



**Cytotoxic activity of *Thelesperma megapotamicum* (Spreng.) Kuntze
on cell MCF-7 (human breast carcinoma cells)**

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ABSTRACT

“In vitro” viability tests were done with organics extracts and organics fractions of *Thelesperma megapotamicum* in search of compounds with antitumoral activity. Human breast tumor cell line (MCF-7) was incubated (6 h) in vitro with whole plant and stoloniferous stem extracts at 0, 50 and 100 ug/ml. Cell viability was determinate by the staining crystal violet. The stoloniferous stem hexane extract was purified by means of chromatography on a Silica gel-60 column with solvents gradient, the fractions obtained were newly incubated on the cell and the viability percentage was calculated. The active fraction (hexane:ethyl ether 1:1 v/v) was submitted to new separation on Silica gel column and three fraction with antiproliferativity activity were found. In future, the active fraction of *Thelesperma megapotamicum* that have shown pronounced cytotoxic activity in this study will be further evaluated for the possible isolation of any active antitumor compounds.

Keywords: Breast cancer; cellular viability; plant extract; *Thelesperma megapotamicum*

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Introduction

The cancer is the second cause of mortality in the world, being characterized for fault proliferation cellular regulating mechanics, with high growth clonal and capacity of invade other tissue. Particularly the breast cancer is the principal reason of feminine morbimortality in Argentine (MS 2006, Indicadores Básicos 2006).

The chemotherapeutic agents are one of the current treatments for this disease being the plant therapeutic agent (taxol) the most used (Kingston DG, 1991). Recent studies over inhibitors tumor compounds derived from vegetable, are providing new structures (Lee KH, 1996; Li Y et al., 2007). According to the World Health Organization (WHO) more 80% of the world populations use the traditional medicine to satisfy their needs of primary care of the health and that great part of the traditional treatments implies the use of extracts plants or their active principles (Bermudez A et al., 2005). The search of natural compounds in the flora native or their semi synthetic derivatives capable to inhibit stages as initiation, promotion or progression of cancer result to be particularly interesting as a contribution in the fight against this disease.

In this respect result obtained from a previous cytotoxic screening, from fifteen plants that were used, *Thelesperma megapotamicum* (Spreng.) Kuntze exhibited pronounced cytotoxic effect (<25% cell viability) at MCF-7 cell line, being a potential anticancer source (Goleniowski M et al., 2007; Bongiovanni G et al., 2006).

Thelesperma megapotamicum is specie of Asteraceae family, knowing as Pampa tea or Indian tea (Barboza GE et al., 2006). Infusions are used against renal, digestive, anti-spasmodic affections and as anaesthesia (Nuñez C et al., 2000; Roig F, 2002).

Several compounds were isolated from this specie as stigmaterol, flavonoids (luteolin, 7-O-Glucósido, marein) and phenylpropanoids (Ateya A et al., 1978; Pathak V et al., 1987). Is kwon the anticancer activity of luteolin, it has inhibitor activity of topoisomerase I in eukaryotic DNA (Chowdhury AR et al., 2002) and DNA protector effect against oxidant agents (Horváthová K et al., 2004).

The aim of this work was to evaluate the "in vitro" cytotoxic activity of the organic extracts and active fractions obtained by bioassay guide of *Thelesperma megapotamicum* on human tumoral breast cell line (MCF-7).

Experimental

Plant material

Plant recollection was carried out at Santa María de Punilla, "Sierras de Córdoba". This side is located at approximately 45 km north of "Córdoba", Argentine. Plant samples were collected during spring 2008, immediately transported to the lab and a voucher specimen was deposited in the International Herbarium of the National University of Rio Cuarto, Argentina (RIOC).

Preparation of raw extracts of whole plant material (12 g) was thoroughly extracted with ethanol, hexane and ethyl ether (50 ml) by maceration at room temperature for 24 h under stirring. A supernatant was obtained by filtration, and concentrated under reduced pressure. The solid extract was dissolved in dimethylsulfoxide (DMSO) at a concentration of 100 mg dry weight/ml.

In vitro viability test

Human breast carcinoma cells (MCF-7, cell line ATCC HTB-22), were cultured in Dulbecco's Modified Eagle's medium (DMEM) with 10% fetal bovine serum, 100 UI/ml penicillin and 100 µg/ml streptomycin, incubated at 37°C in a 5% CO₂ atmosphere. 2 x 10⁴ cells were added per well (in a 96 wells-plate). After 48 hours, plant extract (0, 50 y 100 µg/ml), and DMSO or DMEM were added, and cells were cultured during 360 min (the time of incubation was standardized in previous tests). Cell viability was determined by staining with 0.5% crystal violet in 50% methanol for 15 min. Plate was washed with 50% methanol, and then dried (Mehlen et al., 1995). A solution containing 1% sodium dodecylsulphate in 60% ethanol was added to solubilise the stained cells, and the absorbance of each well was read with an Anthos Labtec 2010 micro ELISA reader at 610 nm, after establishing a 99% correlation. The viability percentage was defined as the relative absorbance of treated versus untreated control cells (100%).

Bioassay-directed fractionation

The hexane stoloniferous stem extract was subjected to chromatography on a Silica gel-60 column (40 cm length, 2 cm i.d.) equilibrated with hexane and eluted with a stepwise gradient of hexane and ethyl ether. 90 fractions of 5 mL each were collected, filtrated and concentrated under reduced pressure. The solid extract was dissolved in dimethylsulfoxide (DMSO) at a concentration of 100 mg dry weight /ml for the "in vitro" viability test.



The tumor cell line was incubated with these fractions and it was calculated the growing inhibition percentage allowing to separate one or more cytotoxic fractions.

The second division column was realized with the previously fraction active on a Silica gel-60 column (40 cm length, 2 cm i.d.) equilibrated with hexane:ethyl ether (9:1 v/v) and eluted with a stepwise gradient of hexane and ethyl ether. 50 fractions of 5 ml each were collected and analyzed by TLC aluminium sheets before it were tested by "in vitro" viability test.

Thin layer chromatography

The fractions eluted of second silica column were subjected to thin layer chromatography (TLC) examination on aluminium sheets pre-coated with silica gel 60 F 254 (Merck). Mobile phase was hexane: ethyl ether (1:1 v/v). The chromatograms were observed without chemical treatment, under UV 254 and UV 365 nm light. On the basis of the TLC profile the similar fractions were pooled into 5 fractions: I, II, III, IV and V, Figure 1.

Microscopic analysis

The cells were observed by means of optical Microscopy after the incubation with the extracts and fractions of *Thelesperma megapotamicum* to analyze changes in the morphology compared with the control cells (cells without treatment, with culture medium DMEM). The observation was realized at 10X and 40X.

Statistical analysis.

Data were reported as means \pm SEM ($n \geq 3$). Differences between treatments were established by ANOVA followed by the LSD Fisher test ($p < 0.05$).

Results and discussion

In order to evaluate the cytotoxic effect of *Thelesperma megapotamicum*, hexane, ethyl ether and ethanol extracts were assayed on the MCF-7. With regard to the whole plant extracts used, all the extracts exhibited a strong cytotoxic effect with not significant different at the concentration and time assayed (Table 1).

On the other hand, hexane and ethanol stoloniferous stem extracts had more cytotoxic activity than the ethyl ether extracts (Table 2).

The hexane stoloniferous stem extracts was eluted in chromatographic column (silica gel) with increasing polarities of hexane:ethyl ether mixture (100:0 to 0:100 v/v). The resulting hexane stoloniferous stem extracts elution (15 fractions) showed an active fraction on MCF-7 cell "in

vitro" culture (hexane:ethyl ether 1:1 v/v fraction).

Table 1: Viability percentage after incubation with different concentration of whole plants extracts (50 and 100 $\mu\text{g/ml}$)

Extraction	50 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$
Hexane	25,42 \pm 0,72 %	27,86 \pm 0,64 %
Ethyl ether	28,20 \pm 1,19 %	29,89 \pm 1,10 %
Ethanol	39,69 \pm 3,58 %	30,06 \pm 2,63 %

Table 2: Viability percentage after incubation with different concentration of stoloniferous stem extracts (50 and 100 $\mu\text{g/ml}$)

Extraction	50 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$
hexane	27,02 \pm 1,98 %	26,43 \pm 0,47 %
Ethyl ether	69,33 \pm 2,90 %	34,79 \pm 4,06 %
Ethanol	24,49 \pm 1,28 %	24,83 \pm 2,06 %

Table 3: Viability percentage after incubation with different concentration of stoloniferous stem fractions (50 and 100 $\mu\text{g/ml}$)

Fraction	50 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$
1	49,14 \pm 8,87 %	31,24 \pm 2,74 %
2	26,35 \pm 1,63 %	29,89 \pm 1,27 %
3	23,31 \pm 0,53 %	24,07 \pm 0,67 %
4	77,09 \pm 20,00 %	66,79 \pm 22,84 %
5	73,21 \pm 7,73 %	48,81 \pm 5,57 %

An additional separation of this active fraction (hexane:ethyl ether 1:1 v/v) was done in silica gel column, guided by thin layer chromatography (TLC) with hexane:ethyl ether 1:1 v/v. The fractions eluted of the column with increasing polarities of hexane:ethyl ether mixture (90:10 to



0:100 v/v) gave five new fractions according to profile of migration by TLC (Fig. 1). Each fraction was formed by several compounds. The fractions I, II and III exhibited a pronounced cytotoxic effect against human tumor cell line, giving a small percentage of viability ($31,24 \pm 2,74$; $29,89 \pm 1,27$ and $24,07 \pm 0,67\%$ respectively) (Table 3). These results suggest that *T. megapotamicum* cytotoxic activity (on at least the hexane:ethyl ether fraction 70:30 v/v) could be attributed at more of one compounds (TLC).

The microscopic photography of the cell MCF-7 incubated with the stoloniferous stem extracts and the fractions derived of column from stoloniferous stem hexane:ethyl ether (1:1 v/v) extracts showed a marked decreased in the viable cell number to respect the control cells (cell without treatment). The cell treated with active extracts (I, II and III) were grouped in cellular conglomerate, it was not observed at the control cell, which were abundant and were distributed in all the visual campo of the microscope (Figure 2 and 3).

Conclusions

In future, the active fraction of *Thelesperma megapotamicum* that have shown pronounced cytotoxic activity in this study will be further evaluated for the possible isolation of active antitumor compounds.

Note: This study was presented at the "II Reunión de Biotecnología Aplicada a Plantas Medicinales y Aromáticas" (Second Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2009.

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Note: Part of this study was presented at the 'II Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (Second Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2009.

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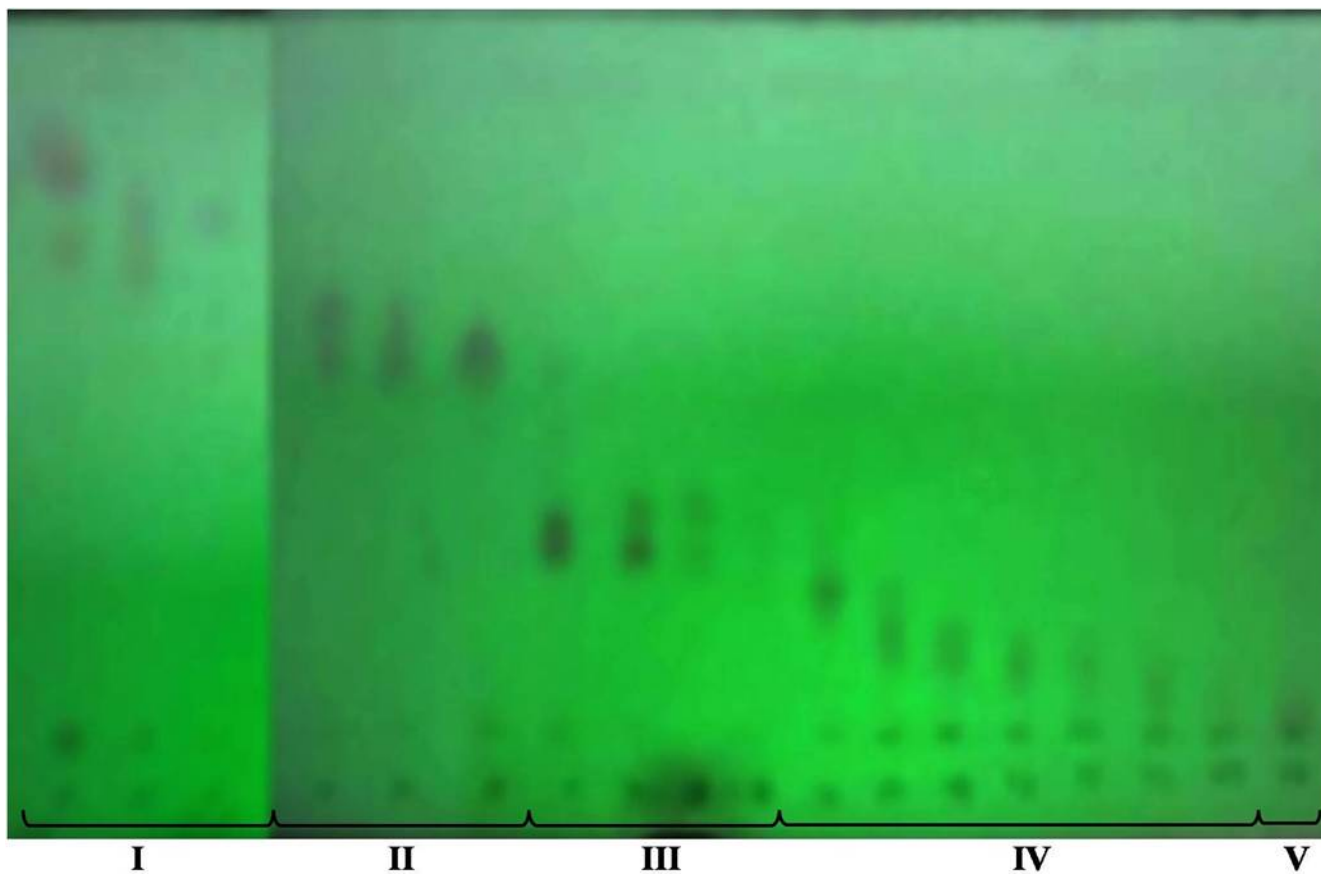
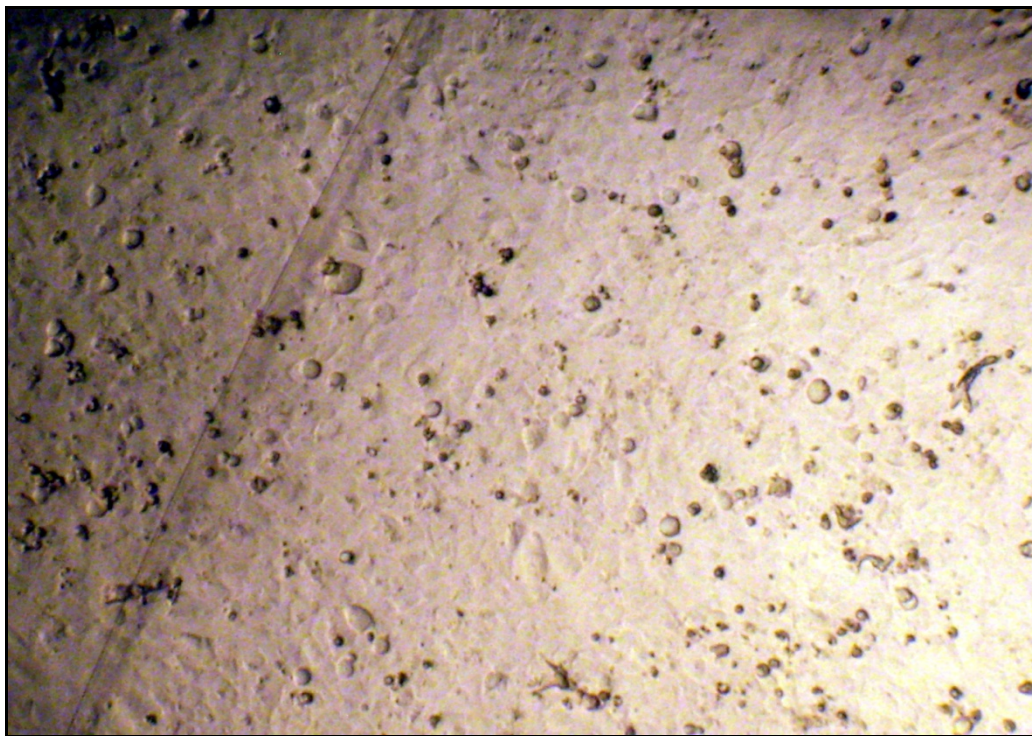
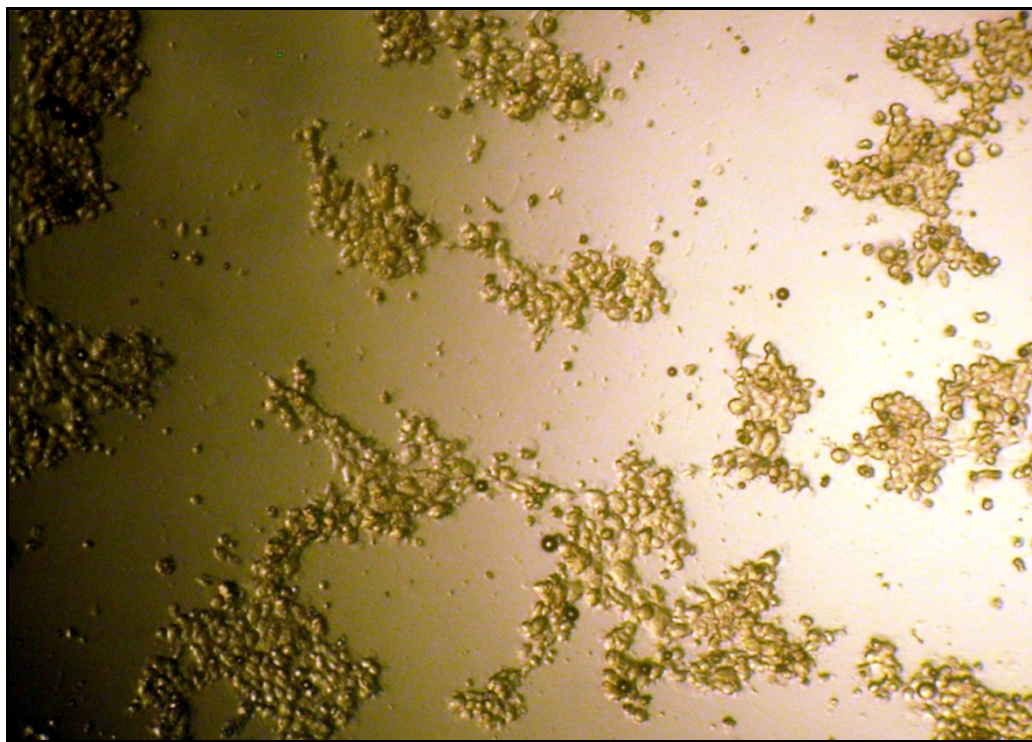


Figure 1: Fractions of stoloniferous stem hexan:ethyl ether extracts eluted of column. TLC profile of migration. The numbers represent the fraction that were pooled



A



B

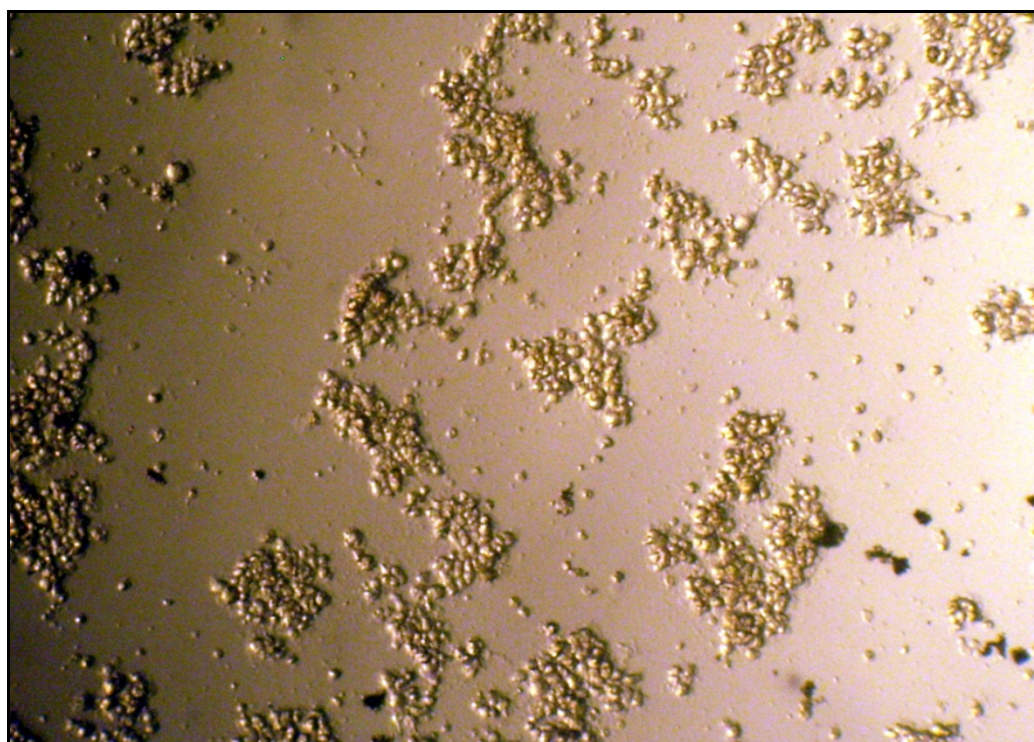
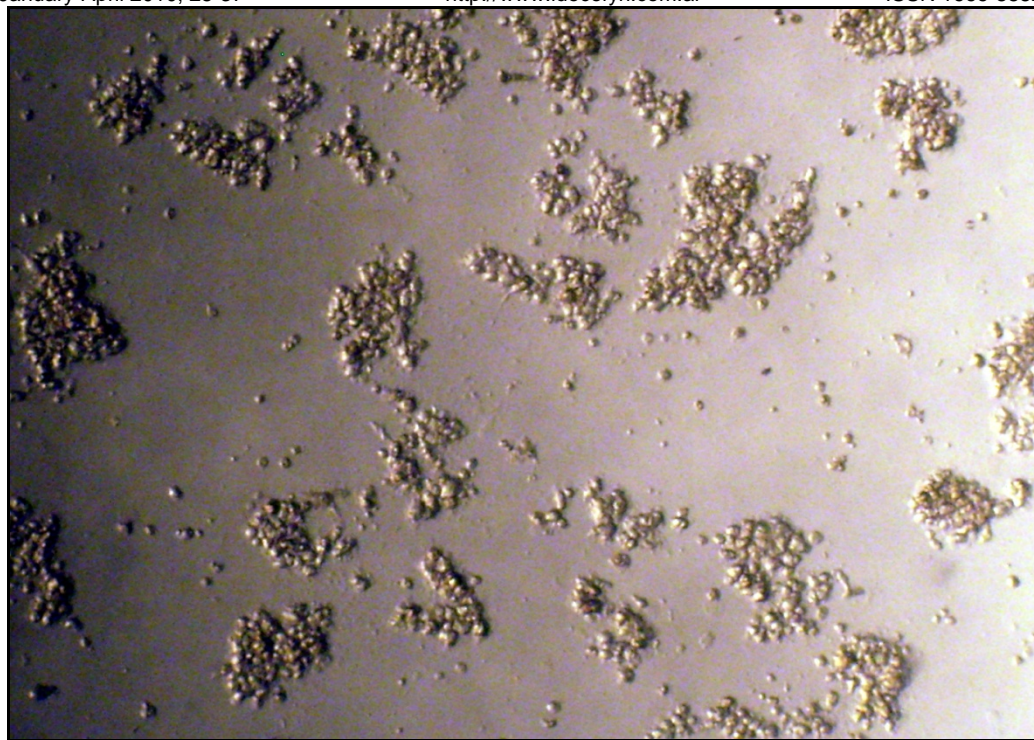
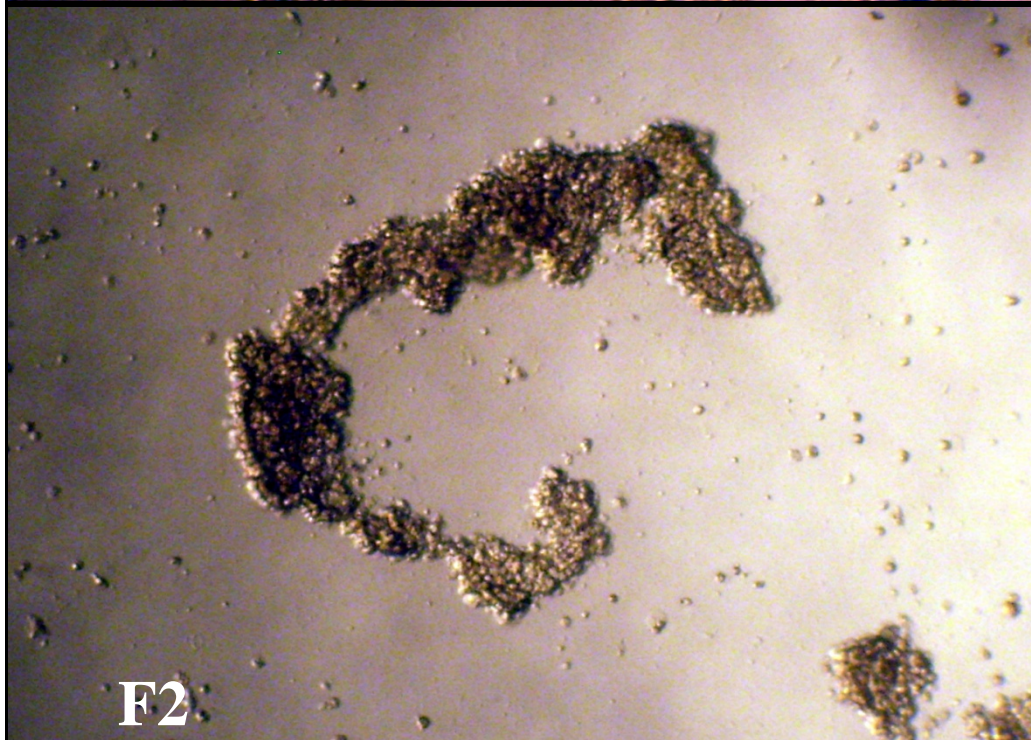
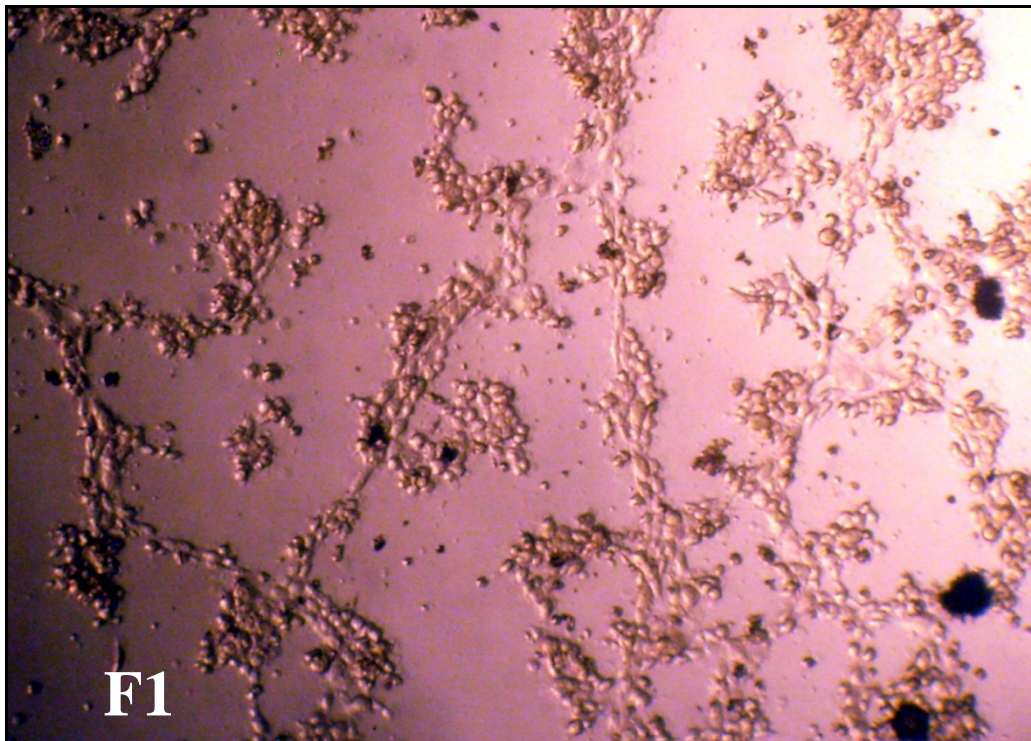
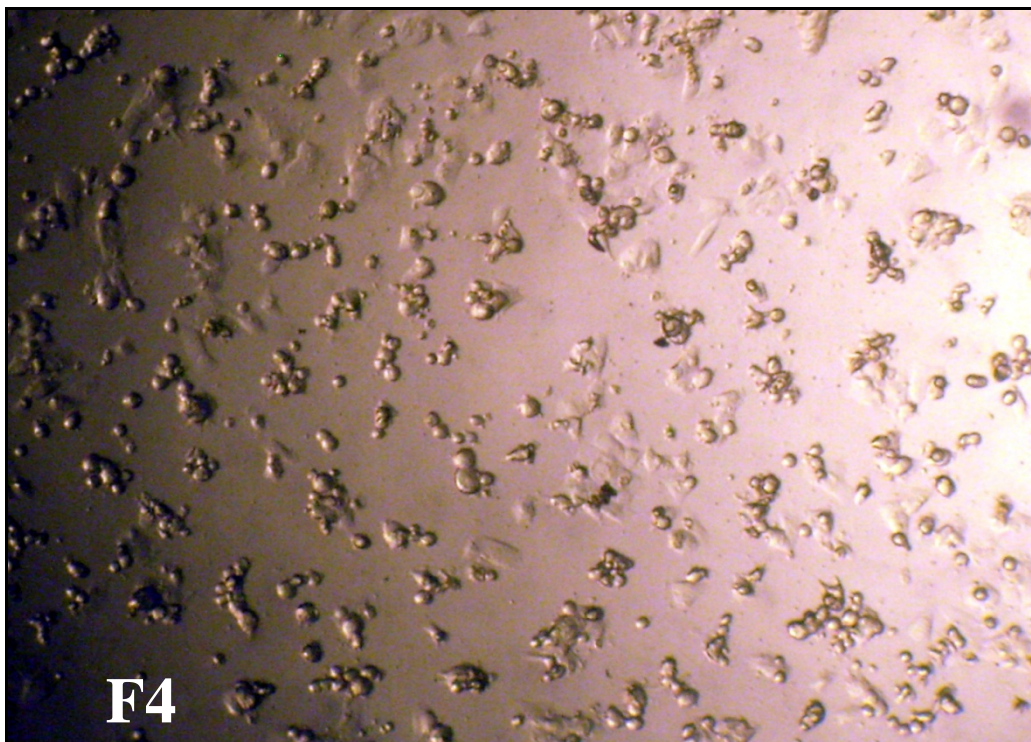
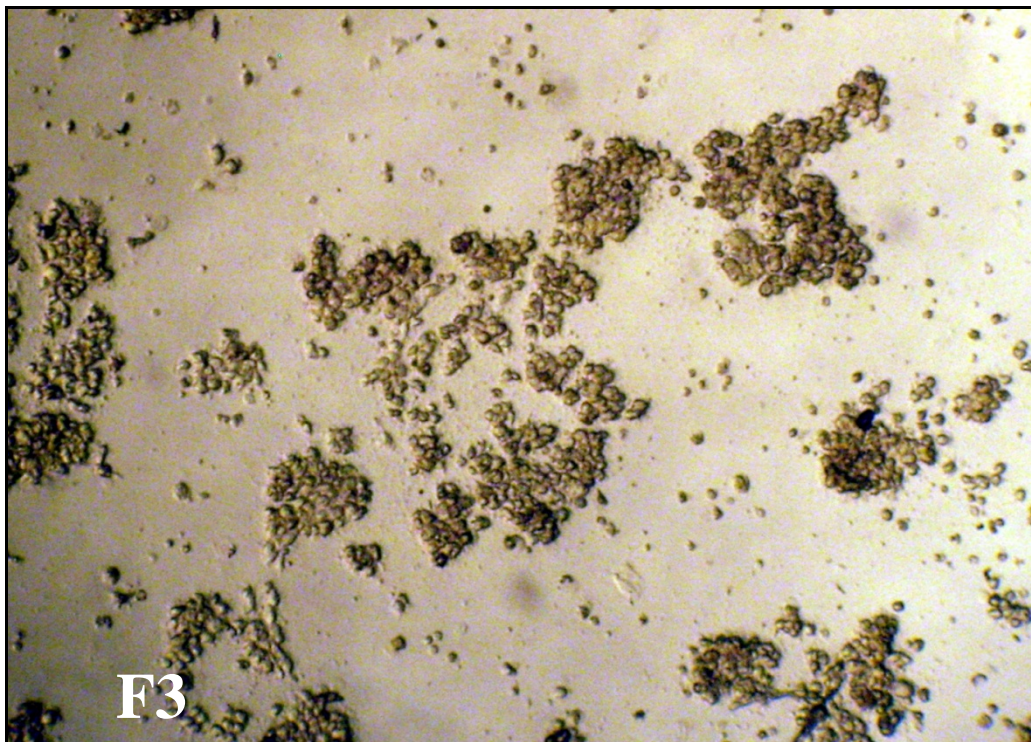


Figure 2: Cells MCF-7 later to the incubation with organics extracts of stoloniferous stems, 100 $\mu\text{g/ml}$. Optical microscopy 10X. Extracts done in A-DMEM, B-hexane, C-ethyl ether, D-ethanol.





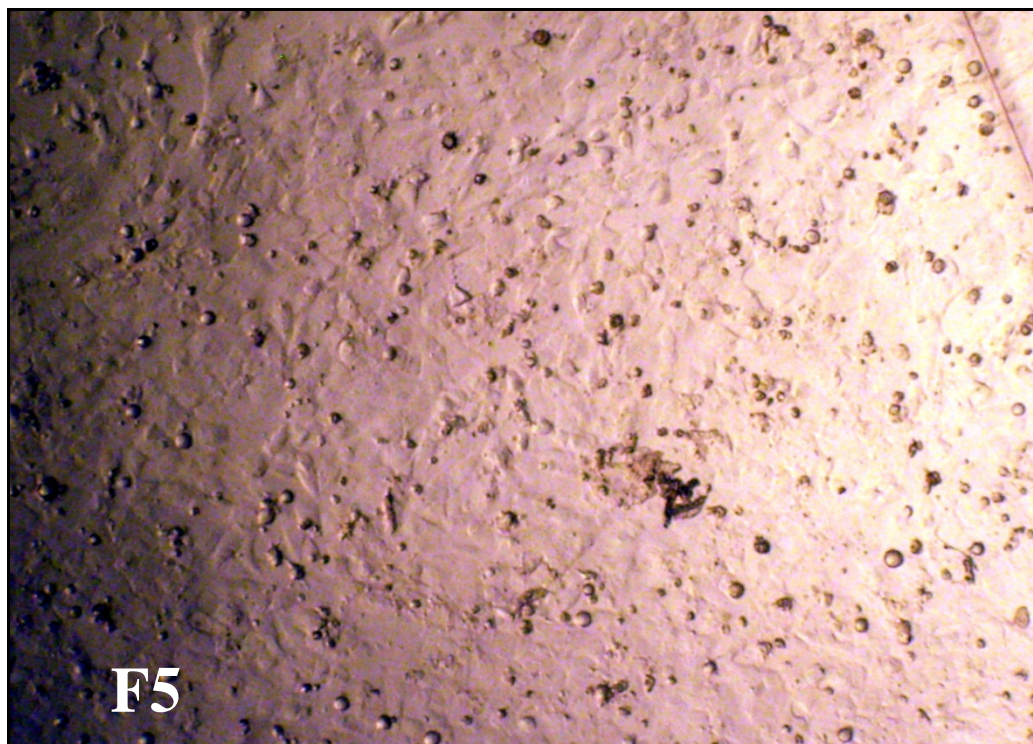


Figure 3: Cells MCF-7 incubated with fractions (F1 to F5) from an extract of stoloniferous stem (hexane:ethyl ether 1:1 v/v) 100 µg/ml. Optical microscopy 10X.