Cytotoxic and genotoxic activity of phenolic fractions from *Ulomoides dermestoides* Fairmaire, 1893 (Coleoptera, Tenebrionidae), in HaCat cells

Dary Luz Mendoza-Meza* and Pierine España-Puccini
Grupo de Investigación en Productos Naturales y Bioquímica de Macromoléculas. Programa de Química, Facultad de Ciencias Básicas, Universidad del Atlántico. Km 7 antigua vía a Puerto Colombia, Departamento del Atlántico, Colombia. E-mail: *darymendoza@mail.uniatlantico.edu.co.

**Abstract**

*Ulomoides dermestoides* (Fairmaire, 1893) is a beetle used in alternative medicine treatments in some South American countries. The objective of this study was to evaluate the cytotoxic and genotoxic effects of phenolic fractions (PF) from *U. dermestoides*. The PF were separated from crude extracts in acetone (PFAc) and ethanol (PFEtOH). The total phenolic content (TPC) was determined by Folin-Ciocalteu test. Volatile and semi-volatile compounds presents in crude extracts were identified by GC-MS; moreover, phenolic fractions were analyzed by HPLC-MS. The cellular viability, after exposition to phenolic fractions, was determined by Trypan blue exclusion test and MTT reduction assay on immortalized human keratinocyte cell line (HaCat); the degree of DNA damage was detected by alkaline comet-DNA assay. The TPC in PFAc and PFEtOH were: 11.34±0.88 mgGAE/g and 6.52±1.28 mgGAE/g, respectively (mean.dif: 4.951; p value = 0.0000). In both samples, HPLC-MS showed a pseudo-molecular ion [M-H]− at m/z 153, tentatively identified as protocatechuic acid. The results of cytotoxic assays suggest that the viability of HaCat cells depends on the concentration and exposure time of each treatment. Furthermore, the comet assay revealed moderate genotoxic effect after 48 hours of exposure to PFAc (40 to 160 µg.mL−1); cytotoxic/genotoxic activity of this fraction could be related to the higher phenol contents.

**Key Words:** beetles, cell viability, cytotoxicity, genotoxicity, phenolic fractions, *Ulomoides dermestoides*.

Actividad citotóxica y genotóxica de fracciones fenólicas de *Ulomoides dermestoides* Fairmaire, 1893 (Coleoptera, Tenebrionidae), en células HaCat

**Resumen**

*Ulomoides dermestoides* (Fairmaire, 1893), es un escarabajo usado en medicina alternativa en algunos países de Sudamérica. El objetivo de este estudio fue evaluar el efecto citotóxico y genotóxico de fracciones fenólicas (FF) de extractos de *U. dermestoides*. Las FF se separaron desde extractos acetónicos (FFAc) y etanólicos (FFEtOH). El contenido de fenoles totales (CFT) se determinó mediante ensayo de Folin-Ciocalteu. Compuestos volátiles y semi-volátiles, presentes en los extractos crudos, se identificaron mediante CG-EM; por otra parte, las FF se analizaron por HPLC-EM. La viabilidad celular, después de exposición a las FF se determinó mediante la prueba de exclusión con azul de tripano y el ensayo de reducción con MTT, usando la línea celular de queratinocitos humanos inmortalizados (HaCat); el grado de daño en el ADN se detectó mediante el ensayo de ADN-cometa alcalino. CFT en FFAc y FFEtOH fueron: 11,34±0,88 mgGAE/g y 6,52±1,28 mgGAE/g, respectivamente (dif.media: 4,951; p value = 0,0000). En ambas muestras, HPLC-EM mostró un ion pseudo-molecular [M-H]− a 153m/z, identificado tentativamente como ácido protocateúico. Los resultados de los ensayos de citotoxicidad sugieren que la viabilidad de células HaCat depende de la concentración y el tiempo de exposición a cada tratamiento. Además, el ensayo cometa reveló efecto genotóxico moderado después de 48 h de exposición a FFAc (40 a 160 µg.mL−1). La actividad citotóxica/genotóxica de esta fracción podría estar relacionada con el contenido más alto de fenoles.

**Palabras Clave:** escarabajos, viabilidad celular, citotoxicidad, genotoxicidad, fracciones fenólicas, *Ulomoides dermestoides*.
**Introduction**

Around the world, many cultures use insects and their products as nutraceuticals. The *Ulomoides dermestoides* Fairmaire, 1893 (Coleoptera, Tenebrionidae, Diaperinae) (synonymy: *Martianers dermestoides; Palembus dermestoides*) is a darkling beetle of Asian origin, known in oriental culture for its aphrodisiac and therapeutic properties; in South America, this beetle is eaten alive as an alternative therapy for bronchial asthma, psoriasis, vitiligo, chronic skin diseases, rheumatoid arthritis, hemorrhoids, diabetes mellitus, inflammation and different types of cancer.

During the last decade, the interest to research the therapeutic effects attributed to the *U. dermestoides* has risen. Santos *et al.*, 2010 showed the anti-inflammatory properties of polar extracts of this beetle, using the carrageenan-induced edema test in mice; Crespo *et al.*, 2011 described cytotoxic and genotoxic effects of dichloromethane extracts from whole body of *U. dermestoides* and the main quinone in their defense secretion (1,4-benzoquinone), on cellular line of lung cancer (A549). Tobón *et al.*, reported depressive activity on the central nervous system (CNS) of albino rats (*Mus musculus* Linnaeus, 1758) because of administration of oil extracted from *U. dermestoides* (3 mg.kg-1, orally).

Additionally, presence of secondary metabolites with antioxidant activity and antioxidant enzymes such as superoxide dismutase on whole body *U. dermestoides* extracts has been described. This is proof of existence of complex systems antifree radical in the beetles, that could be explored in the search of novel therapeutic agents for treatment of human disease associated with oxidative stress. However, it is also necessary to know the effect of *U. dermestoides* extracts or their fractions, on viability of healthy human cells. Skin is an important barrier in protecting the body from external chemicals and HaCat cell are an in vitro model to investigate cytotoxic effects on epidermal tissues. In addition, HaCat cells exhibited a remarkably stable genetic balance over extended culture periods, without shifting to the tumorigenic phenotype, for these reasons HaCat cell in vitro were selected for this research.

The objective of this study was to investigate cytotoxic and/or genotoxic effects of phenolic fractions from *U. dermestoides* whole body extracts, on a line of immortalized human keratinocytes (HaCat cells).

**Materials and methods**

**Sample.** A culture of *U. dermestoides* was established from a broodstock whose taxonomic identity was validated in the entomology department of the Natural Science Institute of the Universidad Nacional, Bogotá, Colombia (collection code ICN-45905). The beetles were bred under controlled temperature conditions (27 ± 2°C) and relative humidity (70-75%) and were fed exclusively with wheat bread and wheat bran. After 90 days of culture, adult individuals were separated from the substratum and were stored at -70°C.

**Materials.** Chemicals and solvents were obtained from Merck (Darmstadt, Germany) unless stated otherwise. Analytical grade chemicals were used. Solvents used in HPLC-MS, extraction and sample preparation were of HPLC grade.

**Extracts.** The frozen beetles were ground by friction with a mortar under a stream of liquid nitrogen until obtaining a pulverized material. Four grams of this material were mixed with 40 mL of extraction solvent, the mixture was submitted to constant agitation at 4°C, during 48 h. The extraction solvents used were: absolute ethanol (EtOH), butanol (BuOH), acetone (Ac) and ethyl acetate (EtAc). Homogenates were then clarified using a fritted funnel and centrifuged at 3,500 g for 20 min at 4°C in a Beckman Refrigerated Benchtop equipment (model GPR), the supernatants were stored in amber glass vials at -20°C.

**Phenolic fractions (PF).** Separation of the PF was done with column chromatography (40 x 2 cm), using 20-60 mesh Amberlite® XAD®- 2 resin (Supelco, 10357), with a solvent flow rate of 10 mL.min⁻¹. It was added to a 1 mL column of the total extract, and then washes were done with 10 mL of distilled water; compounds were eluted from the column with 10 mL of methanol (MeOH)⁴. Eluates were lyophilized at -40°C and 1.33 mBar, in a FreeZone 1-liter Benchtop Freeze Dry System (Labconco, Kansas City, MO).

**Total phenolic content (TPC).** The TPC was determined with Folin-Ciocalteu method described by Ozgen *et al.*, 2008. Samples were prepared by dissolving the lyophilized phenolic fractions to a concentration of 0.2 mg.mL⁻¹. A volume of 2 mL of this solution was mixed with 2.5 mL of the Folin-Ciocalteu reagent (Panreac Quimica S.L.U., Barcelona, España), diluted at 10% v/v in distilled water. The mixture was incubated at room temperature for 2 min, then 2 mL of 7.5% w/v sodium carbonate were added, and the mixture was then incubated at 50°C for 15 min. Absorbance of the sample was read in a Thermo Scientific Genesys 6 spectrophotometer (Madison, WI, USA) at a 765 nm wavelength, the results were interpolated in a gallic acid (Sigma, G7384) standard calibration curve (0.1 – 0.00312 mg.mL⁻¹) and were expressed as milligram of Gallic Acid Equivalent (GAE) by gram of dry weight (mg GEA/g). The calculation was done using the following equation:

\[ \text{mg GEA/g} = \frac{(C \times V)}{M} \]

Where:

- **C** = Concentration, determined by the gallic acid standard curve (mg.mL⁻¹).
- **V** = Volume of sample used in the test (mL).
- **M** = Mass of the dry weight (g).
GC-MS. This procedure was performed at the Chromatography and Mass Spectrometry Laboratory of the Universidad Industrial de Santander. Preparation of the sample (total extracts of Ulomoides dermestoides) was carried out by direct injection of the samples into the chromatography equipment. The analysis was done in an AT 6890 Plus Series (Agilent Technologies, Palo Alto, California, USA.) gas chromatographer, coupled to a selective mass detector (Agilent Technologies, MSD-5975) operated in full scan mode. The columns that were employed in the analysis were DB-5MS (J & W Scientific, Folsom, CA, USA.) [5%phenyl-poly (dimethylsiloxane), 60 m x 0.25 mm x 0.25 μm]. The injection was carried out in Split mode (30:1), VOliss = 2 μL. Tentative identification of registered compounds in the samples was established based on their mass spectrum (EI, 70Ev), using Adams databases, Wiley 138 and NIST 2005 (W*/N05)17.

HPLC-MS. The analysis was performed at the Mass Spectrometry Laboratory at the Universidad de Antioquia (University Research-SIU). Phenolic fractions from U. dermestoides extracts, were analyzed on an Agilent 1100 series LC-MS (Agilent Technologies, Waldbronn, Germany) system equipped with a selective detector with single quadrupole mass (G1956A LC/MSD Quad VL System), a vacuum degassing module (G1379B), a binary pumping module (G1312A), a high performance autosampler (G1367B), a column thermostat (G1316A), and a diode-array detector (G1315A). Data were processed using Agilent LC-MSD ChemStation B.04.03 Data Analysis Software.

An Agilent Zorbax Eclipse Plus Rapid Resolution Stable Bond Poroshell XDB-C18 column 100 mm x 4.6 mm id and 3.5 μm particle size was used for the separation. The analysis was performed as described previously18 with some modifications. Composition of the mobile phase was: Phase A consisting of 10% (v/v) CH3CN in H2O and 0.05% (v/v) of CH3COOH; and phase B consisting of 90% (v/v) CH3CN in H2O and 0.05% (v/v) of CH3COOH. The column elution was carried out with the following linear gradient, 0.0 min: 100% phase A with a flow rate of 0.5 mL min−1; 39.0 min: 100% phase B with a flow rate 0.5 mL min−1; 40.0 min: 100% phase B with a flow rate of 1.0 mL min−1; 41.0 min: 100% phase A with a flow rate of 1.0 mL min−1; 46.0 mn: 100% phase A with a flow rate of 1.0 mL min−1; 47.0 min: 100% phase A with a constant flow rate of 0.5 mL min−1. The detector used was diode arrangement, the temperature of the column at the beginning and end of the test was kept at 25°C and the pressure between 46.8 and 51.3 bar, respectively. The injection volume of the sample was 10 μL.

Mass analysis was performed by electrospray (ESI-API) using an ionization source of negative polarity. Equipment conditions: 60 psi nebulizer pressure; drying gas flow of 13 L min−1; quadrupole temperature at 100 °C; gas temperature at 350°C; source current of 8nA at the beginning and 31nA at the end. The ion scan was carried out in full scan mode.

Cytotoxicity and genotoxicity tests. These were performed on the HaCaT cell line (human keratinocytes), established at the Laboratory of Genetic Toxicology, Universidad de Antioquia, Colombia. Cytotoxic effects of the phenolic fractions (PFAc and PFEtOH) were evaluated by two methods, namely a MTT reduction test and trypan blue exclusion test. The genotoxic effect was assessed using the alkaline comet-DNA test.

Cell Cultures: HaCaT cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Gibco BRL) supplemented with 5% of fetal bovine serum (FBS), penicillin (100 μg.mL−1), streptomycin (100 μg.mL−1) and incubated in a humidified atmosphere of 5% CO2 at 37 °C.

MTT reduction test: This is a colorimetric assay for assessing cell metabolic activity and consist in measure of metabolic reduction of (3(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT reagent). The reaction is catalyzed by mitochondrial enzyme succinate dehydrogenase, producing a blue colored compound (formazan). The test determines the mitochondrial functionality of the treated cells, since the amount of living cells is proportional to the amount of formazan produced.

HaCaT cells were plated at density of 1x104 cells/well in 96-well microplates and maintained under standard culture conditions. After allowing accessions, the treatments were performed for each concentration and each sample; after 24 and 48 h of incubation 10 μL of MTT (Sigma M2128) at 5 mg.mL−1 (final concentration in the well: 0.5 mg.mL−1) were added and samples were incubated for 4h in the dark. Finally, to dissolve the formazan crystals, 100 μL of isopropanol - HCl (0.04 M) were added and absorbance at 570 nm (A570) was measured using a microplate reader Benchmark Plus (Bio-Rad Laboratories). The percentage of viability was calculated as the following equation.

\[
\text{Viable cells (\%)} = \frac{A_{570} \text{ of treated samples }}{A_{570} \text{ of untreated samples }} \times 100
\]

The positive control of the assay was 1 mM H2O2 and the negative control was the sample solvent (EtOH). The results are shown as the mean ± SD (standard deviation) of three biological replicates per experimental group.

Trypan blue exclusion test: This test is based on the fact that living cells have intact cell membranes that exclude certain dyes such as trypan blue, while dead cells do not. HaCat cell were plated (1 x 104 cells/well) into 12-well microplates and were maintained under standard culture conditions. After allowing accessions, treatments were performed for each sample and each concentration. After 24 and 48 h of exposure, the microplates were processed according to the protocol. For this purpose,
cells were detached with a trypsin-EDTA (0.1%) solution and equal volumes of cell suspension and trypan blue 0.4% w/v (Sigma, T6146) were mixed to quantify the cell viability using a hemocytometer. The percentage of living cells was determined by dividing the number total live cells by the total number of cells (both alive and dead cells). For this test, several trials were performed with the purpose of determining the range of concentrations at which cell viability was greater than 80% and thus, based on these resulting ranges, develop the genotoxicity studies. Results are shown as the mean ± SD of three biological replicates per experimental group.

DNA-Comet alkaline test: HaCat cell were plated into 12-well microplates at density of 1 x 10^4 cells/well and were maintained under standard culture conditions. After allowing access, treatments were performed for each sample and each concentration under evaluation; after 48 h exposure, cells were processed under guidelines of International Workshop on Genotoxicity Test Procedures21, adopted as follows: after treatment, aliquots of 2 x 10^4 cells were re-suspended in 20 µL of simple medium (DMEM) and mixed with 80 µL of agarose low melting point (Sigma, A9414 molecular biology grade) at 37°C. Afterwards, the cell/agarose mix was layered on glass microscope slides, pre-coated with agarose of normal fusion point (Sigma, A9539 molecular biology grade). The coated slides were kept at 4°C for 15 min to allow solidification of the agarose. Subsequently, cells were then subjected to digestion for 1 hour in lysis buffer (2.5 M NaCl, 0.1 M Na$_2$EDTA, 10 mM Tris base, 1% N-lauryl sarcosinate, 10% DMSO and 1% Triton X-100 (Sigma, T8787), adjusted at pH 10.0) and 30 min in denaturation buffer (50 mM NaOH/1mM EDTA, pH13). After, slides were place in a horizontal submarine gel electrophoresis system (Bio-Rad Co., Hercules, CA, USA) containing Tris-Borate-EDTA buffer. The electrophoresis conditions were 25 volts and 300 mA. Then, plates were washed three times for 5 min with neutralizing buffer pH 7.4 (0.4M Tris). Finally, cells were dyed with 40 μL of ethidium bromide (20 μg.mL$^{-1}$) (Sigma, E7637) and plates were read in a fluorescent microscope (Leica DMLS, Austria) under magnification 20X, excitation filter 515-560 nm and barrier filter 590 nm. For each treatment, 50 cells were photographed and subsequently analyzed with the Comet Assay Software Project (Casp, http://casp.sourceforge.net) for quantifying percentage of DNA in the queue. According to the amount DNA in the tail, nuclei were classified into one of following categories: a) no damage DNA, b) low damage, c) moderate damage d) high damage. The positive control in this trail was 0.3 mM hydrogen peroxide (30 min exposure) and the negative control was untreated cells.

Statistical analysis. The results are presented as the value of the mean ± standard deviation (SD). Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (SPSS Inc., Chicago, IL, USA). For quantifying total phenolic content, five independent replicates were performed.

One-way analysis of variance (ANOVA) with Tukey’s HSD (Honestly Significant Difference) post-hoc test was used to determine the possible differences among treatment. The cytotoxicity and genotoxicity tests were performed in triplicate for each experimental group and data were analyzed using the Bonferroni’s multiple comparison tests, the mean difference (mean.dif) and the confidence interval at 95% of mean.dif were calculated. $P$ values $< 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Extracts and total phenolic content. The color of the extracts obtained was between yellow to reddish. The ethyl acetate extracts were the ones with the lighter shade, while the darkest were the acetone extracts. Fractions PFAc and PFEtOH had the highest content of phenols (PFAc = 11.34 ± 0.88 mgGAE/g; PFEtOH = 6.52 ± 1.28 mgGAE/g; PFBuOH = 0.713 ± 0.23 mgGAE/g; PFEtAc = 0.15 ± 0.17 mgGAE/g). ANOVA shows that the treatments means aren’t all equal ($p$ value $= 7.18 x 10^{-11}$). In addition, pairwise comparison (Tukey’s HSD test) showed differences between all treatments ($p$ value $< 0.001$), except for PFEtAc - PFBuOH couple (mean.dif: -0.562; $p$ value: 0.679; IC95: -2.042 to 0.917).

GC-MS and HPLC-MS. GC-MS analysis identified two phenolic compounds in the acetone extracts, these are: ethyl-resorcinol and di-tert-butylphenol, with a relative abundance of 2.6% and 0.9% respectively. Other compounds present in ethanolic and acetone extracts were: pentadecene, esters of saturated and unsaturated fatty acids, fatty acid derivatives and sterol derivatives (Table I).

By HPLC-MS, the following compounds were tentatively identified: 3,4-dihydroxybenzoic or protocatechuic acid, dinitrosalicylic acid and ethylresorcinol (Table II). Protocatechuic acid (abbreviation PCA) was common in both fractions (Figure 1); this compound was found in the cuticle of arthropods, such as cockroaches (Blatella orientalis and Periplaneta americana)22-24 and beetles (Pachynoda ephippiata and Tenebrio molitor)25. PCA seems to play the important role in obscuring the cuticle and capturing free radicals. This compound is widely distributed in plant kingdom23, being extracted by their pharmacological properties as antioxidant agent26-27.

Cytotoxicity tests. Results of MTT reduction test using HaCat cells are presented in Figure 2. None of the treatments with PFAc showed significant decrease of cell viability at 24 h exposure. At 48 h, the cell viability decreased by 44.2 ± 6.2% in the treatment with 100 µg.mL$^{-1}$ (IC$_{90}$ = 91.85 ± 12.05 mg.mL$^{-1}$) (Figure 2A).

Moreover, HaCat cells treated with PFEtOH showed survival higher than 100%, at concentrations > 100 µg mL$^{-1}$ (Figure 2B). A cell-free assay showed that PFEtOH react with MTT reagent (data not shown). This behavior has been reported with
Table I. Tentative identification and relative abundance (%) of compounds in acetonic and ethanolic extracts from *Ulomoides dermestoides* beetles, determined by gas chromatography coupled to mass spectrometry (GC-MS).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Acetonic extracts</th>
<th>Ethanolic extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t_R$ min</td>
<td>Relative quantity, %</td>
</tr>
<tr>
<td>Ethyiresorcinol</td>
<td>35.86</td>
<td>2.6</td>
</tr>
<tr>
<td>Pentadecene</td>
<td>38.00</td>
<td>5.9</td>
</tr>
<tr>
<td>Di-tert-butylphenol</td>
<td>38.52</td>
<td>0.9</td>
</tr>
<tr>
<td>Ethyl Tetradecanoate</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Palmitate (Methyl/Ethyl)*</td>
<td>49.14</td>
<td>1.6</td>
</tr>
<tr>
<td>Linoleate (Methyl/Ethyl)*</td>
<td>52.55</td>
<td>1.2</td>
</tr>
<tr>
<td>Oleate (Methyl/Ethyl)*</td>
<td>52.67</td>
<td>0.8</td>
</tr>
<tr>
<td>Stereate (Methyl/Ethyl)*</td>
<td>53.14</td>
<td>3.6</td>
</tr>
<tr>
<td>Isomer of linoleic acid</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Ethyl Eicosanoate</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Heneicosanoate</td>
<td>60.40</td>
<td>1.0</td>
</tr>
<tr>
<td>Fatty acid derivates M+ 348</td>
<td>62.20</td>
<td>15.1</td>
</tr>
<tr>
<td>Mono stearine</td>
<td>65.83</td>
<td>58.1</td>
</tr>
<tr>
<td>Nitrogen compound M+337</td>
<td>67.90</td>
<td>3.6</td>
</tr>
<tr>
<td>Nitrogen compound M+351</td>
<td>71.78</td>
<td>3.9</td>
</tr>
<tr>
<td>Sterol derivates</td>
<td>81.69</td>
<td>4.0</td>
</tr>
</tbody>
</table>

$t_R$ = Retention time. All compounds were tentatively identified according to their pseudomolecular ion, therefore the chemical identity must be verified with standard compounds.

Table II. Relative chemical composition of phenolic fractions of ethanolic extracts (PFEtOH) and acetonic extracts (PFAc) of *Ulomoides dermestoides*, determined by HPLC-MS-ESI in negative ion mode.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Tentative compounds</th>
<th>$t_R$(min)</th>
<th>Molecular Weight (g.mol$^{-1}$)</th>
<th>[M–H]$^-$ m/z</th>
<th>Base peak m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFEtOH</td>
<td>Protocatechuic acid</td>
<td>5.463</td>
<td>154</td>
<td>153</td>
<td>109</td>
</tr>
<tr>
<td>PFEtOH</td>
<td>Dinitrosalicylic acid</td>
<td>4.843</td>
<td>228</td>
<td>227</td>
<td>123</td>
</tr>
<tr>
<td>PFAc</td>
<td>Protocatechuic acid</td>
<td>5.577</td>
<td>154</td>
<td>153</td>
<td>109</td>
</tr>
<tr>
<td>PFAc</td>
<td>Ethyiresorcinol</td>
<td>8.846</td>
<td>138</td>
<td>137</td>
<td>137</td>
</tr>
</tbody>
</table>

$t_R$ = Retention time. All compounds were tentatively identified according to their pseudomolecular ion, therefore the chemical identity must be verified with standard compounds.

certain plant extracts and with redox-active polyphenolic compounds, which may directly reduce tetrazolium salts, even without cells. Although the MTT assay is widely used to measure the cellular proliferation, cell viability and drug toxicity, several studies report compounds that increase MTT reduction with no enhance of cell viability, a fact that should be considered when natural extracts are evaluated, to avoid false results. For this, we propose that MTT reduction test is not a reliable method to assess the cytotoxicity of extracts from *U. dermestoides*.

Results with the trypan blue exclusion test show that viability of HaCaT cells is dependent on the concentration and time of exposure to each treatment (Figure 3). Cell viability, at 24 and
48 h was higher in treatments with PFETOH (Cl<sub>50</sub> of 757.39 ± 134.43 μg.mL<sup>-1</sup> and 627.65 ± 138.67 μg.mL<sup>-1</sup>, respectively). In treatments with PFAc, Cl<sub>50</sub> were 373.72 ± 90.37 μg.mL<sup>-1</sup> and 193.83 ± 20.00 μg.mL<sup>-1</sup>, respectively (Table III).

Genotoxicity tests. Results of DNA-comet alkaline test, showed statistically significant difference (p < 0.05) between the untreated and treated groups with different concentrations of PFAc, with intermediate genotoxic effect to 160 μg.mL<sup>-1</sup> (Mean.dif: -7.646; IC95: -11.65 to -3.641) (Figure 4). On the other hand, treatment with PFETOH resulted in a low genotoxic effect, at concentrations equal to or less than 400 μg.mL<sup>-1</sup>; statistically significant difference between the untreated and treated groups were observed at concentrations ≥ 800 μg.mL<sup>-1</sup> (Mean.dif: -13.52; IC95: -23.71 to -3.323). The positive control
of the trial (0.3 mM H\textsubscript{2}O\textsubscript{2}) produced cores with high damage (% DNA in the tail: 57.55 ± 0.34%); comparatively, the DNA damage in cells exposed to solvent EtOH (negative control) was very low (% DNA in the tail: 2.73 ± 1.02%).

We suggested that the higher cytotoxic and genotoxic activity of PFAc could be related to the higher content of phenolic compounds, as PCA. Several studies report the antioxidant and free radical capturing activity of PCA\textsuperscript{33-36}; however, this compound has also been reported as a pro-oxidant, depending on their concentration\textsuperscript{37}. It has even been shown that in high doses PCA could induce lipid peroxidation dependent on the Fe\textsuperscript{2+}/Fe\textsuperscript{3+} redox cycle and oxidative DNA damage dependent on the Cu\textsuperscript{1+}/Cu\textsuperscript{2+} redox cycle\textsuperscript{38,39}.

A study by Babich \textit{et al.}, 2002 describes oxidative stress inducing in culture of non-tumorigenic gingival epithelial cells (SG) and cultured malignant cells derived from salivary glands (HSG1), exposed to > 10 mM PCA. These authors suggest a mechanism of PCA-mediated activation of tyrosinase enzymes leading to production of an active metabolite (quinone) capable of oxidizing the glutathione (GSH), altering cellular defense systems against ROS\textsuperscript{40}. On the other hand, Galati \textit{et al.}, 2002, proposed that phenolic rings of certain polyphenols could be metabolized by peroxidases to form pro-oxidant phenolic radicals, reactive enough to co-oxidize the GSH and the NADH coenzyme, with the subsequent formation ROS\textsuperscript{41}.

It should also be considered that, under certain experimental conditions, some phenolic compounds could initiate autoxidation reactions, behaving as pro-oxidant molecules in culture media such as DMEM and RPMI\textsuperscript{42}. For example, it has been found that in the presence of DMEM, high doses of (-)-epigallocatechin-3-gallate, (+)-catechin and quercetin produce toxic levels of H\textsubscript{2}O\textsubscript{2} (frequently > 50 µM), semiquinone and quinones\textsuperscript{43-46}. In addition, high doses of phenolic acids were also associated with DNA damage induced by H\textsubscript{2}O\textsubscript{2}\textsuperscript{47}. It has been described that exposure during 6 hour, to doses of up to 50 mmol L\textsuperscript{-1} of

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**Tabla III. Results of the trypan blue exclusion test in HaCaT cells exposed to different concentrations of phenolic fractions of \textit{Ulomoides dermestoides} extracts.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Viability (%) at 24h (Mean ± SD)</th>
<th>Viability (%) at 48h (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>800 (µg.mL\textsuperscript{-1})</td>
<td>0 (µg.mL\textsuperscript{-1})</td>
</tr>
<tr>
<td></td>
<td>0.00 ± 0.00</td>
<td>95.35 ± 1.99</td>
</tr>
<tr>
<td>PFAc</td>
<td>5.23 ± 7.40</td>
<td>94.64 ± 3.19</td>
</tr>
<tr>
<td>PFEtOH</td>
<td>44.65 ± 9.02</td>
<td>95.96 ± 0.02</td>
</tr>
</tbody>
</table>

PFAc = Phenolic fraction of acetonic extract.
PFEtOH = Phenolic fractions of ethanolic extract.

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**Figure 4. DNA damaged in alkaline comet assay using HaCaT cells after 24h incubations with phenolic fractions of (A) acetonic extracts of \textit{Ulomoides dermestoides} and (B) ethanolic extracts of \textit{Ulomoides dermestoides}.**

![Figure 4](image-url)
purified flavonoids, could cause chromosomal translocation and clastogenicity in human cell lines\textsuperscript{48,49}.

Given that our experiments were performed in cells cultured in DMEM, it is possible that the decrease in cell viability is related to $\text{H}_2\text{O}_2$ production from auto-oxidation of phenolic compounds present in extracts of \textit{U. dermestoides} analyzed.

**Conclusions**

Ethanolic and acetonic extracts from whole body of \textit{Ulomoides dermestoides} are a source of redox-reactive phenolic compounds that could be of biomedical interest. In both extracts was tentatively identified the protocatechuic acid, but further analysis with the standard compound would be required to confirm this assignment. Additionally, experimental design developed in this study suggests that the beetle’s ethanol extracts shown low toxicity in HaCaT cell line, at the tested concentrations. We suggest that the greater cytotoxicity/genotoxicity of acetic extracts could be related to a higher concentration of redox-reactive compounds present in the sample and the time of exposure to these compounds.

**References**


23. Whitehead, D.L. Haemocytes play a commensal role in the synthesis


