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# ANTIOXIDATIVE EFFECTS OF *MAYTENUS DASYCLADA* MART. (CELASTRACEAE)

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## ABSTRACT

*Maytenus dasyclada* is a trees belonging to the Celastraceae family. The species of *Maytenus* genus are popularly used to treat gastrointestinal diseases and inflammatory processes as well. The aim of this study was to evaluate the antioxidant activity of hexanic, ethyl acetate and ethanolic extracts from leaves of species, verifying the ability in protect the DNA and remove reactive species , identify and quantify phenolic compounds. The ethyl acetate extracts of species showed the highest amount of total polyphenols (43.65 ± 0.80 mg gallic acid equivalents/g) and the highest content of flavonoids (3.19 ± 0.32 mg quercetin equivalents/g). Quercetin and kaempferol were identified in ethyl acetate and ethanolic extracts. Ethyl acetate and ethanolic extracts of species showed antioxidant activity in the Trolox equivalent antioxidant capacity, in the inhibition of the hydroxyl radical formation, of lipid peroxidation, protein oxidation, and in the protection of damage cellular. The antioxidant activity was related with the antigenotoxic potencial of the extracts. These achieved results showed that extracts of species demonstrated antioxidant activity related to the presence of polyphenols compounds.

**KEYWORDS:** ***Maytenus dasyclada*, Antioxidant, Phenolic compounds and Flavonoids.**

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# INTRODUCTION

*Maytenus* is a largest genus of the family Celastraceae, and there are about 200 recognized species of tropical distribution[1]. In Brazil we find its center of diversity and is represented by 39 species, grouped into two sections: *Maytenus* Mol and *Oxyphylla* Loes[2]. The species of this genus are widely used in traditional and herbal medicine as antiulcerogenics, anti-inflammatory and antitumor[3-7]. Literature cites the presence of triterpenes, such as maitenin, maitefolins A and B[8], friedelin, friedelan-3-ol, lupeol, betulin[6], and polyphenolic compounds, mostly the glycosides of quercetin and kaempferol[9], and catechins such as epicatechin, etilepigalocatechin and 4'-O-ent-methylgallocatechin[10]. Concerning to its pharmacological activity, several studies demonstrated the antioxidant activity of the species of the genus in different assays[11-13]. No chemical or biological work was done previously for the species *M. dasyclada*. Genotoxic alterations are defined as those in which a potentially toxic agent causes any damage to the genetic material of cells (DNA) as a single strand or double breaks, formation of base adducts, chromosomal damage, and others. Among the genotoxic agents can be included free radicals[14]. In biochemistry of free radicals, the oxygen and its radicals are the reagents most important. When addresses the term "reactive oxygen species" (ROS) its included free radicals containing oxygen, such as superoxide anion (•O2-), hydroxyl radical (OH•), peroxyl radical (ROO•) and non-radical species such as hydrogen peroxide ($H_2O_2$) and singlet oxygen ($^1O_2$). These are often generated as byproducts of biological reactions or by exogenous factors. Thus, these reactive oxygen species may be responsible for causing a large number of disorders to react with cellular lipids, proteins and nucleic acids. Both are involved in the aging process, but also in many biological complications, including chronic inflammation, respiratory, neurodegenerative diseases, diabetes mellitus, atherosclerosis, autoimmune diseases of the endocrine glands, mutagenesis and carcinogenesis[11]. Thus, the aim of this study was to quantify total polyphenols and flavonoids, in the hexanic (HE), ethyl acetate (EAE) and ethanolic (EE) extracts obtained from the leaves of *Maytenus dasyclada*, and to evaluate the antioxidant activity by the evaluation of the total reative antioxidant potencial (TRAP) and against nitric oxide and hydroxyl radicals, inhibition of lipid peroxidation (TBARS) and protein oxidation (carbonyl groups) assays, as well as the evaluation of the genotoxic potential through the comet assay. Taking into account phytochemical analysis, the flavonoids in the different extracts were identified by high performance liquid chromatography-diode array detector (HPLC/DAD).

# MATERIALS AND METHODS

All chemicals were purchased from Sigma (Milwaukee, WI, USA), Fluka Chemie (Buchs, Switzerland) and Merck (Darmstadt, Germany) and they were of analytical grade.

### *(i) Plant Collection and Extractions*

The leaves of *Maytenus dasyclada* were collected in October 2008, in the city of Erechim (Rio Grande do Sul, Brazil). A voucher specimen is deposited in the herbarium of the Universidade Regional Integrada do Alto Uruguai e das Missões, and cataloged under the registration number 9949. Plant material was dried in a stove at controlled temperature (38 °C), triturated and subjected to the extraction by soxhlet following the order: hexane, ethyl acetate and ethanol, in cycles of 12 hours consecutives for each solvent. The extracts were concentrated under reduced pressure.

### *(ii) Total Polyphenols Content*

The total phenolic content was determined using the Folin-Ciocalteau method[34]. Samples were prepared at a concentration of 2 mg/mL. The standard curve was plotted using gallic acid (10–50 µg/mL) which regression equation was y = 0.010 x + 0.001 ($R^2$ = 0.9980). The absorbance was measured at 750 nm and the results were

expressed as mg of gallic acid equivalents per gram of dry weight sample (GEA/g). The experiment was conducted in triplicate.

### *(iii)Total Flavonoid Content*

The total flavonoid content was determined according to the colorimetric method, using a 2% aluminum chloride solution. Samples were prepared at a concentration of 2 mg/mL. Absorbance was measured at 420 nm, in triplicate. A standard curve of quercetin was used for quantification, and the results were expressed as mg of quercetin equivalents per g of extract (mg/g QE)[35].

### *(iv) HPLC/DAD Analysis*

HPLC/DAD analysis was performed on a Waters Alliance 2690 Chomatograph Separations Module using a multiple UV wavelength photo-diode array detector (Model 996, Waters) and a Luna $C_{18}$ column (4.6 x 250 mm, 5 µm, Phenomenex, CA, USA) protected by a Bondapak $C_{18}$ guardcolumn (1 x 8 mm, 37-55 Xm, Waters, MA, USA). Empower Software 2002 (Waters) was used for equipment control, data aquisitoin, and processing chromatographic information. Elution was performed using a gradient system, and mobile phase consisted of a water (A) acidified with 0,1 % formic acid (v/v) and acetonitrile (B). The gradient profile was: 0-4 min 12% of B, 4-5 min from 12 to 14% of B, from 5-6 min 14% of B, 6-18 min from 14 to 17% of B, 18-25 min from 17 to 22% of B, from 25-36 min from 22 to 25% of B, 36-46 min from 25 to 50% of B, 46-50 min from 50 to 100% of B, 50-70 min from 100 to 0% of B. Flow rate of 0,7 mL/min, injection volume of 10 µL, and column temperature at 22 ± 2 ºC were used. Stock solutions of quercetin and kaempferol at 2 µg/mL were prepared, and samples of extracts were dissolved in acetonitrile at concentration of 2 mg/mL.

### *(v) Total reactive antioxidant potential (TRAP)*

This method is based on the quenching of luminol-enhanced chemiluminescence (CL) derived from the thermolysis of 2,20-azo-bis (2-amidinopropane)dihydrochloride (AAPH) as free radical source[41]. The stock solution was prepared with AAPH (10 mM) and luminol (8 nM) in a glycine buffer (0.1 M; pH 8.6). After the stabilization time (2 h), 20 µL of Trolox with tests solutions or system (glycine buffer) were placed in a 96 cell-plate. The wells were completed to 200 µL with the stock solution. The count time was 10 s, and CL emission was monitored for 3000 s. Trolox samples (200 nM) were prepared in triplicate using the stock solutions stored at 4 ºC. The antioxidant activity of the samples was compared with a freshly standard Trolox solution. The results were expressed as area under curve (AUC). The tests solutions were 100, 10, 1, 0.1 and 0.01 µg/mL of HE, EAE and EE *M. dasyclada* extracts.

### *(vi) Inhibition of the hydroxyl radical formation (•OH)*

The reaction was started by the addition of Fe(II) (6 mM) to solutions containing 5 mM 2-deoxyribose, 100 µM $H_2O_2$ (both final concentration) and 20 mM phosphate buffer (pH 7.2). The tests solutions were added together at this system (250 µL). Incubation was carried out for 15 min at room temperature (25 ºC) in the dark and were stopped by the addition of 0.5 mL 4% phosphoric acid (v/v) followed by 0.5 mL 1% TBA (w/v, in 50 mM NaOH). After boiling for 15 min, the solutions were cooled and the absorbance of them was measured at 532 nm. Inhibition of the hydroxyl radical formation generated was measured by comparing the absorbance values of the system (control)[24]. The tests solutions were 100, 10, 1 and 0.1 µg/mL of HE, EAE and EE *M. dasyclada* extracts.

### *(vii) Scavenging activity of the nitric oxide radical (NO•)*

Reaction mixture containing 10 mM sodium nitroprusside in phosphate-buffered saline (900 µL) were incubated at 25 ºC for 60 min with the tests solutions (100 µL) in the dark. To 0.4-mL aliquot of the incubated sample was removed and 0.2 mL of 1% sulfanilamide in 5% $H_3PO_4$ and 0.2 mL of 0.1% naphthylethylene diamine dihydrochloride was added. Incubation was carried out for 7 min at room temperature (25 ºC). The absorbance was measured up to 30 min at 540 nm. Inhibition of the nitric oxide

generated was measured by comparing the absorbance values of the control (phosphate-buffered saline)[42]. The tests solutions were 100, 10, 1 and 0.1 µg/mL of HE, EAE and EE *M. dasyclada*.

***(viii) Inhibition of lipid peroxidation (TBARS)***

Thiobarbituric acid reactive substances (TBARS) were determined in plasma by the method of Ohkawa et al [39]. Aliquots of plasma and saline or the tests solutions (150 µL each) were incubated by 6 hours at 37 ºC. After, in brief, samples were incubated at 100 °C for 60 min in acid medium containing 0.45% sodium dodecyl sulfate and 0.6% thiobarbituric acid. After centrifugation, the reaction product was determined at 532 nm using 1,1,3,3-tetramethoxypropane as standard and the results were expressed as nmol MDA/mg protein. The tests solutions were 100, 10, 1 and 0.1 µg/mL of HE, EAE and EE *M. dasyclada* extracts.

***(ix) Inhibition of protein oxidation (carbonyl groups)***

Content protein carbonyl was determined as described by Levine et al [40]. The carbonyl protein presence is indicative of oxidation. Aliquots of plasma and saline or the tests solutions (100 µL each) were incubated by 6 hours at 37 ºC. After, plasma samples were added 0.2 mL of trichloroacetic acid 10% and placed on ice for 5 minutes. After centrifugation (5 min, 8000 g), was added 1 mL of 2,4-dinitrophenylhydrazine (DNPH) in 2M HCl to 10mM and 1 mL samples of 2M HCl in white tubes and incubated for 90 min at 37 ° C. The proteins were dissolved in 6M guanidine and interference was removed after washing with ethanol-ethyl acetate 1:1 (v / v). The extent of the damage will be done by reading absorbance at 370 nm. The results were expressed as nmol cabonyl/mg protein. The tests solutions were 100, 10, 1 and 0.1 µg/mL of HE, EAE and EE *M. dasyclada* extracts.

***(x) Assay comet***

Aliquots of blood and saline or the tests solutions (100 µL each) were incubated by 6 hours at 37 ºC. To 20 µL of cell suspension was added to 100 µL of agarose of low melting point (LPM 0.75% - Low Melting Point) and spread on microscope glass slides pre-covered with 1% agarose. Agarose-coated slides were placed in an appropriate glass vessel protected from light at 4 ° C with cold lysis solution (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10.0 with 1% triton X-100 and 10% DMSO) for 1 hour. Finally, the electrophoresis was performed in alkaline conditions (pH 13) at 25V and a current of 300 mA for 15 minutes. After electrophoresis the samples were neutralized (0.4 M Tris, pH 7.5) and the slides placed in an oven to dry (70 ° C) where they remained for 20 minutes. After, were colored with silver solution (silver nitrate 0.1%; tungstosilicic acid 0.25%, 0.15% formaldehyde) and analyzed in an optical microscope at 100 X. It was analyzed for each sample, 100 randomly selected cells in triplicate [14]. The tests solutions were 100, 10, 1 and 0.1 µg/mL of HE, EAE and EE *M. dasyclada* extracts, which were compared to analysis without extracts addiction.

***(xi) Data Analysis***

Results are expressed as means ± S.D. and statistical significance was determined by One-Way Analysis of Variance (ANOVA, $p < 0.05$).

## RESULTS AND DISCUSSION

***(i) Total polyphenols and flavonoids content***

The contents of total polyphenols and flavonoids are shown in Table 1. The EAE extracts presented the highest content of total polyphenols (53.58 ± 0.43 mg/g gallic acid equivalents (GAE)), and total flavonoids (3.72 ± 0.27 mg/g quercetin equivalents (QE)). Few quantitities of total polyphenols and flavonoids was found in the HE extracts of the species. Hydromethanolic extracts obtained from leaves of *M. ilicifolia* demonstrate 21.96 a 45.92 mg /g (tannic acid equivalents) of total polyphenols, and 1.349 at 3.859 mg of total flavonoids[15].

**Table 1**
***Total polyphenols (TP) and total flavonoids (TF) in the HE, EAE and EE extracts from leaves of M. dasyclada.***

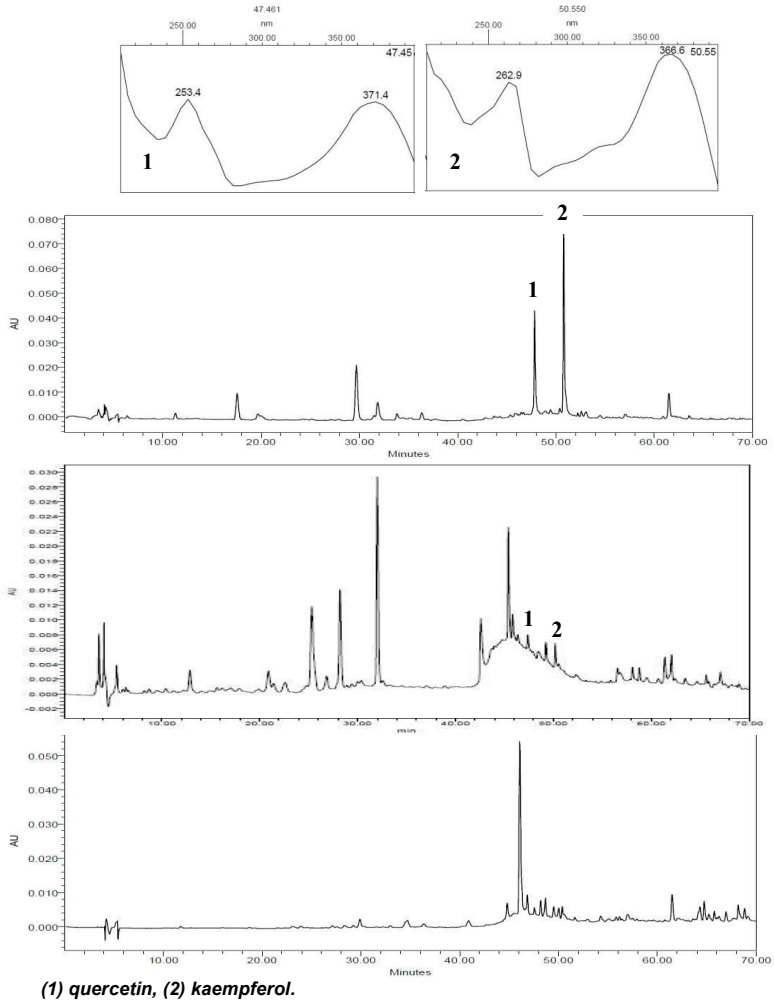
| Species | Extract | TP (mg GAE/g) | TF (mg QE/g) |
|---|---|---|---|
| *M. dasyclada* | HE | 1.54 ± 0.68[a] | 0.86 ± 0.13[a] |
| | EAE | 43.65 ± 0.80[b] | 3.19 ± 0.32[c,d] |
| | EE | 14.59 ± 0.66[c] | 2.36 ± 0.50[b] |

*Values are expressed as mean ± standard deviation. GAE: gallic acid equivalents, QE: quercetin equivalents,*
*[a–d] Means with the different letters in each column are significantly different (p < 0.05), by analysis of variance (One-way ANOVA) (n = 3).*

### *(ii) HPLC/DAD Analysis*

To verify the presence of flavonoids in the different extracts of both species, the samples were subjected to HPLC/DAD analysis. The flavonoids quercetin and kaempferol were identified by comparison of their retention's time and UV spectrum with those of the standards. Quercetin was found in the EAE and EE extracts of species; and kaempferol was found in all extracts of *M. dasyclada*. The chromatograms of the extracts are shown in Figure 1. Flavonoids like quercetin and kaempferol were identified in other species of *Maytenus* genus[9,16]. With exception to the peaks presents in HE of both species, the others unknow peaks show UV's absorbance characteristic of flavonoids and derivatives catechins.

**Figure 1**
***HPLC/DAD phenolics profile of HE (A), EAE (B), EE (C) of M. dasyclada extracts.***




*(1) quercetin, (2) kaempferol.*

***(iii) Total reactive antioxidant potential (TRAP)***

The mechanism of luminol chemiluminescence induced by thermolysis of ABAP [2,20-azobis (2-amidinopropane)] and directed for producing derivatives of luminol radicals generated by the reaction with peroxyl radicals is the most used method to estimate the ability of antioxidant substances *in vitro*. The ability of a substance to inhibit induced luminol chemiluminescence can be related to their ability to capture free radicals derived from luminal[17-18]. Much attention has been focussed on determination of the total antioxidant capacity of compounds using the Trolox equivalent antioxidant capacity (TEAC) test. The most advantage of this assay is determining the "total" antioxidant (or the free radical trapping) capacity of biological fluids, because it can determine the capacity of the system to withstand oxidative stress. Furthermore, decreased values can be associated with pathologies that are determined by (or produce) enhanced amounts (or steady-state concentrations) of free radicals[19-22]. The results of this test are shown in Graphic 1. Can be observed that HE, EAE and EE extracts of species significantly ($p < 0.01$) inhibited the chemiluminescence compared to Trolox, used as positive control, at all concentrations tested. It is further noted that the extracts with concentrations of 100 and 10 mg / mL, had inhibitory chemiluminescence activity significantly ($p < 0.05$) higher than extracts in concentrations of 1, 0.1 and 0.01 mg / mL. The antioxidant activity of the species *M. ilicifolia* was also demonstrated by evaluation of total reactive antioxidant potential (TRAP). Barks were extracted in ethanol and the results showed an $IC_{50}$ of 2.0 ± 0.07 versus 0.7 ± 0.04 by positive control (Trolox)[11]

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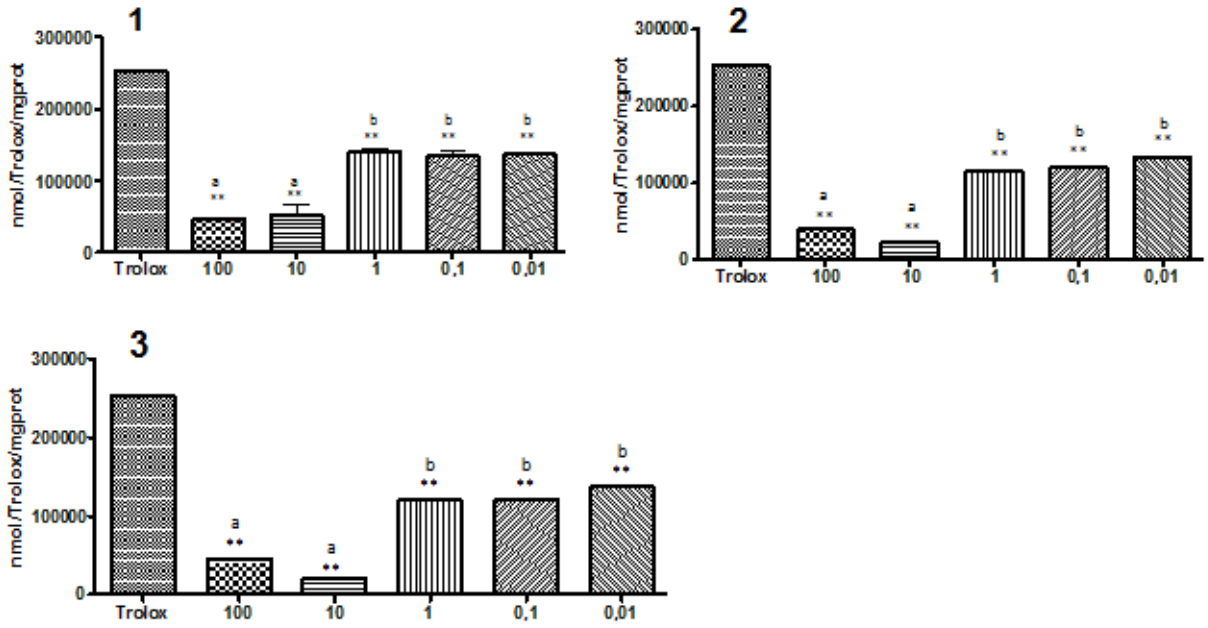
**Graphic 1**

***Total reactive antioxidant reactivity (TRAP, mL/Trolox/mg protein) of extracts (1 = HE, 2 = EAE, 3 = EE M. dasyclada extracts and Trolox.***




*Results are expressed as means ± S.D. Significant differences determined by ANOVA complemented with Tukey's test.*
*** $p < 0.01$ trolox vs. extracts, $^{a-b}$ Means with the different letters in each extract are significantly different ($p < 0.05$).*

***(iv) Inhibition of the hydroxyl radical formation (•OH)***

The Table 2 shows the values of absorbance at 532 nm observed for the control and the different extracts of *Maytenus* tested. All extracts at all concentrations tested demonstrated significant ($p < 0.01$) effect in preventing the degradation of deoxyribose

induced by hydroxyl radicals generated by the Fenton reaction, when compared to control. The percentages of inhibition ranged between 22% (EE in minor concentration) and 54% (HE in concentration of 0.1 µg/mL). It was also observed that significant differences between the tested concentrations of these extracts were visualized only between the lower (0.1 µg / mL) and the highest concentrations (100 and 10 µg / mL) of EE extracts, demonstrating that the different concentrations of the extracts did not caused dose-response effect.

**Table 2**
***Comparison of the efficiencies of extracts and control in preventing 2-deoxyribose degradation.***

| | Extract | Concentration (µg/mL) | Absorbance (532 nm) ± SD | Inhibition degradation of deoxyribose (%) |
|---|---|---|---|---|
| Control | | | 0.252 ± 0.02 | |
| *M. dasyclada* | HE | 100 | 0.145 ± 0.01** | 42.46 |
| | | 10 | 0.118 ± 0.01** | 53.18 |
| | | 1 | 0.115 ± 0.008** | 54.37 |
| | | 0.1 | 0.1373 ± 0.01** | 45.52 |
| | EAE | 100 | 0.133 ± 0.02** | 47.22 |
| | | 10 | 0.147 ± 0.002** | 41.67 |
| | | 1 | 0.170 ± 0.02** | 32.57 |
| | | 0.1 | 0.153 ± 0.02** | 39.29 |
| | EE | 100 | 0.117 ± 0.02**[a] | 53.57 |
| | | 10 | 0.127 ± 0.01**[b] | 49.61 |
| | | 1 | 0.159 ± 0.008** | 36.90 |
| | | 0.1 | 0.196 ± 0.02**[a,b] | 22.22 |

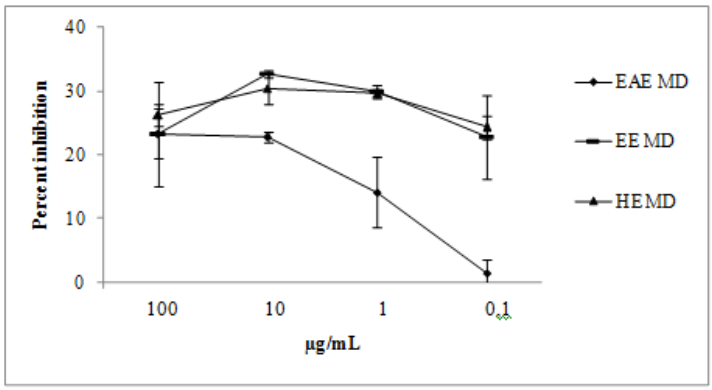
***Values are expressed as mean ± standard deviation. Statistical comparison was performed using one-way ANOVA followed by Tukey's test. ** p <0.01 when compared to the control group,[a–c] Means with the same letters are significantly different (p < 0.01).***

The hydroxyl radicals are the major reactive oxygen species causing lipid oxidation and enormous biological damage. The mixture of ferric chloride incubated with $H_2O_2$ generates a degradation of deoxyribose to form a pink chromogen which can be quantitated by UV absorbance. The tested extracts of *Maytenus* species were effective in capturing the hydroxyl radicals formed which generated the high degradation of deoxyribose. The mechanism of antioxidant action of polyphenols has usually been attributed to •OH scavenging activity[23]. However, several works indicate that polyphenols does not block 2-deoxyribose degradation by simply trapping •OH radicals. It seems that these substances act as an antioxidant by complexing iron, forming complexes with Fe (II) that cannot participate in Fenton reactions[24]. Grinberg et al[25] also concluded that the protective activity of tea polyphenols against •OH dependent salicylate hydroxylation was due to iron chelation.

### *(v) Scavenging activity of the nitric oxide radical (NO•)*

It is well known that nitric oxide has an important role in several types of inflammatory processes. Studies have shown that reactive nitrogen intermediates, such as nitric oxide (NO•), peroxynitrite (ONOO-) and nitrogen dioxide (NO2•), also play an important part in the inflammatory process and possibly in carcinogenesis[26]. The results of this study indicate that different extracts of *M. dasyclada* exerted no significant ($p < 0.05$) inhibitory effect on the production of nitric oxide radical (Graphic 2) when compared with the control (phosphate-buffered saline). These results demonstrate that the extracts of the species has no activity on the mechanism control of antioxidant against the radicals formed by the nitric oxide system. The evaluation of responsiveness to nitric oxide radicals has been demonstrated for the species *M. aquifolium*. The ethanolic extract of the root bark showed an effective antioxidant demonstrated by the inhibitory effect on the oxidation of Griess reagent by nitric oxide (Inhibition = 18.3 ± 0.4%)[27].

**Graphic 2**
***Inhibition of nitric oxide radicals by extracts***

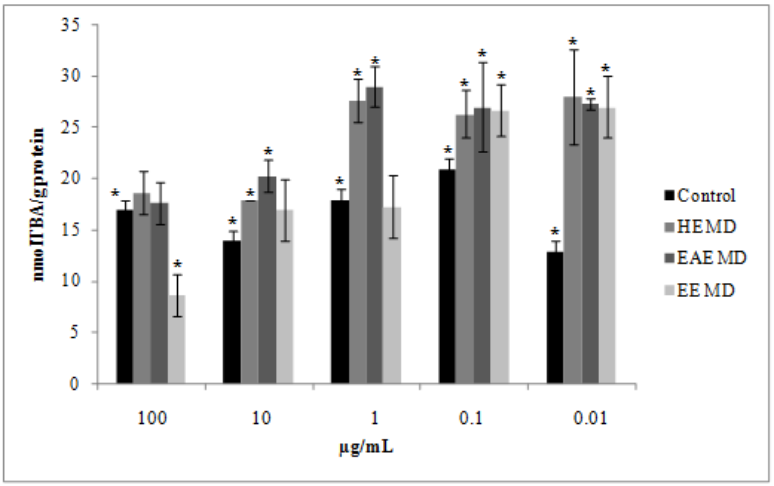



*EAE MD = ethyl acetate extract; EE MD = ethanolic extract; HE MD = hexanic extract. Data are reported as the means ± SD for three measurements. p < 0.05 compared to control (% inhibition by control = 0%; ANOVA followed by the Dunnett's test).*

### *(vi) Inhibition of lipid peroxidation (TBARS)*

The antioxidant potential of the extracts was evaluated through the TBARS assay based on the formation of malondialdehyde (MDA), a subproduct of lipid peroxidation. The lipid peroxidation was stimulated with $FeSO_4$ addition to serum. Only the EE extract at concentration of 100 µg/mL were able to signicantly ($p < 0.05$) decrease of MDA formation (Graphic 3).

**Graphic 3**
***Effect of Maytenus extracts on TBARS production in serum.***




*HE MD = hexanic extract; EAE MD = ethyl acetate extract; EE MD = ethanolic extract. Data are reported as the means ± SD for three measurements. p < 0.05 compared to control (ANOVA followed by the Dunnett's test).*

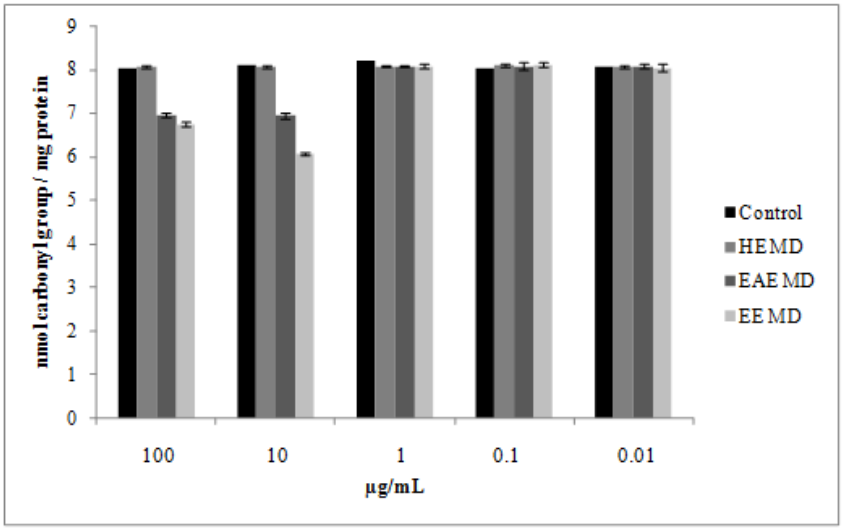
In study of hepatoprotective activity of some plants extract against paracetamol induced hepatotoxicity in rats, the levels of TBARS as an index of lipid peroxidation in liver tissue of paracetamol treated rats were significantly ($p < 0.05$) elevated (by 51.86%) when compared to control animals. Lipid peroxidation level was restored 100% towards their normal value by treatment with *P. niruri* and *M. emarginata* extract on induced toxicity by paracetamol. *Phyllanthus niruri* and *Maytenus emarginata* extract proved to be the best among all the other extracts in protecting liver from peroxidative injury[28].

***(vii) Inhibition of protein oxidation (carbonyl groups)***

Protein carbonyl content is the most commonly used marker of protein oxidation, and its accumulation has been observed in several human diseases, including Alzheimer's disease, diabetes, arthritis and others. ROS-mediated oxidation of proteins leads to the conversion of lysine, arginine, and proline residues. Of particular significance is the fact that oxidation of some amino acid residues leads to the formation of carbonyl derivatives. In addition, carbonyl derivatives of proteins are produced as a consequence of oxidative cleavage of the peptide backbone via α-amidation pathway, or cleavage associated with the oxidation of glutamyl residues. Carbonyl derivatives can also be formed as a consequence of secondary reactions of some amino acid side chains with lipid oxidation products, such as 4-hydroxy-2-nonenal (HNE), or with reducing sugars or their oxidation products[29-31]. The EAE extracts was able to reduce protein carbonyl content in serum compared to the induced group ($H_2O_2$), at the concentration of 100, 10 and 1 µg / mL. The EE extract show effectiveness at concentration of 100 and 10 µg / mL (Graphic 4).

**Graphic 4**

***Effect of Maytenus extracts on protein carbonyl content in serum samples.***




*HE MD = hexanic extract; EAE MD = ethyl acetate extract; EE MD = ethanolic extract. Data are reported as the means ± SD for three measurements. p < 0.05 compared to control (ANOVA followed by the Dunnett's test).*

Molecular studies have revealed that phenolics can exert modulatory actions in cell by interacting with a wide spectrum of molecular targets central to the cell signaling machinery. These include activation of mitogen-activated protein kinase (MAPK), protein kinase C (PKC), serine/threonine protein kinase Akt/PKB, phase II antioxidant detoxifying enzymes, downregulation of proinflammatory enzymes (COX-2 and iNOS) through the activation of peroxisome proliferator-activated receptor gamma (PPARg), regulation of calcium homeostasis, inhibition of phosphoinositide 3-kinase (PI 3-kinase), tyrosine kinases, NF-kB, c-JUN, as well as modulation of several cell survival/cell-cycle genes. The redox status of the cell has a profound effect on the cell signaling pathways, in particular the MAP kinase cascade[32]. Among the antioxidant tests performed in this work we can mention the significant activity of EAE and EE extracts in total reactive antioxidant potential (TRAP) and inhibition of the hydroxyl radical formation (•OH). This may be due to the chemical similarities found in the chromatographic analysis of extracts EAE and EE, and the

predominant presence of quercetin and kaempferol.

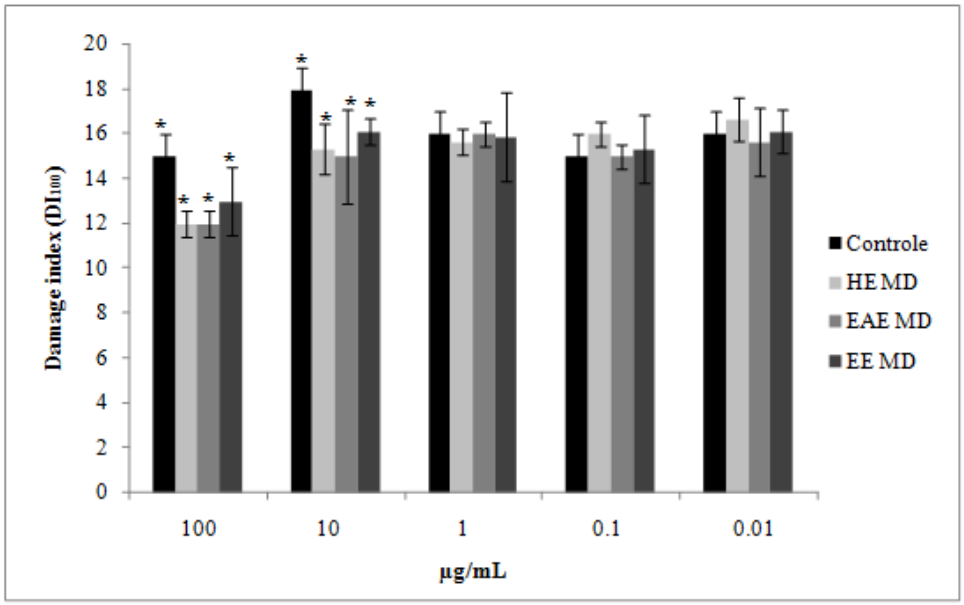
### *(viii) Comet assay*

The comet assay is a test of genotoxicity, therefore, detects the fragmentation of DNA nuclear. A small number of irradiated cells suspended in a thin agarose gel on a microscope slide were lysed, electrophoresed, and stained with a fluorescent DNA binding dye. The electric current pulled the charged DNA from the nucleus such that relaxed and broken DNA fragments migrated further. The resulting images, which were subsequently named for their appearance as 'comets', were measured to determine the extent of DNA damage[14]. The effects of *Maytenus dasyclada* extracts on comet assay are demonstrated in Graphic 5. The HE, EAE and EE extracts in concentrations of 100 and 10 µg/mL significantly ($p < 0.05$) decrease the damage index ($DI_{100}$) when compared to control. It was observed that, in the major concentrations tested, the extracts were more effective in the protection of cells damage. The same extracts were shown to be more effective antioxidants in the assays performed. Thus, can be observed clearly a relationship between antioxidant effects of the extracts and the effects observed in the comet assay.

The DNA damage can be induced by chemical agents from the environment or resulting from chemical reactions that occur in the cells themselves, may affect processes duplication and gene transcription, and yet, epidemiological studies show that nutritional factors as ingredients in the human diet as causing DNA damage[33]. Some flavonoids appear to modulate the repair of DNA alkylated damage. A consistent effect is shown by the flavone apigenin that induces endonucleases able to excise this kind of damage, which may then lead to an apparently faster repair; this is indicated by lower strand breakages at all investigated time points (12, 24, 48 h)[38]. The flavonol quercetin induces nonspecific endonucleases, resulting in a higher strand breakage at a short time; this is consistent with a clastogenic effect of this flavonoid. The effects of the flavanone sakuranetin and the flavan-3-ol epicatechin on DNA repair appear more difficult to explain[38].

**Graphic 5**

***Effect of Maytenus extracts on comet assay expressed in damage index ($DI_{100}$).***




*HE MD = hexanic extract; EAE MD = ethyl acetate extract; EE MD = ethanolic extract. Data are reported as the means ± SD for three measurements. $p < 0.05$ compared to control (ANOVA followed by the Dunnett's test).*

In study with other *Maytenus* species was observed that after 48 hrs of treatment, *M. robusta* hydroalcoholic extract had weak genotoxic effects but no clastogenic effects in mice cells. Three doses (50, 250 and 500 mg/kg body weight) were administered to mice orally 2 times at 24-h intervals. Cytotoxicity was assessed by scoring 200 consecutive total polychromatic and normochromatic erythrocytes to calculate their ratio. Parametric (analysis of variance/Tukey) and non-parametric (Kruskal-Wallis/Dunn post hoc) tests were used to evaluate the results according to the nature of the data distribution. The results showed a significant increase in the frequency of DNA damage on leukocytes at the 2 higher doses tested, but the extract did not enhance micronucleus frequency in bone marrow cells[36]. In other study, *M. rigida* alcoholic and aqueous extract showed no chromosomal abnormalities of any kind in the concentrations tested on cell division in the onion root stem[37].

## CONCLUSION

This study showed the antioxidant activity of the leaves of *M. dasyclada* and contributed to reveal some phytochemical characteristics of this species. The antioxidant activity could be related with the antigenotoxic potential of the extracts. Quercetin and kaempferol were identified in the species by HPLC/DAD and may be involved in the activities shown. Taken together, our results indicate that these plants have antioxidant potential and can be a promising source of natural antioxidants. The literature reports antioxidant activities with the content of polyphenols[43] in different plants.

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