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*Full Length Research Article*

# In vitro cytotoxicity of lignans isolated from *Ficus citrifolia* P.Miller (Moraceae) on C6 glioma cell line

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**Matairesinol and arctigenin, two dibenzylbutyrolactonelignans, were isolated by chromatographic analysis of the $CH_2Cl_2$ extract from the leaves of *Ficus citrifolia.* Structural identification was performed using typical spectroscopic methods, NMR $^1H$ and NMR $^{13}C$, in addition to DEPT, NOE-diff, HETCOR, COSY $^1H$ x $^1H$ and HMBC, confirmed by literature comparison. Furthermore, to date, no reports have described the phytochemical investigation of *Ficus citrifolia*. Previous studies have suggested that dibenzylbutyrolactonelignans exert a preventive effect against different type of cancers such as breast, prostate and colon. In this study, we analyzed the cytotoxic effects of matairesinol and arctigenin and the induction of apoptosis of C6 rat glioma cells. These compounds produced a dose-dependent reduction in the viability of C6 cells, as determined by an MTT assay and were not cytotoxic against astrocyte cell cultures from rat cerebral tissue. For ultrastructural analysis, C6 cells were incubated with 50 µM and 250 µM of arctigenin and matairesinol. Treated cells presented altered morphology, condensation of nuclear chromatin into irregular clumps, swollen mitochondria, and disorganized internal-membrane cristae. However, the nuclear and plasma membranes remained intact. These morphological changes are characteristic of apoptotic cellular death. In addition, these treatments did not significantly affect astrocyte viability. Thus, these results suggest that both dibenzylbutyrolactonelignans have significant anti-proliferative and apoptotic effects on glioma cells and may contribute as selective agents against brain cancer.**

**Keywords:** *Ficus citrifolia*; Moraceae; dibenzylbutyrolactonelignan; matairesinol; arctigenin; cytotoxicity, glioma cells (C6).

## INTRODUCTION

Ethnopharmacological studies have identified many natural products with significant biological activity that could benefit novel drug discovery and development. It is reasonable to consider that a survey of ethnomedical uses of a plant may provide useful clues for drug discovery.

Phytochemical studies are the basis for drug development and are sometimes associated with use for a particular illness, in this case glioblastoma, and could have an important socioeconomic value (Paavilainen, 2005). The genus *Ficus,* better known as the fig, has been cultivated for over 11,000 years, possibly predating cereal grains (Kislev et al., 2006).

*Ficus* consists of approximately 800 species and is one of approximately 40 genera of the mulberry family, Moraceae (Carauta, 1989; Woodland, 1997). The species of the genus are distributed in the tropical and subtropical regions in the world, and 64 are referred to Brazil (Van den Berg, 1982). Plants of this genus are used by the population of the Amazon region to combat numerous maladies, and the leaves and latex are the most commonly used parts (Van den Berg, 1982). This work describes the first phytochemical study of the species *Ficus citrifolia* (Moraceae). Three previously known dibenzylbutyrolactonelignans were isolated from the dichloromethane extract of leaves: matairesinol (1), arctigenin (2) and arctiin (3).

These substances have been isolated and purified using conventional chromatographic techniques, and structural identification was performed using spectroscopic methods, including NMR $^{1}H$ and NMR $^{13}C$, in addition to DEPT, NOE-diff, HETCOR, COSY $^{1}H$ x $^{1}H$ and HMBC. The structures were also confirmed by comparison with literature data. Previous studies suggest that dibenzylbutyrolactonelignans have a preventive effect against hormone-dependent cancers such as breast, prostate and colon (Adlercreutz, 2002) and are potent cytostatic agents against human leukemia cells HL-60 (Hirano et al., 1994); exhibit antiviral activity, being potent inhibitors of HIV type-1 Integrase (Eich et al., 1996; Yang et al.,1996*)*; act as phytoestrogens (Kato et al., 1998; Meagher et al, 1999) and exhibit immunoregulatory (Cho et al., 1999); neuroprotective (Jang et al., 2001; Jang et al., 2002), antioxidative (Willför et al., 2003; Jin et al., 2005), anti-inflammatory (Cho et al., 2002; Jin et al., 2005), anti-asthmatic (Lee et al., 2011) and hypolipidemic properties (Charlton, 1998; Ward, 1999). Therefore, in the present study, we have investigated the effect of matairesinol (1) and arctigenin (2) on the viability of C6 rat glioma cells, primary tumors of the brain that are difficult to treat due to common recurrence (Jovčevska et al., 2013). These data were compared with astrocytes from the cortex of rats used as a control.

## MATERIALS AND METHODS

### Reagents

MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide), penicillin, streptomycin, trypsin and EDTA were obtained from Sigma Chemicals (St. Louis, MO), $CH_2Cl_2$,MeOH, Silica gel, HCl, isopropanol purchased from MERCK, glutaraldehyde type II, paraformaldehyde, sucrose, sodium cacodylate, osmium tetroxide, uranyl acetate, acetone and Epon resin were purchased from Electron Microscopy Sciences (EMS). DMEM F-12 and fetal bovine serum were obtained from Gibco.

### General experimental procedures

NMR spectra were recorded in $CDCl_3$ on a Varian Mercury spectrometer 300 (300 MHz for $^{1}H$ and 75 MHz for $^{13}C$ NMR) using tetramethylsilane (TMS) as internal standard. Splitting patterns are described as singlets (*s*), doublets (*d*), double doublets (*dd*), and multiplets (*m*). Proton and carbon shifts are reported in ð units (ppm) relative to TMS. Column chromatography was performed with Silica gel 60 H (70-230 mesh/ Merck), TLC was performed with Silica gel $GF_{254}$ (Merck) and preparative TLC was performed with Silica gel PP-254. Cytotoxic activity was determined by spectrophotometry (SP-2000 uv-Spectrum). Cells were analyzed using transmission electron microscopy (TEM - Zeiss LEO 906E).

### Plant material

Leaves of *Ficus citrifolia* (Moraceae) were collected in the forest of EMBRAPA, Pará State, Brazil and identified by the Botanic Department of EMBRAPA. A voucher specimen (n° 164987) has been deposited in the Herbarium of the CPATU-EMBRAPA, Pará State, Brazil.

### Extraction and isolation

Dried leaves (3.1 kg) of *Ficus citrifolia* were powdered and successively extracted with *n*-$C_6H_{14}$, $CH_2Cl_2$ and MeOH for 72 h at room temperature. Solvent was evaporated in a vacuum to give crude extracts. The $CH_2Cl_2$ extract (20.0 g) was subjected to column chromatography over silica gel 60 H (70-230 mesh/Merck) using n-$C_6H_{14}$, EtOAc and MeOH as eluents, containing gradually increased amounts. This yielded 56 fractions that were recombined based on thin layer chromatographic analysis. Fractions 58-63 were purified by preparative TLC [Silica gel PP-254, n-$C_6H_{14}$-EtOAc-MeOH (7:2.5:0.5)] in two consecutive elutions. The chromatography techniques applied resulted in the isolation 49.2 mg of the dibenzylbutyrolactonelignan,

matairesinol (1). Fractions 28-29 (3 g) were rechromatographed in a silica gel 60 H (70-230 mesh/Merck) column using n-$C_6H_{14}$, EtOAc and MeOH. After analysis of all 120 fractions, fractions 48-53 (306.7 mg) were rechromatographed using $CH_2Cl_2$ and MeOH as eluents; eluting with gradually increased amounts of solvent resulted in the isolation of 52.3 mg of the dibenzylbutyrolactonelignan, arctigenin (2). Fractions 39-43 were rechromatographed using a silica gel 60 H (70-230 mesh/Merck) column. The column was eluted with n-$C_6H_{14}$, $CH_2Cl_2$ and MeOH, resulting in the isolation of 18.2 mg of the dibenzylbutyrolactonelignan, arctiin (3).

**Cell cultures and drug treatment**

The rat C6 glioma cell line was originally derived from *N*-nitrosomethylurea-induced rat brain tumors (Jacobs et al., 2011; Chesler et al., 2012). C6 cells were seeded in 24-well culture plates at 5 x $10^4$ cells per well and maintained in Dulbecco's modified Eagle medium (DMEM F-12) supplemented with 10 % fetal bovine serum (FBS) and antibiotics (100 units/mL of penicillin and 100 g/ml of streptomycin). Incubation was carried out at 37 ºC in a humidified atmosphere of 5 % $CO_2$ and 95 % air. Astrocytes were isolated from the cerebral cortex of 3-day old rats (Zhang et al., 2013). Briefly, the brain was dissected and meninges removed. The cerebral cortex was separated from other structures and transferred to a solution of trypsin with 0.05 % EDTA for 5 minutes at room temperature. DMEM-F12 was added and supplemented with 10 % FBS (2.5 mL/L penicillin and 2.5 mL/L of streptomycin) to inactivate trypsin. After that, astrocytes were removed from the bottle, and the cells were plated at a density of approximately 5 x $10^4$ cells per well. Experimental protocols and animal care methods in the experiments were approved by the Experimental Animal Committeeat University Federal of Pará (approved number: CEPAC 111-13).

**Cell viability assay**

Cell viability was determined by MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide] using a colorimetric assay (Mosmann, 1983). The cells were seeded on a 24-well plate at a density of 5 x $10^4$ cells/well. DMEM-F12 medium (500 µL) was added to each well, and the plate was incubated at 37 ºC in 5 % $CO_2$. After 24 h of incubation, the medium was discarded and replaced with medium containing different concentrations (50 µM, 100 µM, 250 µM) of matairesinol and arctigenin dissolved in 0.1 % DMSO. Control cells were treated only with 0.1 % DMSO and incubated at 37 ºC in a humidified atmosphere of 5 % $CO_2$ for 72 h. After that, the cells were washed with phosphate buffered saline (PBS) and incubated with 50 µL MTT (0.5 mg/mL) and 500 µL PBS for 4 h. The formazan crystals were solubilized with 50 µL of 0.04N HCl in isopropanol, and the plates were measured using a spectrophotometer at 570 nm. Each experiment was repeated three times in quadruplicate samples.

**Ultrastructural analysis of C6 glioma cells by TEM**

Ultrastructural analysis of C6 cells was performed as previously described (Rodrigues et al., 2011). Briefly, C6 cells were incubated with 50 µM and 250 µM of arctigenin and matairesinol for 72 h at 37 ºC. After that, the controls and treated cultures were washed in PBS and fixed with 2.5 % glutaraldehyde and 4 % freshly prepared formaldehyde in a buffer solution containing 60 mM Pipes, 20 mM Hepes, 10 mM ethyleneglycol-bis-(B-aminoethylether)-N,N,N'-tetraacetic acid, 70 mM KCl and 5 mM $MgCl_2$, pH 6.9, for 1 h at room temperature. Subsequently, cells were washed in the same buffer and post-fixed in a solution containing 1 % osmium tetroxide, 0.8 % ferrocyanide, and 5 mM calcium chloride for 1 h. Cells were washed, dehydrated in graded acetone and embedded in Epon ®. Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss LEO 906E TEM.

**Statistical analysis**

Data were presented as the mean ± standard deviation (SD), and One-way ANOVA followed by the Bonferroni post-hoc test compared the differences between experimental groups.P-values <0.05 were considered statistically significant. All statistical analyses were performed using the application BioEstat 4.0.

## RESULTS

**Structure elucidation**

The structures of compounds 1, 2 and 3 (Figure 1) were elucidated by $^1$H- $^{13}$C NMR, HETCOR, $^1$H-$^1$H COSY, DEPT, NOE-diff and HMBC, and their spectral data were compared with published data (Rahman et al., 1990). Here, we phytochemically investigated the dichloromethane extract of leaves from *Ficus citrifolia* by chemical characterization of three lignans present: matairesinol, arctigenin, and arctiin. These three compounds are dibenzylbutyrolactone-type lignans that were isolated for the first time in the genus *Ficus*. The chemical profile of *Ficus citrifolia* shares similarity with members belonging to the Moraceae family, indicating that this study has contributed to a better understanding of the genus beyond the knowledge of the chemical composition of these species.

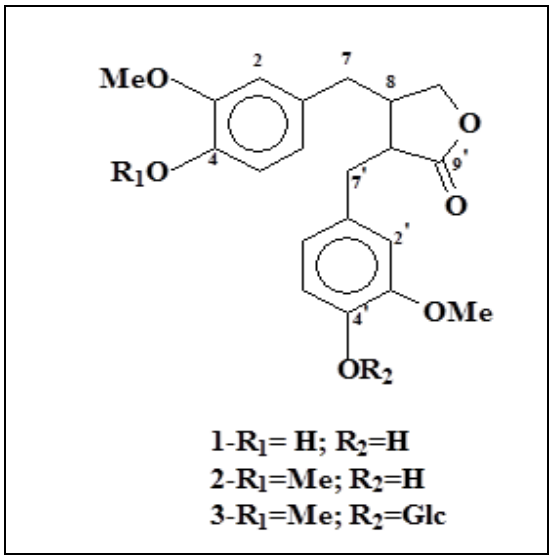


**Figure 1.** Structure of lignans matairesinol (1), arctigenin (2) and arctiin (3) isolated from *Ficus citrifolia*.

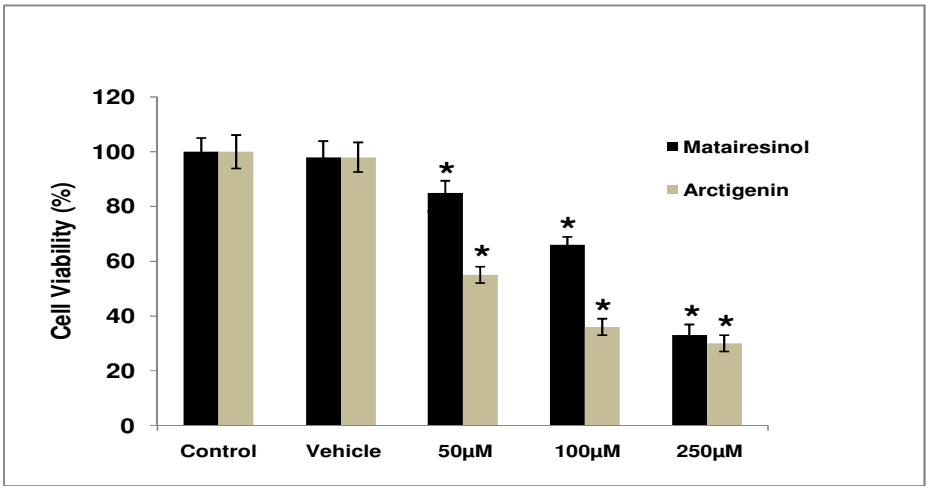


**Figure 2.** Cell viability of C6 cells treated with matairesinol and arctigenin at different concentrations. Cytotoxic effects were evaluated using an MTT assay following 72 h treatments with 50 µM, 100 µM and 250 µM lignan. * p <0.05 compared to control (ANOVA, followed by the Bonferroni post-test).

## Cytotoxicity of matairesinol and arctigenin to C6 glioma cells and rat cortical astrocytes

Viability of rat C6 glioma cells was assessed using an MTT assay after incubation with matairesinol and arctigenin at different concentrations (50 µM, 100 µM, and 250 µM) for 72 h as shown in Fig 2.The lignans matairesinol and arctigenin reduced glioma cell viability in a dose-dependent manner ($IC_{50}$ = 125 µM for matairesinol and $IC_{50}$ = 50 µM for arctigenin).

Under the same conditions, we studied whether the effect of drug exposure is time-dependent at a drug concentration of 250 µM during treatment intervals of 18 h, 36 h, 54 h and 72 h as shown in Fig 3. Cells treated with matairesinol showed a 24 % reduction in cell viability after 18 h, a 41 % reduction in viability after 36 h, a 69 ± 3.6 % reduction in viability at 54 h, and an 81 ± 1.8 % reduction in viability after 72 h. Cells treated with arctigenin showed a 23 % ± 6.0 reduction in viability after 18 h, a 60 ± 6.0 % reduction in viability after 36 h, a 79 ± 3.0 % reduction in viability at 54 h, and an 85 ± 1.0 % reduction in viability after 72 h.

Astrocyte cell cultures from rat cerebral tissue were used as positive controls to evaluate the cytotoxicity of matairesinol and arctigenin at the same concentrations used in previous experiments. It was possible to compare the effects produced by matairesinol (Figure 2) and arctigenin (Figure 3) in C6 rat glioma cells and

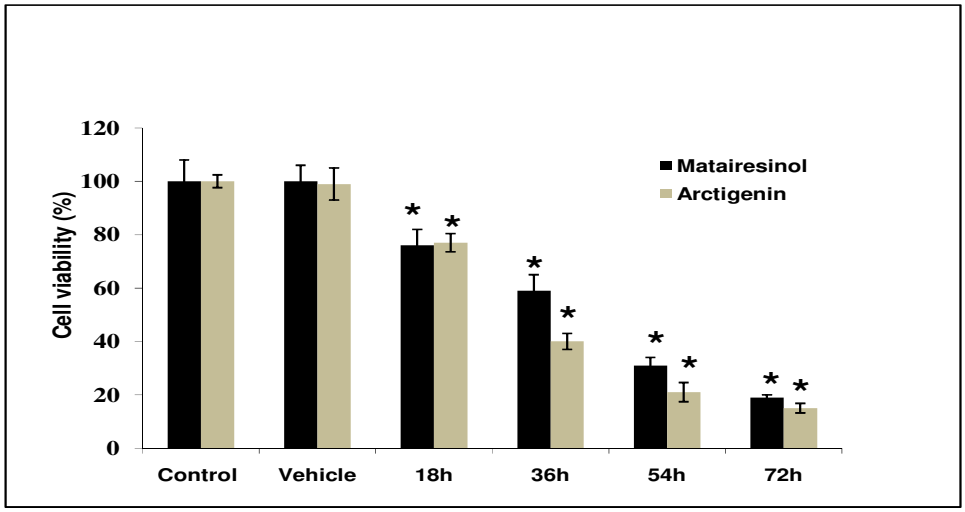


**Figure 3.** Cell viability of C6 cells treated with matairesinol and arctigenin at different time intervals. Cytotoxic effects were evaluated using an MTT assay following 72 h treatment with 250 µM lignan. * p <0.05 compared to control (ANOVA, followed by the Bonferroni post-test).

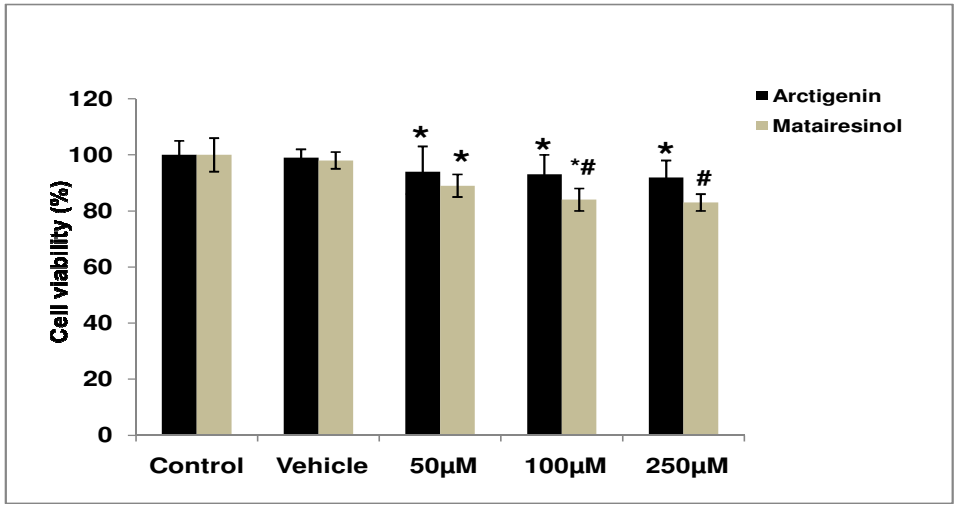


**Figure 4.** Cell viability of rat astrocytes treated with matairesinol and arctigenin at different concentrations. Cytotoxic effects were evaluated using an MTT assay following 72 h treatment with50, 100 and 250 µM lignan. * p <0.05 compared to control and # p <0.05 compared to astrocytes treated in the same concentration (ANOVA, followed by the Bonferroni post-test).

astrocytes from rats (Figure 4). Astrocyte cells used as controls revealed resistance to matairesinol and arctigenin treatment.

## Ultrastructural analysis of C6 glioma cells treated with matairesinol and arctigenin

Ultrastructural analysis showed significant morphological changes in drug-treated cells when compared to untreated cells. Untreated cells are shown in Figure 5A and exhibited well-preserved ultrastructural morphology and elongated shapes. In this case, glioma cells exposed to UV were used as positive controls (Figure 5B) and exhibited rounded shapes. Cells treated with 50 µM and 250 µM of arctigenin (Figures 5C and D, respectively) exhibited rounded shapes; the nucleus showed irregular chromatin condensation, and swollen and disorganized mitochondrial internal membranes were frequently observed (Figures C-D – inset, arrowhead).Cells treated with 50 µM of matairesinol also exhibited rounded shapes similar to what was observed in UV-exposed cells (Figure 5E), and intense disorganized mitochondrial internal membranes were also seen (Figure 5E – inset, arrowhead). However, when cells were treated with 250 µM of matairesinol, few cells maintained their elongated shape (Figure 5F) but with several autophagic vacuoles (Figure 5F – inset, arrow).

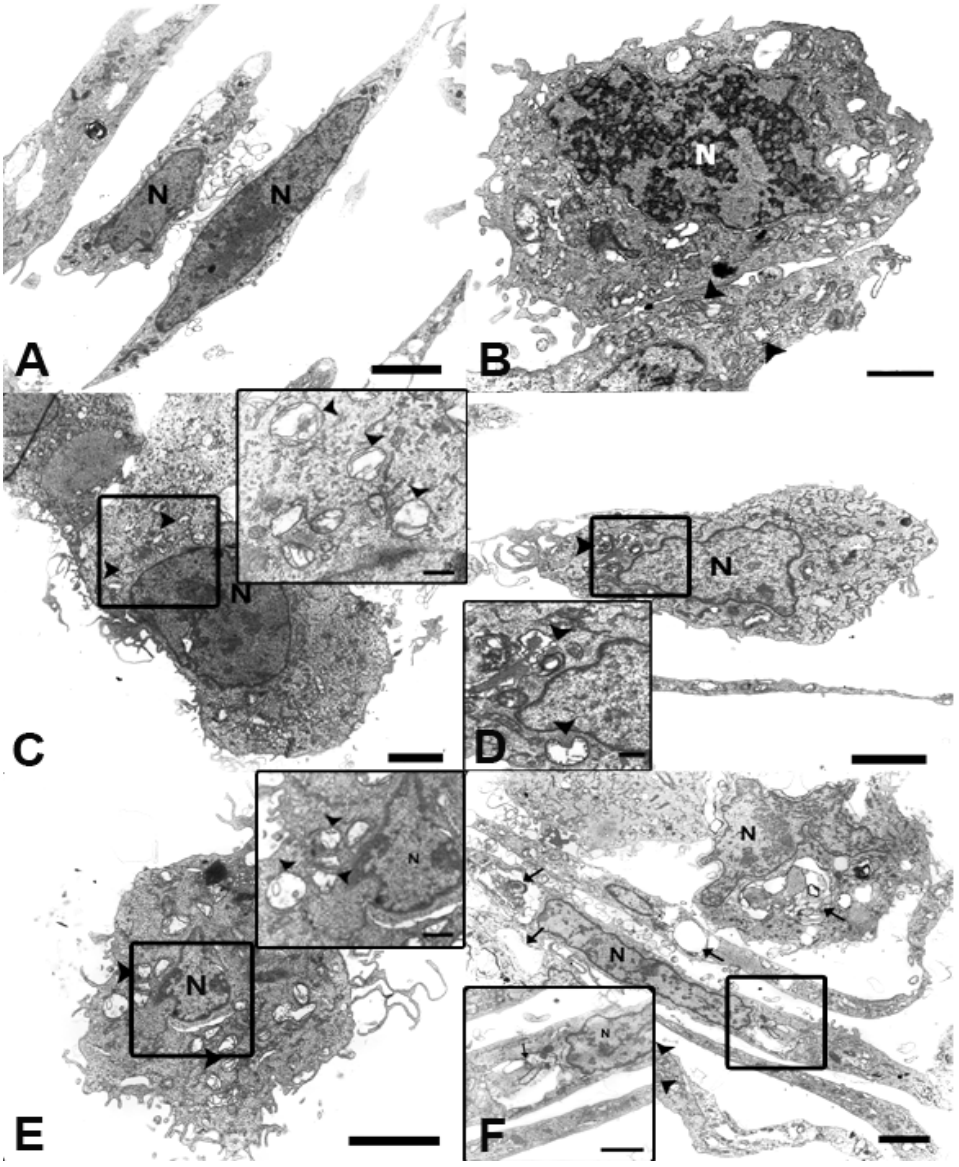


**Figure 5.**Ultrastructural alterations of C6 cells induced by arctigenin and matairesinol lignans. A. Untreated C6 cells with a typical morphology, elongated and with central nuclei. B. C6 cells exposed to UV as positive control, with rounded shape and irregular chromatin condensation. C. C6 cells treated with 50 µM of arctigenin. D. C6 cells treated with 250 µM of arctigenin. Note the rounded shape and disorganized internal mitochondrial membranes (inset-arrowheads). E. C6 cells treated with 50 µM matairesinol. Note the rounded shape with intense disorganized internal mitochondrial membranes and irregular chromatin condensation (inset-arrow heads). F. C6 cells treated with 250 µM matairesinol. Note the rounded and elongated cell shape, intense chromatin condensation in the nucleus and the presence of autophagic vacuoles (inset - arrow). Bars: A: 10 µm; B: 5 µm; C: 2 µm, inset: 10 µm; D-E 10 µm; inset D: 10 µm; inset E: 2 µm; inset F: 5 µm. **N:** nuclei.

## DISCUSSION

Phytochemical investigation of the dichloromethane extract of the leaves of *Ficus citrifolia*, was chemically characterized by the presence of three lignans, matairesinol, arctigenin and arctiin. These three compouds are lignans type dibenzylbutyrolactone and were isolated for the first time in the genus *Ficus*. The chemical profile of *Ficus citrifolia* share similarity with members belonging to the family Moraceae, indicating that this study has contributed to better understanding of the genus, beyond the knowledge of the chemical composition of this species.

Compounds obtained from plants offer perspectives toward the discovery of new active and promising compounds with anticancer activity (Zhang and Liu, 2015; El-Mesallamy et al., 2016). Arctigenin and matairesinol have shown a vast range of biological properties

(Adlercreutz, 2002; Jin et al., 2005). Previous studies have shown that arctigenin protects neural and neuroblastoma cells when treated with H89 via down regulation of the CREB pathway (Zhang et al., 2013). In addition, it is possible that arctigenin and matairesinol as phytoestrogens produce a cytotoxic effect against glioma cells that can be explained by the direct interaction with estrogen receptors in these cells. Similarly, other studies have shown evidence of a possible protective role of female sex hormones against glioma cells (Kabat et al., 2010) and it has recently been shown effects of arctigenin on glioma cells (Maimaitili et al.,2017). However, to date, no reports have described morphological alterations in glioma cells that occur in response to matairesinol and arctigenin treatment.

In the present report, we investigated the effect of arctigenin and matairesinol on proliferation and cellular viability of rat glioma cells culture (C6 cells). We also evaluated the citotoxicity of these compounds against astrocytes cell cultures from rat cerebral tissue. Results showed that 50 µM of arctigenin and 125 µM matairesinol reduced approximately 50% on C6 cells proliferation. Thus, the results showed that arctigenin was more potent than matairesinol regarding of cytotoxicity against glioma cells. Similar concentration of these molecules was not cytotoxic against astrocytes cell culture used as positive control. In contrast to glioma cells, primary rat astrocytes revealed a striking resistance in the same conditions of treatment against arctigenin and matairesinol.

One of the novel findings of the present study is the morphological alteration of cells induced by these lignans. Electron microscopy techniques can be a useful tool in drug studies for the identification of cell surface and ultrastructural alterations, as well as specific organelle targets on the glioma cells. Indeed, identification of morphological changes can help elucidate the mechanism of drug action (Rodrigues et al., 2011). Treatment of C6 cells with 50 µM or 250 µM of arctigenin and matairesinol induced changes in cell morphology and irregular chromatin condensation. For the last ten years, many studies have shown that induction of apoptosis using medicinal plants may provide efficient and therapeutic strategies to suppress cancer cell growth (Formisano et al., 2012; Seo et al., 2011; Solak et al., 2014). On the basis of these considerations, arctigenin and matairesinol demonstrated a cytotoxic effect on C6 cells, inducing cellular death by apoptosis. Apoptosis is a specialized mode of cell death finely regulated at the molecular level and conserved throughout evolution (Seo et al., 2011). Apoptotic cells usually exhibit similar morphological changes including cell shrinkage, nuclear chromatin condensation and DNA fragmentation. In order to maintain homeostasis of multicellular organisms, a strategic intracellular program is initiated, ensuring the fate of unwanted cells. Interference with this program has been implicated in many diseases, including cancer and autoimmune diseases. Therefore, in the search for anticancer drugs, it is very important to find a compound with the ability to induce cellular death by apoptosis. Arctigenin and Matairesinol have significant effects on proliferation and death of C6 glioma cells and may contribute as selective agents against brain cancer. Furthermore, these lignans extracted from *Ficus citrifolia* show potential for chemotherapeutic and/or chemo preventive usage, representing a promising natural alternative source against brain cancer

## CONCLUSION

In conclusion, data presented in this study demonstrate that *Ficus citrifolia* represents an attractive source of secondary metabolites, specifically lignans such as arctigenin and matairesinol, which effectively promote anticancer activity. These substances induce apoptosis and ultrastructural alterations in glioma cells but do not affect rat astrocytes used as controls.

## ACKNOWLEDGMENTS

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### REFERENCES

Adlercreutz H (2002). Phyto-oestrogens and cancer. Lancet Oncol.3: 364-373.

Carauta JPP (1989). Ficus (Moraceae) no Brasil: Conservação e Taxonomia, Albertoa. 2: 1-365.

Charlton JL(1998). Antiviral activity of lignans. J. Nat. Prod. 61: 1447-1451.

Chesler DA, Berger MS, Quinones-Hinojosa A (2012).The potential origin of glioblastoma initiating cells. Front. Biosci. 4: 190-205.

Cho JY, kim AR, Yoo ES, Baik KU, Park MH (1999). Immunomodulatory Effect of Arctigenin, a Lignan Compound, on Tumour Necrosis Factor-a and Nitric Oxide Production, and Lymphocyte Proliferation. J. Pharm. Pharmacol. 51: 1267-1273.

Cho MK, Park JW, Jang YP, Kim YC, Kim SG (2002). Potent inhibition of lipopolysaccharide-inducible nitric oxide synthase expression by dibenzylbutyrolactone lignans through inhibition of I-kBa phosphorylation and of p65 nuclear translocation in macrophages. Int. Immunopharmacol. 2: 105-116.

Eich E, Pertz H, Kaloga M, Schulz J, Fesen MR, Mazumder A, Pommier Y (1996). Arctigenin as a lead structure for inhibitors of human immunodeficiency virus type-1 integrase. J. Med.Chem. 39: 86-95.

El-Mesallamy AMD, Emam SS, Abed El-Azim MHM, Sanad IM (2016). Cytotoxicity and Bioactive Compounds from Diethyl Ether Extract of *Lotus halophilus* Boiss EtSpruner Growing in Egypt. Glo. Adv. Res. J. Med. Med. Sci. 5(3): 74-81.

Formisano C, Rigano D, Russo A, Cardile V, Caggia S, Arnold AN, Maris A, Piacenyes S, Rosselis S, Senatore F, Bruno M (2012). Phytochemical Profile and Apoptotic Activity of Onopordum cynarocephalum. Planta Med. 78: 1651-1660.

Hirano T, Gotoh M, Oka K(1994). Natural flavonoids and lignans are potent cytostatic agents against human leukemic HL-60 cells. Life Science. 55: 1061-1069.

Jacobs VL, Valdes PA, Hickey WF, DeLeo JA (2011). Current review of in vivo GBM rodent models: emphasis on the CNS-1 tumour model. ASN Neuro.3: 171-181.

Jang YP, Kim SR, Kim YC(2001). Neuroprotective Dibenzylbutyrolactone Lignans of Torreya Nucifera. Planta Med. 67: 470-472.

Jang YP, Kim SR, Choi YH, Kim J, Kim SG, Markelonis GJ, Oh TH, Kim YC (2002). Arctigenin protects cultured cortical neurons from glutamate-induced neurodegeneration by binding to kainate receptor. J. Neurosci. Res. 68: 233-240.

Jin DQ, Lim CS, Hwang JK, Ha I, Han JS (2005). Anti-oxidant and anti-inflammatory activities of macelignan in murine hippocampal cell line and primary culture of rat microglial cells.Biochem. Biophys. Res. Commum. 331: 1264-1269.

Jovčevska I, KočevarN, Komel R (2013). Glioma and glioblastoma- how muchdo we (not) know? Mol. Clin. Oncol. 1: 935-941.

Kabat GC, Etgen AM, Rohan TE (2010). Do steroid hormones play a role in the etiology of glioma? Cancer Epidemiol. Biomarkers Prev. 19: 2421-2427.

Kato T, Hirose M, Takahashi S, Hasegawa R,Kohno T,Nishibe S,Kato K,Shirai T (1998). Effects of the lignan, arctiin, on 17-beta ethinyl estradiol promotion of preneoplastic liver cell foci development in rats. Anticancer Res. 18(2A):1053-1057.

Kislev ME, Hartmann A, Bar-Yosef O (2006). Early domesticated fig in the Jordan Valley. Science.312: 1372-1374.

Lee JH, Lee JY, Kim TD, Kim CJ (2011). Antiasthmatic action of dibenzylbutyrolactone lignans from fruits of *Forsythia viridissima* on asthmatic responses to ovalbumin challenge in conscious guinea-pigs. Phytother. Res. 25: 387-395.

Maimaitili A, Shu Z, Cheng X, Kaheerman K, Sikandeer A, Li W (2017). Arctigenin, a natural lignan compound, induces G0/G1 cell cycle arrest and apoptosis in human glioma cells. Oncol. Letters. 13:1007-1013.

Meagher LP, Beecher GF, Flanagan VP, Li BW (1999). Isolation and Characterization of the Lignans, Isolariciresinol and Pinoresinol in flaxseed meal. J. Agric. Food Chem. 47: 3173-3180.

Mosmann T(1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Met. 65: 55-63.

Paavilainen HM (2005). Malaria Drugs in Medieval Medical Literature: An Ethnopharmacological Approach. 5th European Colloquium on Ethnopharmacology. Rev. Fitoterapia. 5(1): 213-216.

Rahman MMA, Dewick PM, Jackson DE, Lucas JA (1990). Lignans of Forsythia intermedia. Phytochem. 29: 1971-1980

Rodrigues APD, Santos AS, Alves CN, Carvalho AS, do Nascimento JLM, Silva EO (2011). Kojic Acid, a secondary metabolite from *Aspergillus*sp., acts as an inducer of macrophage activation. Cell Biol. Int. 35: 335-343.

Seo HS, Choi HS, Choi HS, Choi YK, Um JY, Choi I, Shin YC, Ko SG(2011).Phytoestrogens Induce Apoptosis via Extrinsic Pathway, Inhibiting Nuclear Factor-κB Signaling in HER2-overexpressing Breast Cancer Cells. Anticancer Res. 31: 3301-3314.

Solak KA, Wijnolts FMJ, Nijmeijer SM, Blaauboer BJ, Van den Berg M, Van Duursen MBM (2014). Excessive levels of diverse phytoestrogens can modulate steroidogenesis and cell migration of KGN humangranulosa-derived tumor cells. Toxicol. Reports. 1: 360-372.

Van den Berg ME (1982). Plantas Medicinais da Amazônia - Contribuição ao Conhecimento Sistemático. Ed. CNPq-Programa Trópico Úmido (Brasil). 223p.

Yang LM, Lin SJ, Yang TH, Lee KH (1996). Synthesis and anti-HIV activity of dibenzylbutyrolactone lignans. Bioorganic & Medicinal Chem. Lett. 6: 941-944.

Ward RS (1999). Lignans, neolignans and related compounds.Nat. Prod. Rep. 16: 75-96.

Willför SM, Ahotupa MO, Hemming JE, Reunanen MHT, Eklund PC, Sjöholm RE, Eckerman CSE, Pohjamo SP, Holmbom BR (2003). Antioxidant activity of knotwood extractives and phenoliccompounds of selected tree species. J. Agr. Food Chem. 51: 7600-7606.

Woodland DW (1997). Contemporary plant systematics. Andrews University Press, Berrien Springs; MI, 2nd ed.

Zhang C, Liu Y (2015). Targeting cancer with sesterterpenoids: the new potentialantitumor drugs. J. Nat. Med. 69:255-266.

Zhang N, Wen Q, Ren L, Liang W, Xia Y, Zhang X, Zhao D, Sun D, Hu Y, Hao H, Yan Y, Zhang G, Yang J, Kang T(2013). Neuroprotective Effect of Arctigenin via Upregulation ofP-CREB in Mouse Primary Neurons and Human SH-SY5Y Neuroblastoma Cells. Int. J. Mol. Sci. 14: 18657-18669.