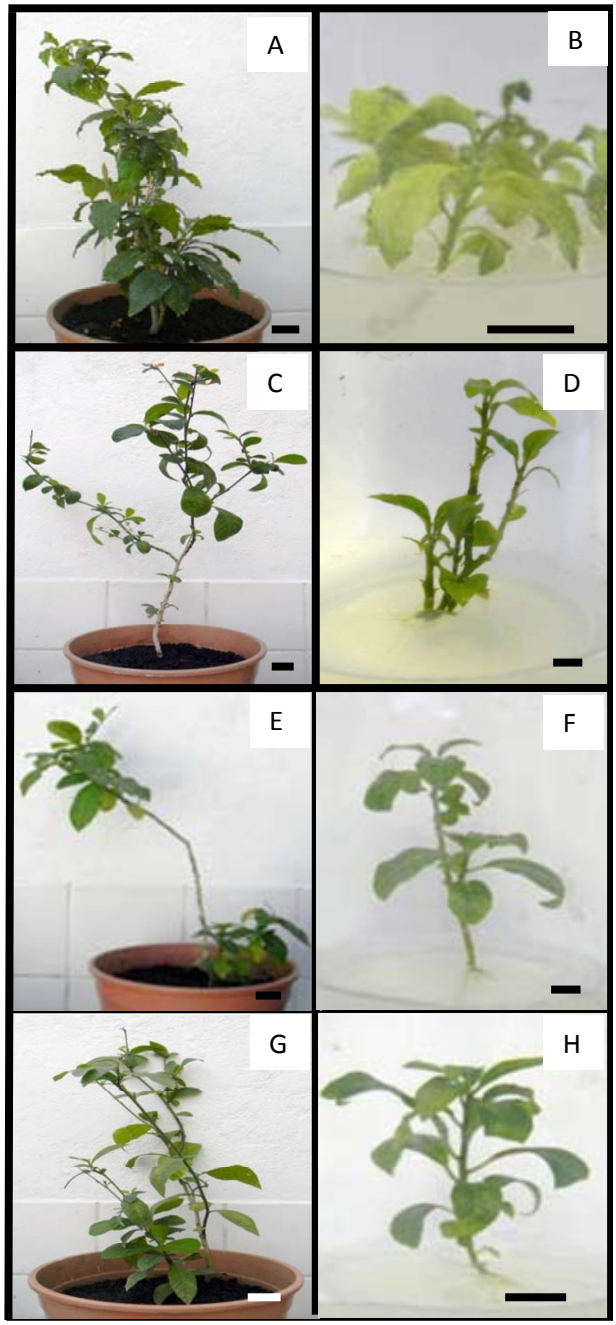

Figure 1. *Petiveria alliacea* L. from field samples: A) MG field; B) MG *in vitro*; C) NT field; D) NT *in vitro*; E) MH field; F) MH *in vitro*; G) VI field; H) VI *in vitro*. Bar = 1 cm

of DNA strand breaks when plasmid DNA molecules were challenged with $SnCl_2$. This experimental protocol is also based on plasmid DNA mobility through agarose gel submitted to an electrical field. The plasmid DNA (pUC 9.1) treated with $SnCl_2$ led to the DNA conversion from Form I to Forms II and III (De Mattos et al., 2000), a pattern that was used as positive control for this type of injury (Figure 2, lane 2). As verified by comparing lanes 6 and 8 in Figure 2B, a protective effect was observed in the presence of NT extracts from *in vitro* plants derived from this population. This effect was also detected in MH field samples (Figure 2E, lanes 7 and 8) and VI field samples (Figure 2G, Lanes 6 and 7). However, in the MG *in vitro* samples, this effect either did not appear (Figure 2D) or was inconclusive (Figure 2F, lanes 6, 7 and 8). The genotoxic/antigenotoxic effects of extracts were measured by densitometry (Figure 2) and further analyzed through Poisson distribution (Figure 3).

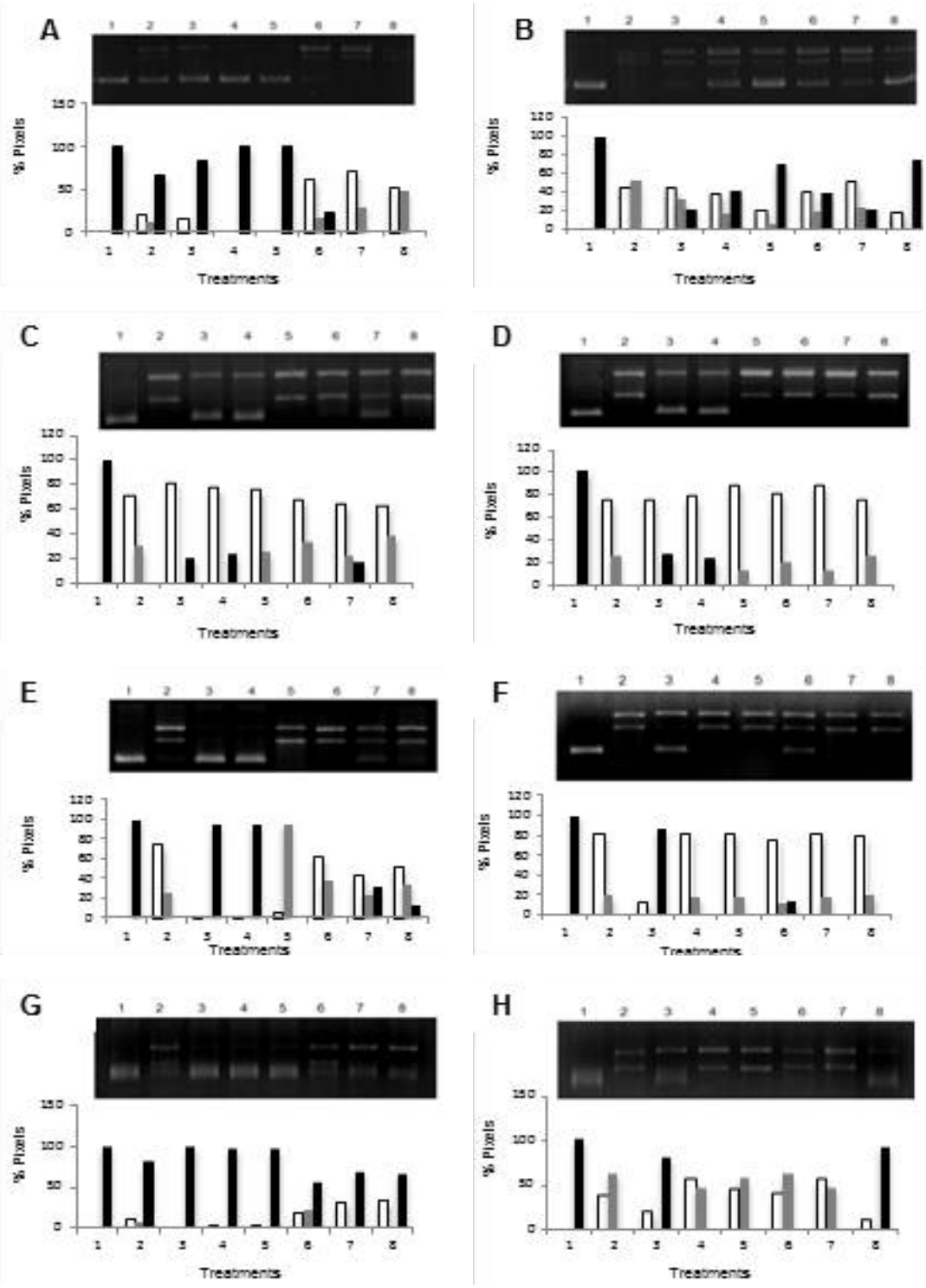

Figure 2. Qualitative and semi-quantitative evaluation of the effects of *P. alliacea* aqueous extract on plasmid pUC 9.1 DNA. Bands corresponding to aliquots of the suspension of plasmid (100 ng) treated with extracts (0-1.0-5.0-10 mg/mL). Lanes 1 and 2 respectively show negative and positive controls. Lanes 3, 4 and 5 correspond to the genotoxic potential of the aqueous extract, while lanes 6, 7 and 8 show the antigenotoxic effect of the extract against stannous chloride: A) NT field plant; B) NT i*n vitro* plant; C) MG field plant; D) MG *in vitro* plant; E) MH field plant; F) MH i*n vitro* plant; G) VI field plant; H) VI *in vitro* plant. Graphical bars represent DNA densitometric measurements by Image J. Bars correspond to lanes: 1 – pUC 9.1; 2 – p UC 9.1 + 1.11 mM Sn Cl_2; 3 – p UC 9.1 + 1 mg/ml extract; 4 – pUC 9.1 + 5 mg/ml extract; 5 – pUC 9.1 + 10mg/L extract; 6 – pUC 9.1 + 1 mg/ml extract + Sn CL2; 7 - pUC 9.1 + 5 mg/ml extract + $SnCl_2$, 8 - pUC 9.1 +10 mg/ml extract + $SnCl_2$.

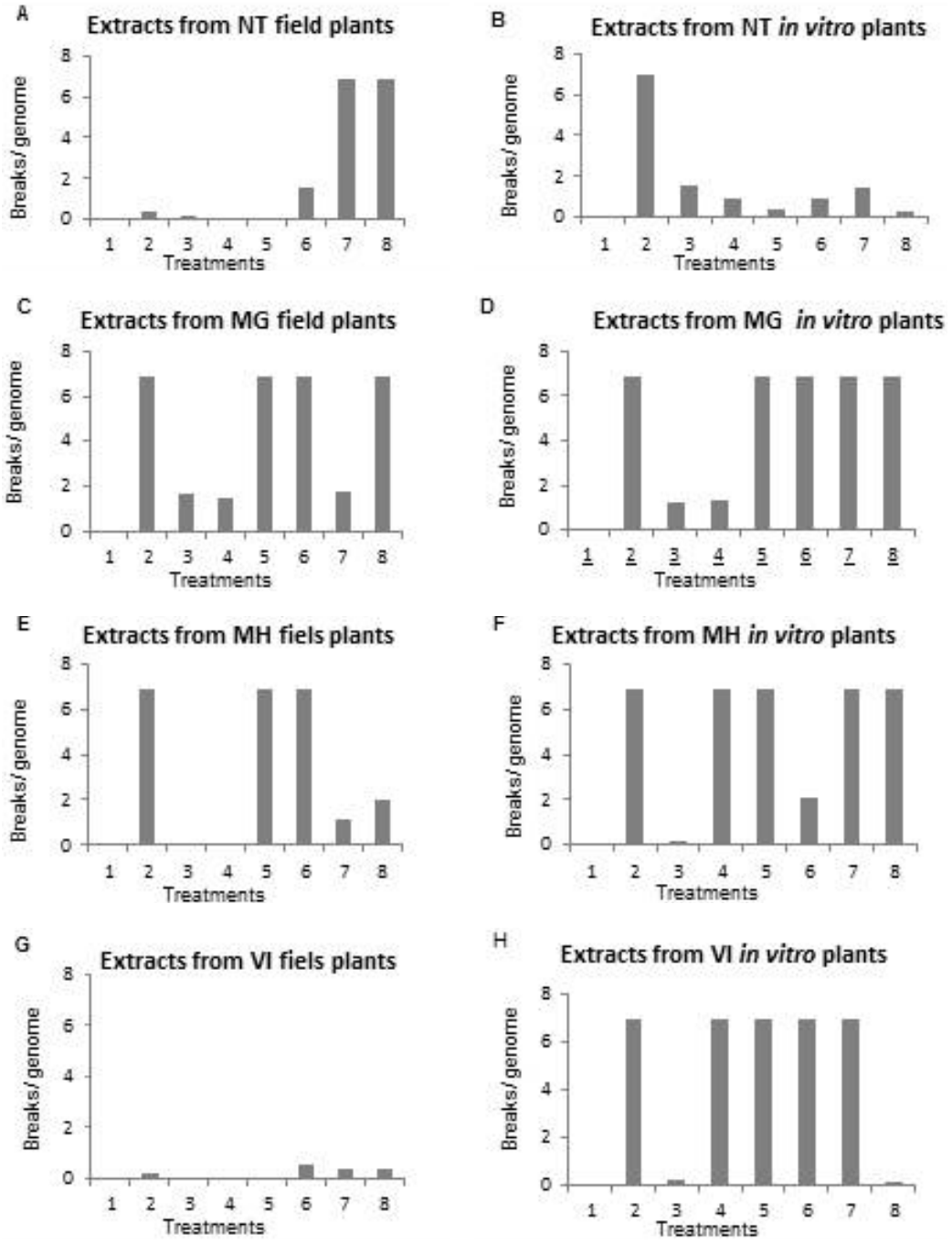

Figure 3. Number of single-strand breaks/genome in plasmid DNA treated with extracts. Bands corresponding to aliquots of the suspension of plasmid (100 ng) treated with extracts (0 to 1.0 to 5.0 to 10 mg/ml). Columns 1 and 2, respectively show negative and positive controls. Columns 3, 4 and 5 correspond to the genotoxic potential of the aqueous extract, while lanes 6, 7 and 8 show the antigenotoxic effect of the extract against stannous chloride. Analysis of densitometric data performed by Image J gave the null events percentage, that is, no breaks, for each of the treatments tested. Using Poisson distribution, we have obtained the mean value of breaks for each of the concentrations, as follows: $\mu = -\ln p(0; \mu)$. Number of single-strand breaks/genome represents the means of three isolated experiments.

The data presented as the DNA-single-strand breaks (DNA-SSB) per kilobase of plasmidal DNA (mean value) is according to the genotoxic/antigenotoxic potential of the extracts. (Figure 3A, B, C, D, E, F, G, H).

DISCUSSION

The methods based on the activation of preformed meristems (shoot tips and axillary buds), which retain the potential to recover true-to-type plants, are desirable for many biotechnological purposes. Thus, micropropagation of various pathogen-free plant species, including many medicinal plants, has been reported (Tripathi and Tripathi, 2003). *In vitro* cultures have been considered an important tool for both mass production of phytochemicals and pharmacological studies (Borgaud et al., 2001). In this way, previous reports have already described the production of plants by amplifying nodal segments on *P. alliacea* (Castellar et al., 2011). In this study, *in vitro* plants were derived from four different regions of Rio de Janeiro State, Brazil, and they were established after germination of their seeds in order to maintain intraspecific diversity, thus generating the four representative samples of the populations studied. Evaluation of electrophoretic mobility has been used to study the oxidant/antioxidant properties of different natural products (Reiniger et al., 1999; Biso et al., 2010; Hamedt et al., 2013). This approach could be used to monitor the quality of plants produced by culture for pharmacological purposes. Thus, it is possible to follow the occurrence of different types of injury (single- or double-strand breaks) based on the intensity and position of the bands produced by electrophoresis. Accordingly, the band corresponding to supercoiled DNA (Form I) becomes less intense when DNA strand breaks occur, generating the characteristic bands corresponding to Forms II (open circle) and/or Form III (linear) (De Mattos et al., 2004).

Some studies have reported on the pro-oxidant effects of this species in different experimental models (Salim, 2011; Andrade et al., 2012). Using a sea urchin model, a moderate genotoxic activity, based on cytogenetic changes, was reported (Hoyos et al., 1992), but to our knowledge, this is the first study on the effect of extracts of *P. alliacea* on the DNA molecule. Based on the evaluation of genotoxic potential, the results obtained in the present study demonstrate that aqueous extracts from *P. alliacea* have components that induce lesions in the DNA molecule. This is also the first study to evaluate the direct effects of extracts of *P. alliacea* on the DNA molecule. It is well known that many biological effects are caused by chemical substances produced in the plant metabolism, which under natural conditions are subject to climate changes, pathogens and predators, justifying the high chemical diversity produced through evolution. In artificial culture, plants are also subjected to various stress conditions, as represented by the artificial environment of culture, a known inducer of specific adaptations (Smulders and Klerk, 2010). These responses are often reflected by increased synthesis of certain substances, or even the synthesis of new substances not detected in field plants (Tripathi and Tripathi, 2003; Vanisree et al., 2004).

In this work, genotoxic effects were stronger with extracts from *in vitro* plants than those obtained from field samples. This is an interesting result since we know that the synthesis of secondary metabolites is highly induced under both natural and artificial conditions. Thus, it is possible that stress conditions in artificial cultures could be more intense than those in natural conditions. Thus, metabolites induced in the culture cannot be formed in the field due to the silencing of some genes. Moreover, the possibility of specific responses cannot be ruled out. The present results demonstrate that aqueous extracts have components capable of inducing breaks and/or alkali-labile injuries in the DNA molecule. Moreover, lesions can lead to cell inactivation, as well as mutagenic potential (Friedberg et al., 2006), justifying the need for further such studies in other experimental models, such as bacteria and eukaryotic cells. In addition, some studies suggest that various extracts or compounds of this species exhibit antioxidant or pro-oxidant activity (Carlini, 2003; Andrade et al., 2012). In this work, the antigenotoxic effect was influenced by plant origin, suggesting, in turn, the influence of some genetic variation. This antioxidant activity could also result from the different flavonoids already reported in this species (Delle-Monache et al., 1996; Okada et al., 2008).

Indeed, many studies are currently focused on the antioxidant activity of medicinal plants (Kumar et al., 2010). Most active antioxidant compounds are phenols, flavonoids, isoflavones, alpha-tocopherol and anthocyanins, and a direct relationship between antioxidant activity and phenolic compounds in plant extracts has been reported by many researchers (Gollucke-Boiago et al., 2008; Chirinos et al., 2008; Sharififar et al. 2009; Du et al. 2009; Conforti et al., 2009). The ability of different plant extracts to protect supercoiled plasmid DNA against the deleterious effects of hydroxyl radicals generated during metabolism was evaluated by a test that detects changes in the structural conformation of plasmids. This test has been widely used, with modifications to evaluate the antigenotoxicity of several plant species (De Mattos et al., 2000; Lee et al., 2002; Kumar et al., 2010). Specifically, the addition of stannous chloride in the reac-tion mixture results in the formation of hydroxyl radicals (OH•) that react with plasmid DNA, thus producing single- and double-strand breaks (Forms II and III). As such, it is possible to follow the occurrence and type of injury (single- or double-strand break) by the intensity and position of the bands produced in the agarose gel. Accordingly, the band on the supercoiled DNA becomes less intense when the DNA is broken by OH radicals, and the characteristic bands of this type of injury are more intense (De Mattos et al., 2004).

In assessing the potential antigenotoxicity of extract samples used in this work, the change in band position obtained in relation to DNA damaged by stannous chloride showed a protective effect of the extract in

agreement with the results obtained for other species (De Mattos et al., 2000, 2004). The antioxidant and antineoplastic activity of *P. alliacea* could result from the presence of different substances, including the flavonoids already reported in the species (Delle-Monache and Suarez, 1992; Delle-Monache et al., 1996; Okada et al., 2008; Soares et al., 2013). Characterization of the arrays and the possibility of applying different methods of eliciting and monitoring the material produced in culture can guarantee the safe *in vitro* production of this material, making possible the therapeutic application of herbal and natural remedies.

Conclusion

The assessment of the genotoxic potential in *P. alliacea* demonstrated the occurrence of changes in plasmid topology influenced by the concentration of extracts and genotype of the plant; however, genotoxic effects were stronger with extracts from *in vitro* plants than those obtained from field samples, possibly because of the differences between *in vivo* and *in vitro* environmental factors. On the other hand, the assessment of potential antigenotoxicity of aqueous field plant extracts and *in vitro* cultures demonstrated a protective effect against injuries caused by $SnCl_2$.

ACKNOWLEDGMENTS

This work was supported by the National Council of Scientific and Technological Development (CNPq), Foundation for the Coordination of Improvement of Higher Education Personnel (CAPES), and the Carlos Chagas Filho Foundation for Research Support at State of Rio de Janeiro (FAPERJ).

Conflict of Interest

Authors have not declare any conflict of interest.

REFERENCES

Andrade TM, Melo AS, Dias RGC, Varela ELP, Oliveira FR, Vieira JLF, Andrade MA, Baetas AC, Monteiro MC, Maia CSF (2012). Potential behavioral and pro-oxidant effects of *Petiveria alliacea* L. extract in adult rats. J. Ethnopharmacol. 143:604-610.

Azevedo SKS, Silva IM (2006). Medical and religious plants in conventional and open-air markets of Rio de Janeiro State, Brazil. Acta Bot. Bras. 20:185-194.

Benevides PJC, Young MCM, Giesbrecht AM, Roque, NF, Bolzani VDS (2001). Antifungal polysulphides from *Petiveria alliacea* L. Phytochemistry 57:743-747.

Biso FI, Rodrigues CM, Rinaldo D, Reis MB, Bernardi CC, De Mattos JCP, Caldeira- de-Araújo A, Vilegas W, Cólus IMS, Varanda EA (2010). Assessment of DNA damage induced by extracts, fractions and isolated compounds of *Davilla nitida* and *Davilla elliptica* (Dilleniaceae). Mutat. Res. Genet.Toxicol. Environ. Mutagen. 702:92-99.

Borgaud F, Gravot A, Milesi S, Gontier E (2001). Production of plant secondary metabolites: a historical perspective. Plant Sci. 161:839-851.

Carlini EA (2003). Plants and the central nervous system. Pharmacol. Biochem. Behav. 75:501-512.

Castellar A, Gagliardi RF, Mansur E (2011). *In vitro* propagation and establishment of callus and cell suspension cultures of *Petiveria alliacea* L., a valuable medicinal plant. J. Med. Plants Res. 5:1113-1120.

Chirinos R, Campos D, Warnier M, Pedreschi R, Rees J, Larondelle Y (2008). Antioxidant properties of mashua (*Tropaeolum tuberosum*) phenolic extracts against oxidative damage using biological *in vitro* assays. Food Chem. 111:98-105.

Cifuentes CM, Serranillos MPG, Iglesias I, Fresno AMV (2001). Neuropharmacological profile of ethnomedicinal plants of Guatemala. J. Ethnopharmacol. 76:223-228.

Conforti F, Sosa S, Marrelli M, Menichini F, Statti GA, Uzunov D, Tubaro A, Menichini F (2009). The protective ability of Mediterranean dietary plants against the oxidative damage: The role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents. Food Chem. 112:587-594.

De Mattos JCP, Dantas FJS, Bezerra RJAC, Bernardo-Filho M, Cabral-Neto JB, Lage CAS, Leitão AC, Caldeira-de-Araujo A (2000). Damage induced by stannous chloride in plasmid DNA. Toxicol. Let. 116:159-163.

De Mattos JCP, Dantas FJS, Caldeira-de-Araujo A, Moraes MO (2004). Agarose gel electrophoresis in the classroom. Biochem. Mol. Biol. Educ. 4:254-257.

De Sousa JR, Demuner AJ, Pinheiro JA, Bretmair E, Cassels BK (1990). Dibenzyl Trisulphyde and Trans-N-methyl-4-methoxyproline from *Petiveria alliacea*. Phytochemistry 29:3653-3655.

Delle-Monache F, Menichini F, Suarez LEC (1996). Substances from *Petiveria alliacea*: II further flavanonoides and triterpenes. Gaz Chim. Ital. 126:275-278.

Delle-Monache F, Suarez LEC (1992). 6-c-formil and 6-c-hydroxymethyl flavanones from *Petiveria alliacea*. Phytochemistry 31:2481-2482.

Di Stasi LC, Costa M, Mendacolli LJ, Kirizawa M, Gomes C, Trolin GJ (1988). Screening in mice of some medicinal plants used for analgesic purposes in the state of São Paulo. J. Ethnopharmacol. 2:205-208.

Du M, Li M, Ma F, Liang D (2009). Antioxidant capacity and the relationship with polyphenol and vitamin C in Actinidia fruits. Food Chem. 113:557-562.

Friedberg EC, Walker GC, Siede W, Wood RD, Schultz RA, Ellenberger T (2006). DNA Repair and Mutagenesis Washington, DC, ASM Press. pp. 1,118.

Gollucke-Boiago AP, Souza CJ, Queiroz TD (2008). (+)-Catechin and (-)-epicatechin levels of concentrated and ready to drink through storage. Int. J. Food Sci. Technol. 43:1855-1859.

Gomes PB, Noronha EC, Melo CT, Bezerra JN, Neto MA, Lino CS, Vasconcelos SM, Viana GS, Sousa FC (2008). Central effects of isolated fractions from the root of *Petiveria alliacea* L. (tipi) in mice. J. Ethnopharmacol. 120:209-214.

Hamedt AL, Ortiz IC, Huertas PAG, Saenz J, Caldeira-de-Araujo A, De Mattos JCP, Rodriguez-Gasquez MA, Chavez OT (2013). Cytotoxic, mutagenic and genotoxic evaluation of crude extracts and fractions from *Piper jericoense* with trypanocidal action. Acta Trop. 131:92-97.

Hoyos LS, Au WW, Heo MY, Morris DL, Legator MS (1992). Evaluation of the genotoxic effects of a folk medicine *Petiveria alliacea* (anamu). Mut. Res. 280:29-34.

Johnson L, Williams LAD, Roberts EV (1997). An insecticidal and acaricidal polysulfide metabolite from the roots of *Petiveria alliacea*. Pest Sci. 50:228-232.

Kubec R, Kim S, Musah RA (2002). S-Substitued cysteine derivatives and thiosulfinate formation in *Petiveria alliacea*-Part II. Phytochemistry 61:675-680.

Kubec R, Kim S, Musah RA (2003). The lachrymatory principle of *Petiveria alliacea*. Phytochemistry 63:37-40.

Kumar A, Kaur R, Arora S (2010). Free radical scavenging potential of some Indian medicinal plants. J. Med. Plants Res. 4:2034-2042.

Lee J, Kim H, Kim J, Jang Y (2002). Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. Saboten. J. Agric.

Food Chem. 50:6490-6496.
Lima SS, Moreira DL, Affonso VR, Henriques AB, Sato A, Esquibel MA, Leitão GG, Lage CLS (2010). Micro propagation of *Polygonum acre* Kunth var. *aquatile* (Mart.) Meisn and seasonal variation of tannins in acclimatized plants. J. Med. Plants Res. 4:573-578.
Marchioretto MS (2010). Phytolaccaceae in: List of species of Flora of Brazil. Botanical Garden of Rio de Janeiro.
Mata-Greenwood E, Ito A, Westenburg H, Cui BL, Mehta RG, Kinghorn AD, Pezzuto JN (2001). Discovery of novel inducers of cellular differentiation using HL-60 promyelocytic cells. Anticancer Res. 21:1763-1770.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
Okada Y, Tanaka K, Satoc E, Okajima H (2008). Antioxidant activity of the new thiosulfinate derivative, S-benzyl phenylmethanethiosulfinate, from *Petiveria alliacea* L. Organ. Biomol. Chem. 6:1097-1102.
Ponte JJ, Franco A, Silveira-Filho J (1996). Preliminary investigation on the nematocide potentiality of Guine's Plants (*Petiveria alliacea*). Phytopathol. Venezuela 9:14-15.
Quadros MR, Souza-Brito ARM, Queiroz MLS (1999). *Petiveria alliacea* L. extracts protects mice against *Listeria monocytogenes* infection-effects on bone marrow progenitor cells. Immunopharmacol. Immunotoxycol. 21:109-124.
Reiniger IW, Silva CRS, Felzenszwalb I, De Mattos JCP, Oliveira JF, Dantas FJS, Bezerra RJAC, Caldeira-de-Araujo A, Bernardo-Filho M (1999) Boldine action against the stannous chloride effect. J. Ethnopharmacol. 68:345-348.
Remington RD, Schor MA (1985). Some important distributions. In: Johnson RA, Wichern DW (Eds.), Statistics with Applications to the Biological and Health Sciences. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. P 415.
Rosner H, Williams LAD, Jung A, Kraus W (2001). Disassembly of microtubules and inhibition of neurite out growth, neuroblastoma cell proliferation, and MAP kinase tyrosine dephosphorylation by dibenzyl trisulphide. Biochim. Bioph. Acta 1540:166-177.
Rout GR, Das SSP (2000). *In vitro* manipulation and propagation of medicinal plants. Biotechnol. Adv. 18:91-120.
Ruffa MJ, Ferraro G, Wagner ML, Calcagno ML, Campos RH, Cavallaro L (2002). Cytotoxic effect of Argentine medicinal plant extracts on human hepatocellular carcinoma cell line. J. Ethnopharmacol. 79:335-339.
Salim S (2011). Oxidative stress in anxiety: implications for pharmacotherapy. Am. J. Integr. Med. 1:11-21.
Sharififar F, Dehghn-Nudeh G, Mirtajaldini M. (2009). Major flavonoids with antioxidant activity from *Teucrium polium* L. Food Chem. 112:885-888.
Smulders MJM, Klerk GJ (2010). Epigenetics in plant tissue culture. Plant Growth Regul. 63:137-146.
Soares BO, Fernandes DC, Cantelmo L, Rocha LP, Pettinelli JA, Christo AG, Coelho MGP, Gagliardi RF (2013). Botanical characterization of *Petiveria alliacea* L. from Rio de Janeiro, Brazil: Systematic and functional implications. Plant Biosys. 147:411-417.
Szczepan CV, Zgorzela P, Hoyer GA (1972). Isolation, structure elucidation, and synthesis of an antimicrobial substance from *Petiveria alliacea* L. Drug Res. 22:1975-1976.
Tripathi L, Tripathi JN (2003). Role of biotechnology in medicinal plants. Trop. J. Pharm. Res. 2:243-253.
Vanisree M, Lee CY, Lo SF, Nalawade SM, Lin CY, Tsay HS (2004). Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures. Bot. Bull. Acad. Sin. 45:1-22.
Webster SA, Mitchell SA, Gallimore WA, Williams LAD, Ahmad MH (2008). Biosynthesis of Dibenzyl Trisulfide (DTS) from somatic embryos and rhizogenous/embryogenic callus derived from Guinea hen weed (*Petiveria alliacea* L.) leaf explants. *In Vitro* Cell. Dev. Biol. Plant 44:112-118.
Williams LAD, Rosner H, Levy HG, Barton EN (2007). A critical review of the therapeutic potential of dibenzyl trisulphide isolated from *Petiveria alliacea L* (guinea hen weed, anamu). West Indian Med. J. 56(1):17-21.
Williams LAD, Vasquez E, Klaiber I, Kraus W, Rosner H (2003). A sulfonic anhydride derivative from dybenzil trisulphide with agro-chemical activities. Chemosphere 51:701-706.