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Leaf Anatomical-Histophytochemical Study and Evaluation of the Cytotoxicity of *Ottonia frutescens* Trel (Piperaceae)

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Abstract: To investigate the leaf histochemistry and micromorphology of *Ottonia frutescens* Schltdl Trel (Piperaceae), the antioxidant potential, and *in vitro* cytotoxic bioactivity of the leaf extract of this species against human leukemia and colorectal cells. Leaf micromorphology and histoanatomy were detailed and recorded by optical and transmission and scanning electron microscopy. The histochemical results reveal classes of secondary phytometabolites such as alkaloids, anthraquinones, coumarins, polyphenols, and saponins. Cardiotonics, flavonoids, and triterpenes compounds were not detected. All concentrations of leaf extracts showed a significant dose-dependent and time-dependent apoptotic effect with more than 90% apoptosis of U937 cells and about 60% apoptosis of COLO-205 cells in 24 h of culture under 200 µg/mL of leaf extract. In addition, the phenolic compounds present in the fractions of the sample contribute to its strong antioxidant capacity. *Ottonia frutescens* exhibit anatomical and histochemical characteristics similar to other Piperaceae, and for the first time, provides a new source for secondary metabolite classes that exhibit high antioxidant potential and cytotoxic bioactivity, reduce cell viability and induce apoptosis in U937 leukemic cells and COLO-205 colorectal cells.

Keywords: *Ottonia frutescens*; Piperaceae; Leukemia; Colorectal cancer; Phytochemical.

1. Introduction

Leukemia is among the ten most common cancers in the number of deaths worldwide [1], and most of the currently used chemotherapy drugs for cancers are known to develop resistance and restrictions by dose-limiting side effects [2]. Colorectal cancer is the fourth leading cause of cancer-related deaths worldwide [1,3]. The WHO Traditional Medicine Strategy 2014-2023 aims to develop proactive practices and implement action plans that will strengthen the role that traditional and complementary medicine plays in keeping populations healthy [4].

Plant-derived bioactive compounds, especially polyphenolic, have shown to reduce tumorigenesis, interfering in the initiation, promotion, and progression of cancer by modulating

different enzymes and receptors in signal transduction pathways related to cellular proliferation, differentiation, apoptosis, inflammation, angiogenesis, metastasis and reversal of multidrug resistance [5–9].

The Piperaceae family has approximately 2000 species distributed mainly in tropical areas and exhibits potential as a drug source based on the use of some species in traditional medicine [10–13]. Many species exhibit classes of secondary metabolites with highly significant pharmacological potential. Iridoids, anthraquinones, triterpenes, alkaloids, flavonoids, phenolic derivates, and terpenoids stand out [11,13–15] with molecular mechanisms in the biochemical pathways that trigger the cell death investigated [16–19]. Research has pointed to the *in vivo* and *in vitro* anti-tumor potential of many species of the Piperaceae family [19–22], including leukemia [14,19,23] and colorectal cancer [16,20,23]. This is the first scientific investigation of the anti-tumor action of *Ottonia frutescens* (Piperaceae) and the first anatomical-histophytochemical and potential antioxidant study of this species. This plant provides a new source for classes of secondary metabolites, exhibits antioxidant potential and anti-tumor cytotoxic effect.

2. Materials and Methods

2.1. Plant material.

The botanical material was collected in a region in the Atlantic Forest in the ombrophyla vegetation in red-yellow latosol on the Santa Rita farm located in the Rio Paraíba do Sul watershed, in the West Forest Zone of the State of Minas Gerais (20º 46' S and 42° 02' W). The annual temperature varies from 16 °C to 28 °C, and the annual average rainfall is 2268 mm. Leaves of adult specimens of *Ottonia frutescens* (Piperaceae) collected with the aid of pruning shears were duly identified, and a voucher specimen was deposited in the Guido Pabst - GFJP herbarium, Itaperuna - RJ, Brazil, registered under GFJP-204.

2.2. Optical microscopy.

Leaf fragments of the selected species were placed in an aqueous solution containing 2.5% glutaraldehyde and 4.0% formaldehyde diluted in 0.05 M of sodium cacodylate buffer, pH~7.2, at room temperature for 2 h. After washing the fragments for 45 min in the same buffer, they were then placed in an aqueous solution containing 1% osmium tetroxide diluted in 0.05 M of sodium cacodylate buffer, pH~7.2, at room temperature for 1 hour with the absence of light. After three 45 min washes in the same buffer, the fragments were submitted to an ascending ketone series [50%, 70%, 90%, 100% (3x)] for 1 hour at each step for dehydration. Next, the fragments were infiltrated with epoxy resin (Epon PolibedTM), using an increasing resin series in propanone. The polymerization of the resin was performed at 60 °C. Semi-thin sections (1 μm thick) were obtained with ultramicrotome glass cutters (Reichert Ultracut-S®). The staining was performed with a 1% toluidine blue aqueous solution, plus 0.1% of Borax. Permanent blades were assembled with Entellan® for optical digital documentation (Axioplan Zeiss Canon® Power Shot 14 mpixel; Oberkohen, Germany).

2.3. Transmission electron microscopy.

Ultra-thin sections (~70 nm thick) were obtained using a diamond knife (Diatome®) in ultramicrotome (Leica Reichert Ultracut-S®, Germany), collected in 300 mesh copper grids.

The staining was performed in a saturated aqueous solution of uranyl acetate (40 min) followed by washing in distilled water (5 min) in a 1% lead citrate [24]. The images were obtained through transmission electron microscopy (Zeiss® EM900) under an accelerating voltage of 80 kV, with the aid of the iTEM (Olympus®) program.

2.4. Scanning electron microscopy.

After dehydration, the samples were dried by the CO_2 critical point method (Bal-Tec® SCD-050 Sputter Coater, Liechtenstein). Dry leaf fragments were attached to appropriate stands with double-face carbon adhesive tape (3M) and carbon glue, covered by sputtering a layer of approximately 20 nm of gold (Bal-Tec® SCD-050 Sputter Coater, Liechtenstein). Samples were observed under a scanning electron microscope (DSM 962 and EVO 40-Zeiss®) at an accelerating voltage of 15 kV or 25 kV.

2.5. Histochemical analysis of the plant sample.

Histochemical tests of freshly collected leaf material were performed using freehand vegetable cuttings. Acid and base compounds were evaluated from exposure of the plant sections for 10 sec to a toluidine blue 0.05% aqueous solution at pH 6.8 [25]. Alkaloids were evaluated by Dragendorff's reagent for 5 to 10 min and washed quickly in sodium nitrite, 5%, and subsequently in water [26]. Total proteins were evaluated by Coomassie brilliant blue G, 0.25% (acetic acid, 5% for 30 min), and washed in 5% aqueous acetic acid three times for 5 min at each stage; semi-permanent slides were assembled with glycerin, 50%. Phenolic compounds were detected by an aqueous solution of ferric chloride (10%, 5 min); peptic substances were detected using an aqueous solution of ruthenium red (0.05%, 10 sec). Acid intracellular compartments were evaluated by an aqueous solution of acridine orange (0.01%, 5 min) [27]. Lipids were evaluated by the Sudan IV test, 0.03% [28].

2.6. Plant extract.

O. frutescens leaves were submitted to forced ventilation drying, at a constant temperature of 40 °C, until stabilization of the weight, to obtain dry plant matter; 350 g of leaves were ground with a mortar and pestle in the presence of liquid nitrogen. The material with reduced particle size was deposited in an amber flask and extracted by maceration in 10% (w/v) methanol for seven days at room temperature. The period for exhaustive extraction was 21 days. Every seven days, the fluid extractor was filtered, and a new fluid extractor was added. The obtained extract was concentrated in a rotary evaporator, and the drying process of the extract was finalized under direct ventilation (40 to 50 °C). Finally, the dried extract was verified and stored in an opaque plastic container in the presence of a desiccant agent.

2.7. Phytochemical prospecting of extracts.

The phytochemical analysis of the methanolic extract of the *O. frutescens* leaves was performed in thin layer chromatography [29]. The following groups of secondary metabolites were evaluated: Alkaloid, Anthraquinones, Cardiotonics, Coumarins, Flavonoids, Polyphenols (Tannins), Saponin, and Triterpene. In the chromatographic process, mobile phases, developers, and specific reference standards were used for each of the groups of secondary metabolites (Table 1).

Table 1. Phytochemical prospection of the extract of the *O. frutescens* leaves

Metabolite group	Mobile phase	Developer	Standard	Reference
Alkaloids	Ethyl methanol-water acetate (100:14:10)	Dragendorff's Reagent	Quinine	[26]
Anthraquinones	Ethyl methanol-water acetate (100:17: 13)	5% KOH solution in ethanol	1,8-dihydroxy-anthraquinone	[29]
Cardiotonics	Ethyl methanol-water acetate (81:11:8)	Kedde's Reagent	Digitoxin	[27]
Coumarins	Acetic acid-toluene-diethyl ether-water (50:25:25:5)	5% KOH solution in ethanol	Benzopyrone	[26]
Flavonoids	Ethylacetate-acetic acid-acetic acid-water (55:5:5:1)	5% $AlCl_3$ solution in ethanol	Routine	[29]
Polyphenols (Tannins)	Toluene-butanol-acetic acid (80:40:16)	Barton's Reagent	Pirogalic acid	[28]
Saponin	Chloroform-glacial acetic acid-methanol-water (15:8:3:2)	Sulfuric anisaldehyde	18-β-glycyretin	[28]

2.8. Evaluation of the antioxidant activity of the extract.

The antioxidant capacity of the extract was determined using the stable radical method 2,2-diphenyl-1-picrylhydrazyl (DPPH), and is based on the free radical reduction DPPH [30]. The solution prepared daily was stored away from the light and kept at 4°C until use. The final concentrations of the sample were 3, 6, 12, 18, 24 µg/mL. The samples were *diluted in* methanol at five different concentrations: 30, 60, 120, 180, 240 µg/mL. 300 µL of each of the five concentrations was added in 2.7 mL of a DPPH methanolic solution (60 µM). The control was prepared from 2.7 mL of the solution of DPPH plus 300 µL of methanol. A blank was performed with the extract, fractions, and controls to reduce the contribution of the color to the absorbance. The absorbance of the samples was measured at 0, 15, 30, and 45 min (515 nm) times in quartz cuvettes. The antioxidant activity of the sample, in the percentage of DPPH (Absorbance, Ab intake), was obtained by the formula [Ab control - (Ab sample - Ab white) / Ab control] x 100.

2.9. Evaluation of the extract effect on the cell line' viability.

U937 leukemia human cells of lymphoid origin (ATCC® CRL1593.2™) and COLO-205 colorectal adenocarcinoma human cells (ATCC® CCL222™) were cultured in D-MEM F12 medium (Gibco®, BRL) supplemented with 20 µg/mL of gentamicin (Gibco®, BRL) and 10% of fetal bovine serum (Gibco®, BRL). Cultures were replicated every 2 days and kept in an incubator (Forma Scientific® Inc., model 3159) at 37 °C, with 5% of CO2 and controlled humidity.

Cell viability was assessed from the assays with 3-(4.5-dimethyl-2-thiazole) 2.5-diphenyl-2-H-bromide of tetrazolium, MTT [31] and from release levels of the lactic dehydrogenase enzyme [32]. Cells were plated in a volume of 100 µL/well ($1x10^6$ cells /mL) in 96-well plates and exposed to the extracts of *O. frutescens* at the final concentrations of 50, 100, 200 and 400 µg/mL and kept in an incubator at 37 °C, with 5% CO2 and controlled humidity. After 24 h under cell culture with the extracts, cell viability assays were performed. For this, 10 µL of MTT (Sigma®) in a 100 µL culture was added for further spectrophotometric reading (Thermo Scientific Multiskan-Ex®) at 570 nm.

2.10. Evaluation of lactate dehydrogenase (LDH).

To evaluate LDH release (Doles® Kit, Brazil), 50 µM of the supernatant was transferred to another 96-well plate containing 100 µM of solution A (250 µM of ferric alum solution + 400 µM of the substrate) and kept in an incubator for 3 min. Afterward, 100 µM of solution B (225 µM FMS / NAD^+ 4275 µM solution of distilled water) were added and kept in an incubator for 7 min for subsequent spectrophotometric readings at 492 nm (Thermo Scientific Multiskan-Ex®). As negative controls, cells were incubated with DMEM F12 medium (Gibco®, BRL) supplemented with 20 µg/mL of gentamicin (Gibco®, BRL) and 10% fetal bovine serum (Gibco®, BRL). For the assays with the extracts, the cells were incubated with 1% DMSO (Sigma®). The experiments were carried out in triplicates.

2.11. Evaluation of apoptosis through fluorescence microscopy.

The leukemic cells U937 and colorectal COLO-205 cells exposed at 12, 24 and 36 h with different concentrations of the extract were stained with 10 µg/mL of acridine orange solution (Sigma®) and 10 µg/mL of ethidium bromide (Sigma®). In random fluorescence microscopy (Axioplan Zeiss®) fields, ~300 cells were evaluated for apoptosis and necrosis. Duplicates were made for each condition, and the experiments were repeated at least twice.

2.12. Statistical analysis.

The results were expressed as mean ± standard deviation, analyzed independently and evaluated by one-way ANOVA followed by the Tukey test (GraphPad 5.0 Software, USA). All experiment samples were made into triplicates and repeated at least three times. Statistically significant differences were defined for $p<0.05$, $p<0.01$, and $p<0.001$.

3. Results and Discussion

3.1. Foliar micromorphology and anatomy.

O. frutescens exhibits a regular abaxial surface, epidermal cells with irregularly contoured anticline walls, and a straight external periclinal wall with layered epicuticular wax; the notable presence of secretory trichomes and multicellular tectors (Figure 1 AB), a botanical characteristic common to the Piperaceae family [33–35]. The leaves are hypostomatic with rare paracytic stomata.

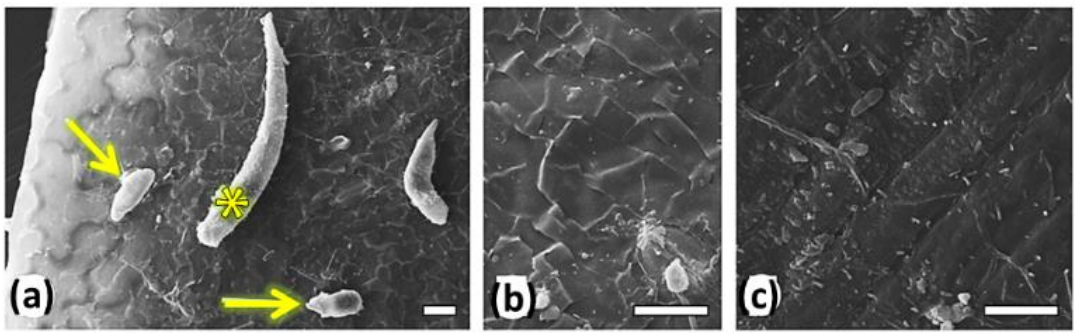

Figure 1. Scanning electron microscopy of *O. frutescens* leaf. (a) Frontal view of the abaxial epidermis showing the presence of multicellular tector type trichomes (*) and secretory trichomes (arrows); Bar: 20 µm. (b) Detail of the abaxial surface covered by layered wax; Bar: 200 µm. (c) Adaxial surface covered by smooth to slightly granular wax; Bar: 10 µm.

The adaxial surface is glabrous with periclinal cell walls and anticlinal wax covered with mild granulation in some areas (Figure 1C). Leaves of *O. frutescens* display micromorphological characteristics common to the Piperaceae family, similar to those described for *O. martiana* [33,36], *Piper mosenii* [34], *Peperomia pellucida* and *Piper longum* [35].

The leaf blade of *O. frutescens* presents both an epidermis constituted by a single layer of isodiametric cells with varied sizes and a straight periclinal wall, the abaxial cell face being smaller than the adaxial ones (Figure 2), botanical characteristics also observed in other species of the Piperaceae family [35], while other species exhibit multiple epidermal tissues, an adaptive botanical characteristic of heliophylous [33]. In this study, no adaxial or abaxial monoserial subepidermal was observed, photoprotective tissue described in other Piperaceae species [33,37,38], including *O. martiana* [36]. The mesophile is dorsiventral consisting of palisade parenchyma formed by a cell layer rich in chloroplasts and lacunar parenchyma consisting of 4 to 6 isodiametric cell layers with small intercellular spaces. The leaf anatomy of *O. frutescens* display characteristics common to the Piperaceae family [33–36].

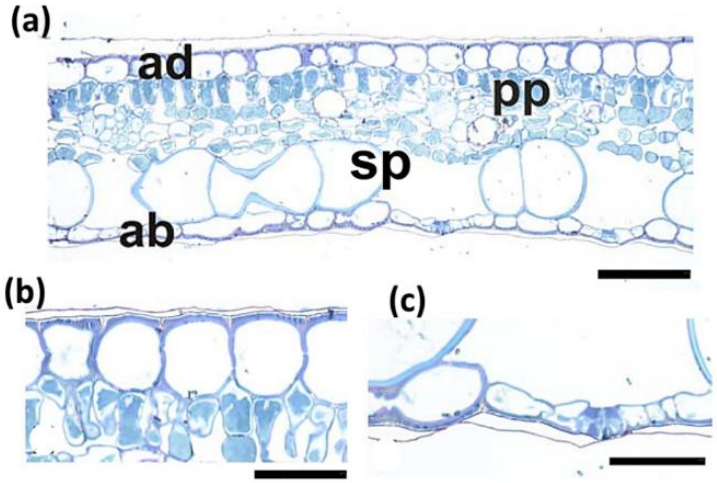

Figure 2. Leaf-blade of *O. frutescens* in the transverse section under optical microscopy (a), with detail of uniserial adaxial epidermis (b) and abaxial epidermis (c). Abbreviations: ad, adaxial surface; ab, abaxial surface; pp, palisade parenchyma; sp, spongy parenchyma. Bars: A: 50 μm; BC: 25 μm.

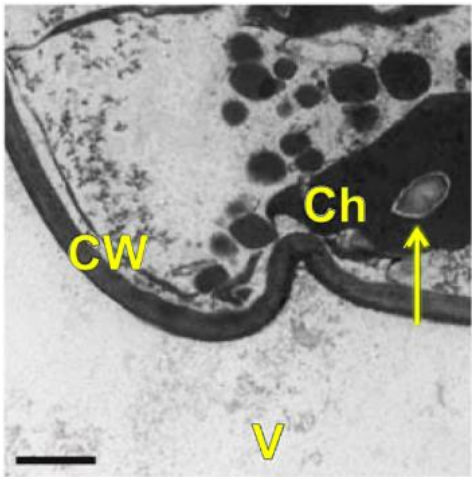

Figure 3. Transmission electron microscopy of *O. frutescens* leaf. A cell of the spongy parenchyma showing a large vacuole (V), thin cell wall (CW), and chloroplast with starch accumulation (arrow). Bar: 1 μm.

Under transmission electron microscopy, it was possible to observe that the external periclinal wall of *O. frutescens* presents a basal polysaccharide layer followed by cuticular extracts divided into arborescent (closest to the polysaccharide layer) and reticulated (more above this extract). Protoplast observation of mesophilic cells revealed common plant cytological features such as the presence of large central vacuoles, cytoplasm occupying a relatively small and peripheral volume, and abundant chloroplasts with starch accumulation (Figure 3).

3.2. Foliar histochemistry.

The Coomassie blue test for proteins and Dragendorff reagent for alkaloids revealed intense markings in the palisade and spongy parenchyma of *O. frutescens*. The ferric chloride test also exhibited markings mainly on the palisade parenchyma (Figure 4A). The staining with acridine orange revealed a predominance of the markings in the adaxial and abaxial epidermis, and in the chlorophyllic parenchyma (Figure 4B).

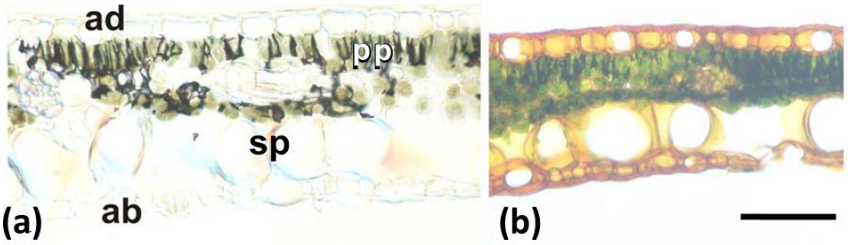

Figure 4. Optical microscopy of the leaf sections of *O. frutescens* submitted to histochemical tests of ferric chloride (a) and acridine orange (b). Abbreviations: ad - adaxial surface; ab - abaxial surface; pp - palisade parenchyma; sp - spongy parenchyma. Bar: 50 μm.

Phytocomposites such as proteins, phenolic compounds, and alkaloids are more concentrated in the leaf parenchymatic region. The leaf epidermis exhibits reactive staining much more acid compartments and pectins than other sites on the leaf, but without reactive epidermal detection of alkaloids and phenolic compounds, lipids and proteins are poorly detected in epidermal cells (Table 2).

Table 2. Histochemistry of the *O. frutescens* leaf blade.

			Leaf Mesophyll		
Reagent	Target Compound	Adaxial Epidermis	Palisade Parenchyma	Spongy Parenchyma	Abaxial Epidermis
Coomassie Blue	Proteins	-	+ +	+ +	+
Ferric Chloride	Phenolic Compounds	-	+ + +	+ +	-
Dragendorff	Alkaloids	-	+ + +	+ + +	-
Acridine orange	Acid Compartments	+ + +	-	+	+ + +
Ruthenium Red	Pectins	+ +	-	-	+ + +
Sudan IV	Lipids	+	+	+	+

Legend: +++ Very strong marking; ++ Moderately strong marking; + Poor marking; - Unmarked

3.3. Phytochemical prospecting.

Phytochemical prospection of foliar extracts of *O. frutescens* reveals the presence of alkaloids, anthraquinones, coumarins, polyphenols, and saponins, attributing recognized anti-tumor effects [39–41] including leukemic cells [42,43] and colorectal cancer [43–45].

Cardiotonics, flavonoids, and triterpenes compound not were detected. However, they have already been identified in considerable concentrations in other species of the Piperaceae family [13,15,19]. With similar results, tannins and coumarins have also been found in the phytochemical screening of extracts from other Piperaceae species, but with alkaloids, flavonoids and saponins also showing interspecific variations [10,12]. In addition to the interspecific characteristic, phytochemical diversity is related to several plant ecophysiological functions, including protection against biotic and abiotic environmental stresses [46–49], and it can change according to the geographical origin of the species and ecological characteristics, accounting for variations in bioactivity [15].

3.4. Determination of the antioxidant activity of the extract.

The antioxidant activity of the leaf extract of *O. frutescens* was evaluated (Table 3). The results were better than the antioxidant activity exhibited by other species of the family Piperaceae [13,15,22,50].

Table 3. Percentage of the antioxidant activity of foliar extract of *O. frutescens* by the DPPH method, under final concentrations of 3, 6, 12, 18, 24 µg/mL, for 30 min. Mean ± standard deviation obtained by triplicate.

Extract Concentration (µg/mL)				
3	6	12	18	24
2.7 ± 0.7	9.6 ± 0.6	12.5 ± 0.6	31.2 ± 0.8	38.3 ± 0.6

The phenolic compounds present in the fractions of the sample contribute to its antioxidant capacity, as observed for other Piperaceae [13,14,20,21]. In *Piper sarmentosum*, the strong antioxidant effect is highly correlated mainly to phytochemicals such as flavonoids and phenolic compounds whose concentrations can vary according to the geographical origin of the plant, interfering in the antioxidant potential of experimental leaf extracts [15].

3.5. Effect of extracts on cell viability.

The cytotoxic effect of the *O. frutescens* extract on human leukemic cell line U937, and human COLO-205 colorectal cells were evaluated. All concentrations tested showed a significant inhibitory effect in a dose-dependent relationship on cell viability, with greater than 90% inhibition of U937 cells and about 75% inhibition of COLO-205 cells in a concentration of 400 µg/mL after 24 h of incubation (Figure 5). The determination of the lethal dose 50 (LD50) was 230.9 ± 1.73 µg/mL under 12 h of culture, 112.9 ± 1.47 µg/mL in 24 h and 86.34 ± 2.32 µg/mL after 36 h of exposure of the U937 cells to the extract, and <400 µg/mL under 12 h of culture, 280.0 ± 1.19 µg/mL in 24 h and 257.5 ± 1.31 µg/mL after 36 h of exposure of the COLO-205 cells to the leaf extract.

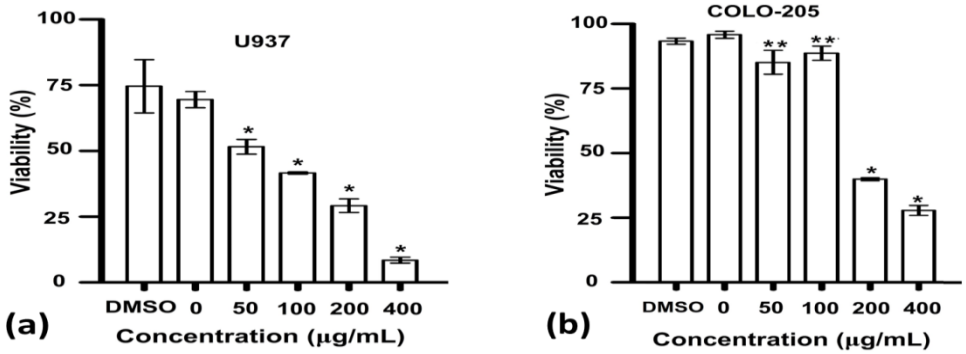

Figure 5. Cytotoxic effect of *O. frutescens* extracts on (a) human leukemic cell line U937 and (b) human colorectal cell line COLO-205 after 24 h incubation. ; (0): negative control; DMSO: positive control. * P<0.05, n = 3. ANOVA, One-way Tukey.

Although other Piperaceae species exhibit confirmed *in vivo* and *in vitro* anticarcinogenic action [19,21,51], selectively with minimal cytotoxic effect on normal cells [10], this is the first investigative study on the anti-tumor bioactivity of leaf extract in the genus *Ottonia*, which somewhat limits the safe inference of the results obtained. On the other hand, it will contribute as a source of primary data for future scientific studies involving this genus.

Further studies at our research center will be conducted in order to understand the chemical composition of fractionated bioactive substances present in the plant extract and their effects on oncogenesis.

3.6. Quantification of lactate dehydrogenase.

As indicative of cell membrane damage and a key feature of cells undergoing apoptosis, necrosis, and other forms of cellular damage, the activity of the cytosolic enzyme Lactate Dehydrogenase (LDH) was evaluated. Statistically significant results were shown by the U937 leukemic cells for all tested concentrations of the *O. frutescens* foliar extract in a dose-dependent effect, 200 µg/mL after 24 h of culture being the best result found for COLO-205 colorectal cells (Figure 6).

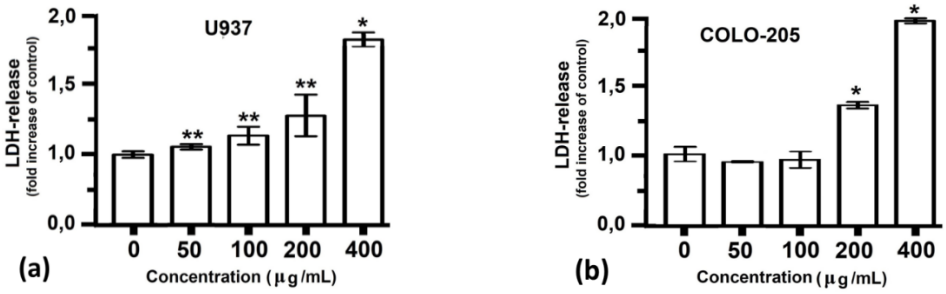

Figure 6. Evaluation of LDH release by (a) U937 leukemic cells and (b) COLO-205 colorectal cells after 24 h of incubation exposed to *O. frutescens* leaf extract. The results were presented as a fold increase of the control cells (0). *P <0.05; ** P> 0.05, n = 3. ANOVA One-way Tukey.

3.7. Evaluation of apoptosis induction and cellular necrosis.

To investigate the anti-tumor activity of *O. frutescens* leaf extract on U937 leukemic and COLO-205 colorectal cells, cell death was evaluated by fluorescence microscopy. All concentrations tested showed a significant dose-dependent and time-dependent apoptotic effect with more than 90% apoptosis of U937 cells and about 60% apoptosis of COLO-205 cells in 24 h of culture under 200 µg/mL of leaf extract (Figure 7).

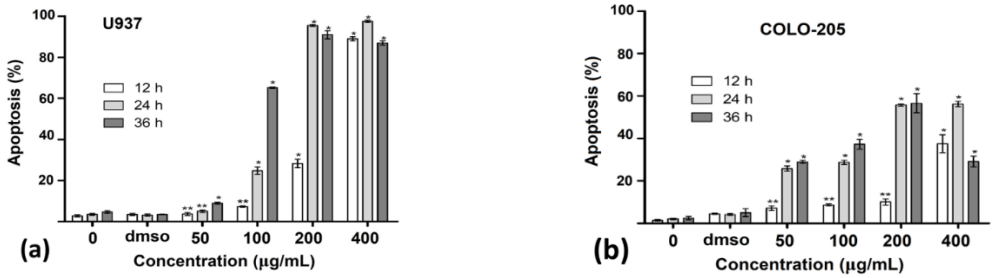

Figure 7. Percentage of apoptosis of U937 leukemic and COLO-205 colorectal human cells exposed to different concentrations of leaf extract of *O. frutescens* at 12, 24, and 36 h of culture; (0): negative control; DMSO: positive control. *P <0.05, ** P> 0.05, n = 2. ANOVA, One-way Tukey.

There was a rate of less than 3% of U937 cell necrosis only at the concentration of 400 µg/mL under 36 h of culture. There was 20% necrosis of COLO-205 cells already starting at 50 µg/mL under 36 h (data not shown). Other Piperaceae species traditionally used in different parts of the world and phytochemically investigated exhibit apoptotic effect in different tumor lines cells [21], including leukemia and colorectal [19,51].

Phytochemical studies of different Piperaceae species have drawn scientific interest [12,14], and this is the first histophytochemical and anatomical study of *O. frutescens*, in particular focusing on tumor cells. Its cytotoxic effects on U937 leukemic and colorectal COLO-205 cells indicate a potential alternative in the treatment of these types of cancer. This plant provides a new source for classes of secondary metabolites: alkaloid, anthraquinones, coumarins, polyphenols, saponins, flavonoids and terpenes, which might be capable of inducing cancer cell apoptotic death [42,51,52]. Perspectives of scientific studies can be directed to studies on the interference of different environments in which *O. frutescens* is adapted, the histochemical and anatomical foliar variations and the relation with the phytochemical bioactivity of this Piperaceae and the different plant parts to U937 leukemic cells and COLO-205 colorectal cells and other lineages of human tumor cells.

Tests with crude extracts of plant organs provide good indications of the plant's bioactivity and the prospect of isolation of promising biocompounds for the development of new future anti-tumor drugs. However, there is a limitation of predicting whether the isolation of a certain *O. frutescens* biocompounds will present interesting experimental results if there is a synergy between the components of the leaf extract. Another limitation is to safely predict which chemical class most affected the viability of human leukemia and colorectal tumor cells. The experimental use of two cell types of cancers so different also brought some limitation in defining the comparative tests with a physiologically compatible cell control, since one cancer exhibits blood characteristics and other cancer with solid tumor characteristics. In addition, tests with extracts using different tumor cells also limit a safe interpretation of the results in relation to the possible mechanism of action, since they are different tumorigenic biochemical pathways for each type of cancer.

4. Conclusions

This, the first report, reveals that *Ottonia frutescens* exhibit anatomical and histochemical characteristics similar to other Piperaceae. This plant provides a new source for classes of secondary metabolites: alkaloid, anthraquinones, coumarins, polyphenols, saponins, flavonoids and terpenes. The leaf extracts exhibit high antioxidant potential and cytotoxic bioactivity, reduce cell viability, and induce apoptosis in U937 leukemic cells and COLO-205 colorectal cells. This plant should be further examined in different test systems to understand its potential cytotoxic properties and the biochemical pathways that interfere with tumor cell lethality.

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Conflicts of Interest

The authors declare no conflict of interest.

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