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Borojoa patinoi CELL CULTURE FOR THE PRODUCTION OF COMPOUNDS WITH ANTICANCER ACTIVITY

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Abstract – Borojó (*Borojoa patinoi*) is an arboreal species native to Colombia, used ancestrally for wound healing, pulmonary problems and hypertension control. In this research an *in vitro* culture of borojó cells in suspension was established in order to evaluate its potential in the production of metabolites of pharmaceutical interest, specifically in cancer. Thus, different culture media (MS, B5, and White) and different plugs in the shake flask (cotton, foam, and aluminum foil plugs) were evaluated for *B. patinoi* cell culture growth *in vitro*. No differences in cell growth were found using the culture media and plugs evaluated. The specific growth rate was 0.11 $\pm$ 0.01 day^{-1} with a doubling time of 6.9 $\pm$ 0.9 days. MTT test showed the cytotoxic effect of crude extracts of *B. patinoi* cells on Jurkat and MCF7 tumor cell lines. The extracts obtained with hexane and ethyl acetate at 150 µg/mL reduced cancer cells viability between 45 and 57%. Selectivity towards tumor cells was observed, presenting a greater effect in cancer cells. Furthermore, significant clastogenic damage (27.8%) to Jurkat cells DNA. Finally, chlorogenic acid, coumarins, terpenes, saponins, and sesquiterpene lactones, secondary metabolites of pharmaceutical interest were identified in *B. patinoi* cells. To our knowledge, this is the first report on the establishment of B. *patinoi* cell cultures in suspension and evaluation of its potential for obtaining compounds, specifically anticancer.

INTRODUCTION

Cancer is the second leading cause of death in the world after cardiovascular diseases (Park, 2012). Every year, cancer is responsible for approximately 8.2 million deaths. It is estimated that by the year 2030, 21.7 million new cases of cancer will be presented annually, which will cause 13.0 million deaths per year (American Cancer Society, 2015). In 2011, cancer treatment in the United States cost 88,700 million dollars, 11% corresponding to prescription drug. In this sense, there is an urgent need for more effective new drugs which reduce the cost of cancer treatments. Many of these drugs could be obtained from plants, which are a significant source of compounds of interest in the pharmaceutical industry. Currently more than 60% of chemotherapy drugs are derived directly or indirectly from these natural sources (Newman *et al.*, 2007). Flavonoids, terpenes, saponins, coumarins, anthraquinones, and alkaloids are an example. Despite the phytochemical potential of bioactive secondary metabolites of plants, they have complex chemical structures and in most cases, their concentration is significantly low in the wild plant. Low yields can lead to plants overexploitation in natural habitat, endangering species in this process (Lalaleo *et al.*, 2016). *In vitro* cultures of plant cells arise as a biotechnological alternative for the production of these compounds, because it is possible to establish contralateral conditions for cell growth and development, which favor yield when obtaining the compound, independently of climatic conditions or geopolitical factors.

The great variety of biologically active natural compounds and their wide diversity cause that a constant interest to find new metabolites with biological activity persists. There are about 400,000 species of higher plants in the world. Colombia, thanks to its geographical location in the equatorial zone, is one of the 12 richest countries in biodiversity worldwide, hosting around 15% of plant species on the planet (Romero *et al.*, 2008). One of these species is borojó (*Borojoa patinoi*), which was used ancestrally by indigenous communities for healing wounds, pulmonary and diuretic problems,

and hypertension control. *B. patinoi* is an arboreal species belonging to the Rubieaceae family, native to the tropical humid forest of the Pacific Panama and western Colombia (biogeographic Chocó). The fruit is recognized for its great nutritional value (Moreno and Medina, 2007). Additionally, the presence of polyphenols, organic acids such as ascorbic and oxalic (Contreras *et al.*, 2011), triterpenes, flavonoids and phenols has been reported, with a total polyphenol concentration (800 mg AGEs/100g) higher than that reported for other species of the same family. In preliminary evaluations, the presence of sesquiterpenlactones was identified by our research group (data not shown).

The main purpose of plant cells culture *in vitro* is the biotechnological production of metabolites of interest on an industrial or commercial scale. However, to achieve this, system optimization is required. It can be achieved by improving biomass production, system productivity, and the release of the compound of interest to the culture medium. For this, highly productive cell lines must be selected, medium composition and culture conditions must be varied, inducers and precursors must be added, a good bioreactor design must be sought, and the extraction and purification processes of the product must be improved (Cusido *et al.*, 2014). The objectives of this research were to establish an *in vitro* culture of *B. patinoi* cells to evaluate cellular growth in borojó suspension using different media of basal culture and plug types in Erlenmeyer flask, and to evaluate the cytotoxic activity and genotoxicity of *B. patinoi* cell suspension. There are no previous reports of the establishment of *B. patinoi* cell cultures in suspension, or the evaluation of the bioactive potential of its compounds. This work is a starting point for the development of new investigations with *B. patinoi in vitro* cultures that allow identifying and characterizing compounds with biological activity. It is expected that this crop become a platform for the production of chemopreventive drugs or as therapeutics for cancer treatment.

MATERIALS AND METHODS

Establishment of *B. patinoi* culture *in vitro*

For the establishment of *in vitro* culture, fruit seeds collected in the municipality of San Carlos, Antioquia, Colombia, were used. Seed was disinfected with sodium hypochlorite (2% v/v, 20 min), ethanol solution (70% v/v, 5 min) and rinsed with sterile distilled water. Disinfected seeds were placed in solid culture medium, composed of Murashige and Skoog basic salts and vitamins (MS) (Murashige and Skoog, 1962) with 20 g/L of sucrose. Callus of *B. patinoi* were obtained from leaves stemming from seeds germinated *in vitro*, in MS culture medium, 40 g/L of sucrose, 1 g/L casein hydrolyzate, 0.3 mg/L of BAP (6 - benzylaminopurine), 0.1 mg/L of 2,4-D (2,4 D-dichlorophenoxyacetic acid) and 7.0 g/L agar-agar. pH 6.3 before sterilizing. Media was sterilized in autoclave at 121 °C, 20 min. Calluses were kept in dark at 26 °C. For the establishment of *B. patinoi* cell suspensions, the same culture medium used for calluses formation without the gelling agent (agar-agar) was used. The calluses were macerated and cultured in 500 mL Erlenmeyer flasks with cotton plug, containing 20% of the culture medium. Cell suspensions were grown in dark, 25 °C, shaking at 120 rpm. Every 12 days the suspension subculture was carried out.

Evaluation of cell growth on culture medium and plug type in Erlenmeyer flask

Cell growth was evaluated by means of the kinetics in liquid culture media MS, B5 (Gamborg *et al.*, 1976), and White (White, 1951) in Erlenmeyer flask using a cotton plug. On the other hand, three different plug types; cotton (3.3 g), polyurethane foam and aluminum were evaluated, using the previous culture medium without agar. Biomass concentration was determined by dry weight; 5 ml of homogenous sample were filtered in vacuum and dried at 60 °C for 24 h. Cell viability was determined evaluating cell membrane integrity by Evans blue dye test (Gaff and Okong O-ogola, 1971).

Obtaining extracts

Cell suspensions were used in exponential phase (12 days) grown in MS medium, cotton plug, and in dark. After filtering suspensions in vacuous and separating cells from culture medium, biomass was lyophilized and macerated. The extraction was carried out in a Soxhlet extractor (Tecnivilab 100 mL) with organic solvents of different polarity: methanol, ethanol, acetone, ethyl acetate, dichloromethane, chloroform, and hexane. 2.0 g of biomass per 100 mL of solvent were used and a total of 4 cycles in the extractor were performed. Subsequently, extracts were concentrated under vacuum and to dryness in a rotary evaporator (RV10 IKA). Finally, extracts were dissolved in 99.5%

dimethylsulfoxide (DMSO) and stored away from light at -20 °C until use.

Determination of cytotoxic activity

MTT test (3-bromide (4, 5-dimethylthiazol-2-yl)-2-5-diphenyltetrazole) was performed using CHO-K1 (cells isolated from Chinese hamster ovaries), Jurkat (acute lymphoid leukemia cells), and MCF7 (isolated line of breast adenocarcinoma) cell lines. Cells were cultured with RPMI-1640 culture medium (Sigma-Aldrich) supplemented with 5% fetal bovine serum (SFB) (Eurobio), 100 µg/mL streptomycin (Sigma-Aldrich) at 37 °C, and a humid atmosphere of CO_2 to 5%. Cultures with a confluence and cell viability greater than 80 and 95% were used. Treatments lasted 24 hours. To determine cell viability with MTT (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazole bromide) the methodology proposed by Hansen (1989)was followed with modifications. 10 µL of MTT (5 mg/mL) were added to each well and incubated for 4 hours under dark conditions. Next, isopropanol acid was added to dissolve formazan crystals. Finally, absorbance was read at 570 nm on a spectrophotometer (Thermo Scientific Genesys 20). Additionally, the hemolytic activity described by Torres-Domínguez (2005) was evaluated.

Determination of genotoxic activity

To perform the genotoxic assay using comet test, cell cultures with a confluence and cell viability higher than 80 and 95%, respectively, were used. 2500 µL of cell suspension were added to each well with approximately 1 x 10^5 cells in RPMI medium, 5% SFB, and 1% streptomycin. Cells were incubated for 24 h at 37 ° C and an atmosphere of 5% CO_2. After this culture time, 250 µL of the extracts to be evaluated were added in such a way that the final concentration of extract in each well was 10 µg/mL. The treatment lasted 24 hours. The protocol described by Singh (1988) was followed. The CaspLab program (version 1.2.3 beta 2) was used to determine DNA damage percentage, tail length of 50 nucleoids was analyzed for each of the evaluated extracts, including a solvent control (DMSO 1.0%) and a positive control (25 mM hydrogen peroxide).

RESULTS AND DISCUSSION

In vitro culture evaluation of culture media and plug type in Erlenmeyer flask

Germination time of the borojó seed *in vitro* was 6 months (seedling, Figure 1). Different culture media were evaluated for callus formation (data not shown). However, the best combination was the one described above with BAP and 2,4D. Callus formation was evidenced after 9 months of leaves inoculation in the semi solid culture medium with the previous growth regulators. Cell suspensions were formed by inoculating anterior friable calli in liquid medium with periodic subcultures and continuous agitation at 120 rpm.

According to Gamborg (1976), culture medium is a determining factor in cell growth. Optimal concentration and proportion of mineral salts are critical in cell growth and development from suspension cell cultures (Ramachandra Rao and Ravishankar, 2002). The culture media developed for plant cells *in vitro* cultures have the purpose of supplying macro and micronutrient requirements necessary for cell growth and development. Figure 2 shows cell growth kinetics in MS medium. The initial biomass concentration was 2.41 ± 0.17 g DW/L and the maximum concentration of biomass reached was 23.76 ± 0.99 g DW/L, obtained on day 21. Initial cell viability was 61.9% and a slight decrease was observed in the first 6 days of culture.

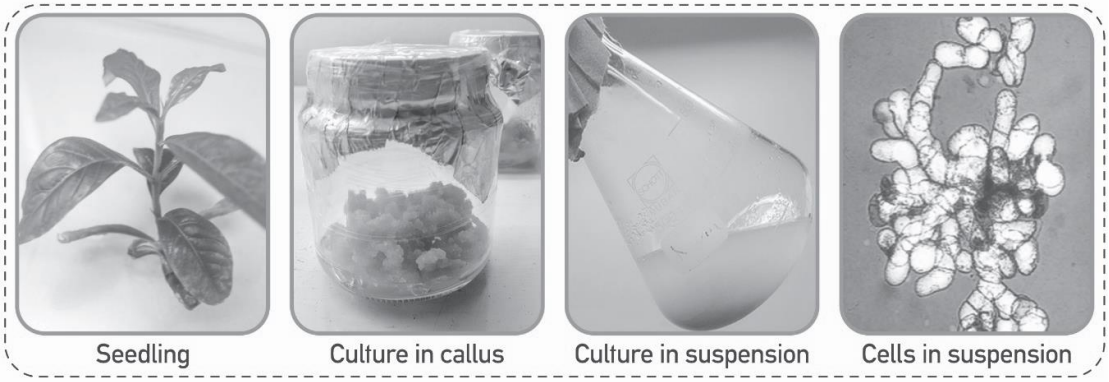

Fig. 1. *B. patinoi in vitro* culture of seedlings, callus and cells in suspension.

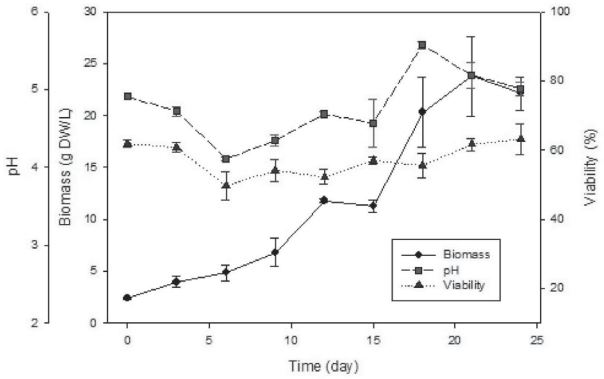

Fig. 2. Growth kinetics, pH and cell viability of *B. patinoi* cell culture in suspension. MS culture medium and cotton plug.

However, from that day, viability increased until reaching 24 values close to the initial day (65%). At the end of the exponential phase, viability increased, even though pH decreased from 5.6 to 5.0. Then, cellular concentration began to become constant. The difference in the composition of MS, B5 and White culture media macro and micronutrients is vast. However, there were no statistically significant differences in cell growth in borojó suspension in these three media. Possibly, requirements of each vitamin, macro and micronutrient provided by culture media evaluated at the lowest concentrations were sufficient to satisfy cell growth and development.

When cell growth in *B. patinoi* suspension was evaluated using the three plug types, no significant differences were found in its kinetic parameters. In agitated cultures oxygen availability is a crucial factor for the metabolic development of each system. In this sense, oxygen transfer in shaken flasks is an important aspect that could affect growth and secondary metabolism of cell culture (Orozco, 2009). The plug type used in plant cell cultures in shake flasks may significantly influence gas exchange between the culture atmosphere and the exterior of the flask (Lee and Shuler, 1991). The three plug types used show differences in gas exchange capacity between the flask and the environment. However, specific growth rate was on average 0.10 day $^{-1}$ with a doubling time of 6.9 days for the three plugs. There was also no evidence of a latency phase; the duration of exponential phase was 14 days and cell death phase started on day 28 of cell culture. Similar growth of *B.patinoi* cells with the three conditions of oxygen transfer could be due to the fact that oxygen requirements are not high and cells grow adequately with the low supply of oxygen provided by the aluminum plug. A low oxygen demand by plant cells is also shown by *Thevetia peruviana* (Arias, 2013), *Uncaria tomentosa* (Trejo-Tapia and Rodríguez-Monroy, 2007) and *Beta vulgaris* (Rodríguez-Monroy and Galindo, 1999) cell cultures. The use of aluminum plug in these cultures does not present limitations in cell growth. In contrast to what happened with *Azadirachta indica* cells, where the use of aluminum plugs significantly reduced cell growth and azadirachtins production (Orozco-Sánchez *et al.*, 2011).

Cytotoxic evaluation

Due to the limited information available on the bioactive properties or content of specific secondary metabolites of borojó, a screening of solvents covering a wide range of polarity was carried out, so that the compounds or metabolites with biological activity present in the cells can be extracted differentially. Extraction solvents were evaporated completely and the biological activity shown corresponds exclusively to the cell extract (Fig. 3).

Cytotoxic activity of medium-low polarity crude extracts on Jurkat cells increased when increasing concentration. The greatest effect on cell viability was evidenced by ethyl acetate extract at 150 µg/mL, which reduced Jurkat cells viability to 43%, followed by acetone, hexane, and dichloromethane, which reduced cancer cells viability to approximately 60%. On the other hand, in MCF7 cells the highest cytotoxic activity was presented with hexane extract, which reduced cells viability by 52%, followed by ethyl acetate 48%, and dichloromethane 47%. In this case, solvent control with 1.0% DMSO showed a decrease of 25% in cell viability. However, they did not show statistically significant differences with negative control. Unlike cytotoxic activity in Jurkat, methanolic and ethanolic extracts in MCF7 cell line showed a decrease in cell viability of 22 and 26% respectively. Additionally, in MCF7 cell line, there was a particularity in the dose-response relationship between treatment concentration and cell viability percentage in all extracts except acetone. In these extracts, dichloromethane and hexane, treatments with the lowest concentration (10 µg/mL), generated a greater decrease in cell viability. On the other hand, in CHO K1 cell line the extract with greatest effect was ethyl acetate at 150 µg/mL, which decreased cell viability to 33.8%. Extracts that also had an important cytotoxic activity were acetone, methanol, and ethanol.

One of the main characteristics desired when looking for new compounds or metabolites with a view to chemotherapeutic drugs production is the selectivity they may have to differentiate a cancer cell from a non-cancer cell. Currently, most of the research in this field is focused on the search for new more effective drugs in disease treatment which entail fewer side effects and achieve neoplasms elimination, minimizing genotoxic damage in patients (Ghantous *et al.*, 2010). Results described above, clearly show that crude extracts of borojó cells in suspension have a cytotoxic effect on Jurkat, MCF7 and CHO cell lines. However, Figure 3 shows that there were statistically significant differences in cell viability reduction in cancer cells with growth in suspension (Jurkat), in comparison with adherent (MCF7 and CHO). In contrast to cytotoxic activity in Jurkat cells, CHO, and MCF7 showed a decrease in

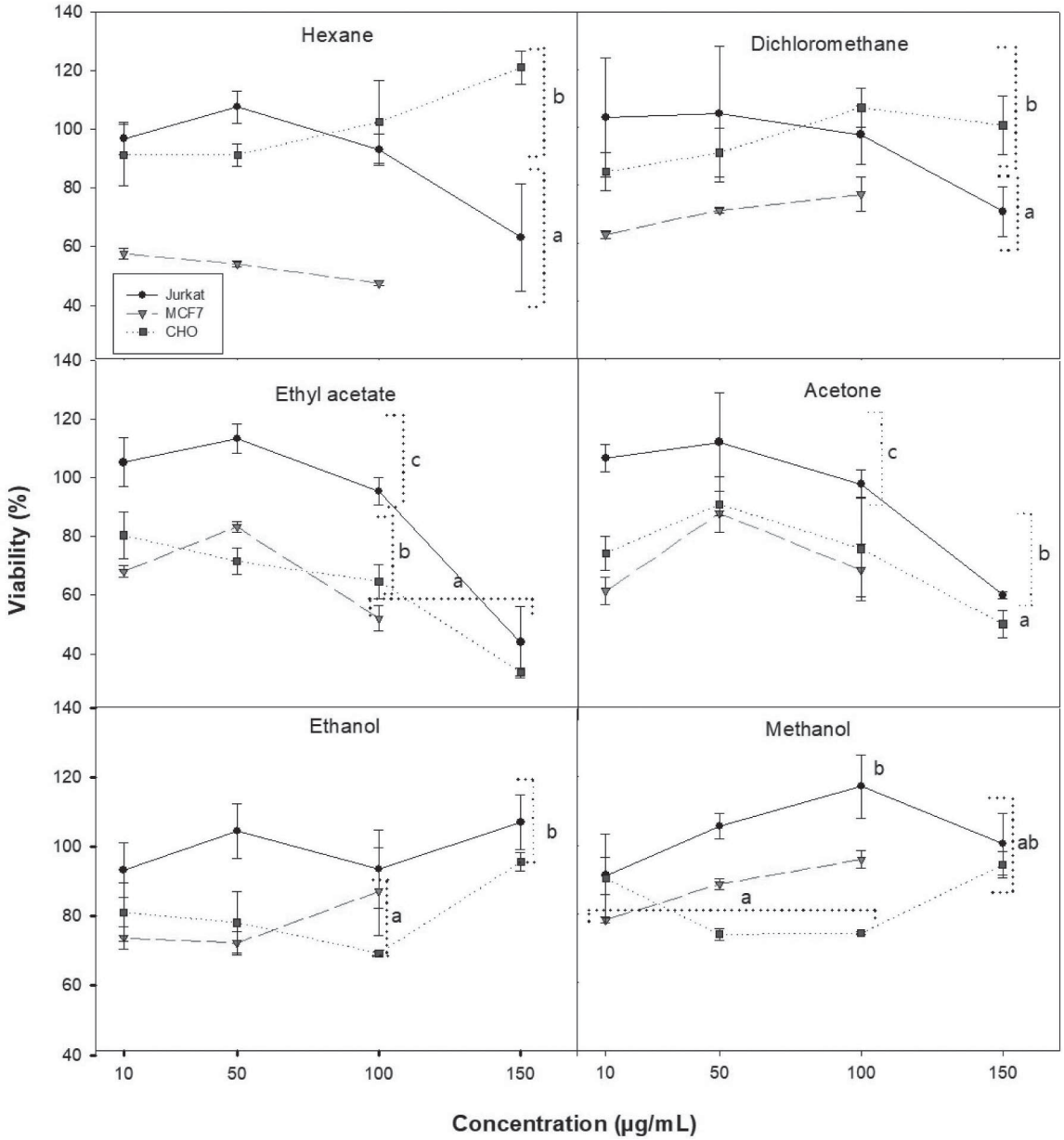

Fig. 3. Effect of crude extracts of *B. patinoi* cell culture obtained with different organic solvents (hexane, ethyl acetate, acetone, dichloromethane, methanol and ethanol) on the viability of CHO, MCF7 and Jurkat cells by MTT assay. Different letters represent statistically significant differences ($p < 0.5$, Fisher's LSD). Data is shown as the average of 4 replicates ± SE

cell viability with ethyl acetate, acetone, and ethanol extracts. Possibly these extracts have compounds or metabolites which act differentially on adhered and suspended cells.

According to Damiano *et al.* (1999); to establish differences in cytotoxic activity caused by a potential active compound in cancer or non-cancer cells, it is important to take into account the nature of the cell line, that is, whether it grows in monolayer or suspension. Cell Adhesion Molecules (CAMs) present in cell-cell interactions of adherent cell lines influence survival and can prevent programmed cell death. It has been shown that cell adhesion mediated by integrin fibronectin (CAM) confers a survival advantage for myeloma cells exposed to cytotoxic drugs, which inhibit apoptosis induced by these drugs (Bates *et al.,* 1994; Damiano *et al.,* 1999). In our case, at a concentration equal to or less than 100 µg/mL of hexane, ethyl acetate, dichloromethane, and acetone extracts, they had greater cytotoxic activity in MCF7 (adhered) cell line in comparison with Jurkat cell line (suspension). The IC_{50} for hexane extracts (62.63 ± 7.25) and ethyl acetate (101.70 ± 15.34) is lower in MCF7 cells compared to Jurkat cells (124.89 ± 4.58, 166.07 ± 5.51, respectively). Possibly, these extracts contain metabolites acting directly and with greater effectiveness on cell-CAM interactions, allowing the entry of this compound into the cell. This income can generate a decrease in mitochondrial metabolic activity, which would eventually lead to cell viability reduction. This behavior has been reported with *H. obovatus, K. coriácea and C. sylvestris* extracts where the IC_{50} reported for glioblastoma SF-295 cells (adherent cells) is lower than Leukemia HL-60 cells growth in suspension (de Mesquita *et al.,* 2009).

Additionally, with ethyl acetate, dichloromethane, and with greater differences in hexane extracts, a desired selectivity was presented to reduce cell viability of MCF-7 cancer cells and, in a lesser percentage, in non-cancerous cells. This selective behavior in cells with monolayer growth has been reported by Jaunky and collaborators (2016). These researchers performed compound identification with selective toxicity, directed towards MCF-7 cells (breast cancer) compared to non-cancerous cells HFF-1 (foreskin fibroblasts). Unlike what happens in HFF-1 cells, where mitotic spindle is correctly organized, in cervical cancer cells (HeLa) and MCF-7 the compound binds directly to tubulin subunits, modifying microtubules assembly dynamics, which ultimately trigger cell arrest in mitosis.

To determine whether compounds present in crude extracts of *B. patinoi* cells in suspension cause cytotoxicity in normal human cells, the ability to cause disruption to cell membrane of human erythrocytes by induction of cell lysis was evaluated. Hemolysis assay showed that none of the evaluated extracts (500 µg/mL) caused damage to human erythrocytes membrane. The maximum permissible value to consider a compound with proven biological activity to be innocuous in human erythrocytes is 10%. The extract that showed highest hemolytic activity on human erythrocytes was hexane with 4.23 ± 0.66%.

Genotoxic evaluation

Comet assay showed that crude extracts of borojó cells in suspension caused significant clastogenic damage to acute lymphoid leukemia cells DNA (Jurkat cell line). The three evaluated extracts presented significant differences with solvent control ($p < 0.5$, Fisher 's LSD). Hexane extract generated a greater damage in DNA 27.82%. However, no differences were observed with ethyl acetate and dichloromethane extracts 24.16 and 23.88%, respectively (Fig. 4).

There are few studies that report *in vitro* genotoxic activity of crude extracts of species belonging to the Rubiaceae family on different cancer models. The clastogenic activity of *Uncaria tomentosa* on colon adenocarcinoma cells HT29 (De Oliveira *et al.,* 2014) and *Oldenlandia diffusa* on HL60 leukemia cells (Willimott *et al.,* 2007) are highlighted. Given the genotoxic damage generated by crude extracts of borojó cell suspensions in Jurkat cells, it could be said that *B.patinoi* is emerging as another species exhibiting this type of biological activity.

Phytochemical analysis

The presence of chlorogenic acids (CGA), coumarins, terpenes, steroids, and saponins was identified in crude extracts of borojó cell culture, that showed greater cytotoxic activity on Jurkat and MCF7 cancer cell lines (Table 1). The presence of phenolic compounds, triterpenes, flavonoids, tannins, alkaloids, and saponins has been identified in the pulp of borojo fruit (Sotelo *et al.,* 2010). In our case, for *in vitro* culture, no flavonoid compounds were identified in the evaluated non-polar extracts, which have no affinity for flavonoids. On the other hand, epidemiological studies have shown that

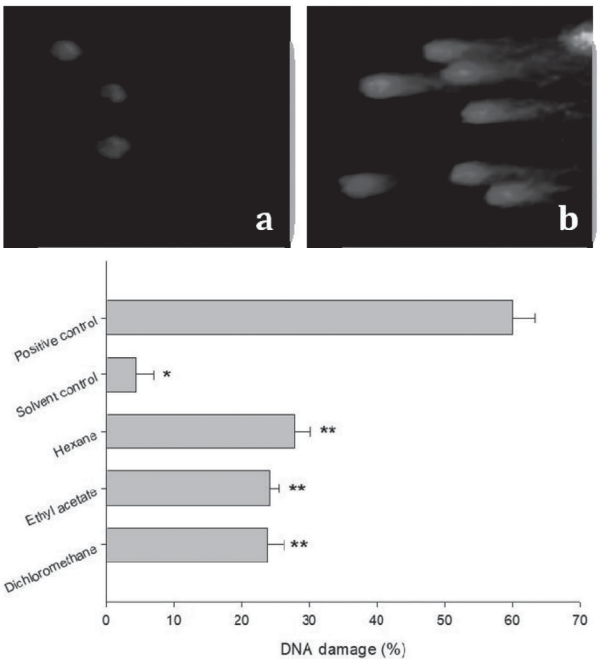

Fig. 4. Clastogenic effect in Jurkat cells treated with crude extracts of B. patinoi cell culture (10 µg/mL) obtained with different organic solvents. Solvent control (DMSO 1.0%), positive control (H_2O_2 25 mM).
a-Comet test solvent control. b- hexane comet test test. Asterisks (*) represent statistically significant differences with solvent control (p <0.5, Fisher's LSD). Data is shown as the average of 3 replicates ± SE.

Table 1. Phytochemical analysis of crude extracts of *B. patinoi* cell culture. (-) Absence of metabolites at detection level of the test; (±) Inconclusive answer; (+) Mild positive response; (++) Moderate positive response; (+++) High positive response.

Metabolite	Extract	
	Ethyl acetate	Hexane
Flavonoids	-	-
Chlorogenic acids	++	+
Coumarins	++	+
Terpenes and/or steroids	+++	++
Sesquiterpene lactones	+++	+++
Alkaloids	±	±
Lignans	-	-
Cardiotonic glycosides	-	-
Saponins	++	+

CGA has biological properties beneficial to health, such as reduced risk of cardiovascular disease, type 2 diabetes, Alzheimer, antiviral, antibacterial, and anti-inflammatory properties (Ji*et al.*, 2013; Shi *et al.*, 2013; Yun *et al.*, 2012). CGAs inhibited the proliferation of HepG2 cells (human liver cancer cell line) *in vitro* and the progression of HepG2 from xenograft *in vivo* (Yan *et al.*, 2017). As hexane and ethyl acetate extracts reduced the viability of MCF-7 cells, CGAs could be responsible for this activity.

Another type of metabolites identified in crude extracts of borojó cells in suspension were coumarins. More than 1,300 coumarins have been identified, some with anti-microbial, anti-HIV, anti-cancer, anti-retroviral or anti-tuberculosis properties (Keri *et al.*, 2015; Revankar *et al.*, 2017). According to Riveiro (2010) due to the antitumor, apoptotic, and effects that induce cellular differentiation, coumarins are promising compounds for cancer treatment. However, at present most of these compounds are chemically synthesized, presenting limitations in the toxic effect they may have on cancer models, since a strict dependence between chemical structure and biological activity has been found (Vázquez *et al.*, 2012).

On the other hand, terpenes have a significant economic value due to their widespread use in several industries, including pharmaceutical, food, and cosmetic sectors. A wide range of therapeutic drugs has been reported, such as anticancer, analgesic, anti-inflammatory, neuroprotective agents, immune modulators, and wound healers (Lima *et al.*, 2016). Fernandes (2013) reports cytotoxic activity of hexanic extracts of *Jatropharibifoli* roots on MCF7 breast cancer cell line, caused by terpenes present in *J. ribifoli.* In the same way, Bourgou (2017) identified terpenes and sesquiterpenlactones (SLs) in *Artemisia herba* extracts with biological activity on breast cancer cells (MCF7). Clinical trials have shown that SLs have selective properties that affect target cells and cancer stem cells, while causing no effect on normal cells (Zhou and Zhang, 2008). Ethyl acetate, dichloromethane and hexane extracts showed selectivity to reduce MCF-7 cancer cells viability and to a lesser extent the non-cancerous CHO. Additionally, as in the three extracts SLs type compounds were identified, it could be said that cytotoxic activity of crude extracts of *B. patinoi* cells in suspension is mediated by SLs. According to Ghantous (2010); the main disadvantages for the production of drugs with SLs as active compound, are structural complexity, chemical synthesis can be restricted, and low concentration available in plants, making natural resources be limited.

Although it is necessary to carry out a more

detailed identification of the compounds present in borojó cells cultured *in vitro,* cytotoxic and genotoxic activity observed on cancer cells could also be generated by the coumarins or sesquiterpenlactones detected in the extracts. In this sense, it could be said that cytotoxic activity presented by the extracts of cell culture could be partially mediated by terpenoid-like compounds. No reports are known about the biotechnological and medicinal potential of *B. patinoi* extracts, nor of plants grown *in vivo*, nor from cell cultures. In our study, *B. patinoi* cell culture was established as a possible productive system scalable at industrial level. In this way, borojó cells culture becomes a promising platform for the production of compounds with anticancer activity on a large scale.

CONCLUSION

In the present investigation an *in vitro* culture of *B. patinoi* cells in suspension in Erlenmeyer flask was established. No differences were found in cell growth using MS, B5, and White culture media. Cell growth was not affected by the plug type used in rough crops. When evaluating different extracts, it was found that the greatest effect on cell viability was caused by ethyl acetate extract at 150 µg/mL. This extract reduced Jurkat cells viability by 60%, followed by acetone, hexane and dichloromethane which reduced it approximately 40 %. In the case of MCF7 cells, the highest cytotoxic activity occurred with hexane extract, which reduced cell viability to 52%, followed by ethyl acetate 48%, and dichloromethane 47%. A greater cytotoxic effect on MCF7 cells adhering compared to growing Jurkat cells suspended was shown. Additionally, ethyl acetate, dichloromethane, and hexane extracts showed selectivity to reduce MCF-7 cancer cells viability and to a lesser extent the non-cancerous CHO. Similarly, dichloromethane caused significant clastogenic damage to Jurkat cells DNA. Furthermore, none of the extracts generated cytotoxicity in human erythrocytes at a concentration of 500 µg/mL. Finally, chlorogenic acid, coumarins, terpenes, saponins, and sesquiterpene lactones were identified in borojó cells in suspension. These compounds could possibly be affecting mitochondrial activity of Jurkat and MCF7 cancer cells, triggering their death or reducing their cell viability.

Based on our development and findings, *B. patinoi* cell culture is proposed as a biotechnological platform for obtaining compounds. These have a potential for drug production due to the observed properties, which could allow them to attack cancer cells without affecting the normal ones. This is the first report on the establishment of *B. patinoi* cell cultures in suspension, the cytotoxic and genotoxic evaluation of their extracts with a selective effect on cancer cells. In this way, *B. patinoi* becomes a promising species for the production of anti-cancer therapeutic compounds.

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REFERENCES

American Cancer Society. 2015. *Cancer Facts & Figures 2017*. https://doi.org/10.1101/gad.1593107

Arias, J. P. 2013. *Optimización de parámetros de cultivo en suspensión de células de la especie vegetal Thevetia peruviana y su efecto en la producción de metabolitos secundarios*. Universidad Nacional de Colombia.

Bates, R. C., Buret, A., Van Helden, D. F., Horton, M. A. and Burns, G. F. 1994. Apoptosis induced by inhibition of intercellular contact. *Journal of Cell Biology*. 125 (2) : 403–415.

Bourgou, S., Bettaieb, I., Mkadmini, K., Isoda, H., Ksouri, R. and Ksouri, W. M. 2017. LC-ESI-TOF-MS and GC-MS profiling of Artemisia herba-alba and evaluation of its bioactive properties. *Food Research International*. 99(1) : 702–712.

Contreras, J., Calderón, L., Guerra, E. and García, B. 2011. Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. *Food Research International*. 44 (7) : 2047–2053.

Cusido, R. M., Onrubia, M., Sabater-Jara, A. B., Moyano, E., Bonfill, M., Goossens, A. and Palazon, J. 2014. A rational approach to improving the biotechnological production of taxanes in plant cell cultures of Taxus spp. *Biotechnology Advances*. 32(6) : 1157–1167.

Damiano, J. ason S., Cress, A. E., Hazlehurst, L. A., Shtil, A. A. and Dalton, W. S. 1999. Cell adhesion mediated drug resistance (CAM-DR): role of integrins and

resistance to apoptosis in human myeloma cell lines. *Blood*. 93(5) : 1658–1667.

de Mesquita, M. L., de Paula, J. E., Pessoa, C., de Moraes, M. O., Costa-Lotufo, L. V., Grougnet, R. and Espindola, L. S. 2009. Cytotoxic activity of *Brazilian Cerrado* plants used in traditional medicine against cancer cell lines. *Journal of Ethnopharmacology*. 123(3): 439–445.

De Oliveira, L. Z., Farias, I. L. G., Rigo, M. L., Glanzner, W. G., Gonçalves, P. B. D., Cadoná, F. C. and Leal, D. B.R. 2014. Effect of *Uncaria tomentosa* extract on apoptosis triggered by oxaliplatin exposure on HT29 cells. *Evidence-Based Complementary and Alternative Medicine*. 2014(5) : 1–10.

Fernandes, E. de S., Rodrigues, F. A., Tófoli, D., Imamura, P. M., de Carvalho, J. E., Ruiz, A. L. T. G. and Silva, R. C. L. 2013. Isolation, structural identification and cytotoxic activity of hexanic extract, cyperenoic acid, and jatrophone terpenes from *Jatropha ribifolia* roots. *Revista Brasileira de Farmacognosia*. 23(3) : 441–446.

Gaff, D. F. and Okong O-ogola, O. 1971. The Use of Non-permeating Pigments for Testing the Survival of Cells. *Journal of Experimental Botany*. 22(72) : 756–758. Gamborg, O., Murashige, T., Thorpe, T. A. and Vasil, A. 1976. Plant tissue culture media. *In Vitro*. 12(15) : 473–478.

Ghantous, A., Gali-Muhtasib, H., Vuorela, H., Saliba, N. a, and Darwiche, N. 2010. What made sesquiterpene lactones reach cancer clinical trials? *Drug Discovery Today*. 15(15–16) : 668–678.

Hansen, M., Nielsen, S. and Berg, K. 1989. Re-examination and further development of a precise and rapid dye method for measuring cell growth / cell kill. *Jurnal of Immunological Methods*. 119(2) : 203–210.

Jaunky, D. B., Forgione, P. and Piekny, A. 2016. Abstract 3808: Novel compound conferring selectivity for cancer cells. *Cancer Research*. 76(14 Supplement), 3808.

Ji, L., Jiang, P., Lu, B., Sheng, Y., Wang, X. and Wang, Z. 2013. Chlorogenic acid, a dietary polyphenol, protects acetaminophen-induced liver injury and its mechanism. *The Journal of Nutritional Biochemistry*. 24(11) : 1911-1919.

Keri, R. S., B.S., S., Nagaraja, B. M. and Santos, M. A. 2015. Recent progress in the drug development of coumarin derivatives as potent antituberculosis agents. *European Journal of Medicinal Chemistry*. 100: 257–269.

Lalaleo, L., Khojasteh, A., Fattahi, M., Bonfill, M., Cusido, R. M. and Palazon, J. 2016. Plant Anti-cancer Agents and their Biotechnological Production in Plant Cell Biofactories. *Current Medicinal Chemistry*. 23(39) : 4418–4441.

Lee, C. W. T. and Shuler, M. L. 1991. Different shake flask closures alter gas phase composition and ajmalicine production in *Catharanthus roseus* cell suspensions. *Biotechnology Techniques*. 5(3) : 173–178.

Lima, P. S. S., Lucchese, A. M., Araújo-Filho, H. G., Menezes, P.P., Araújo, A. A. S., Quintans-Júnior, L. J. and Quintans, J. S. S. 2016. Inclusion of terpenes in cyclodextrins: Preparation, characterization and pharmacological approaches. *Carbohydrate Polymers*. 151 : 965–987.

Moreno, J. R. and Medina, M. 2007. Respuesta cultural y morfogenética de tres explantes de borojó *(Borojó patinoi* CUATRECASAS). *Revista Institucional Universidad Tecnológica Del Chocó*. 26 : 66–78.

Murashige, T. and Skoog, F. 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum*. 15(3) : 473–497.

Newman, D. J., Cragg, G. M. and Snader, K. 2007. Natural products as sources of new drugs over the last 25 years. *Natural Products*. 70(0163–3864) : 461–477.

Orozco-Sánchez, F., Sepúlveda-Jiménez, G., Trejo-Tapia, G., Zamilpa, A. and Rodríguez-Monroy, M. 2011. Oxygen limitations to grow *Azadirachta indica* cell culture in shake flasks. *Revista Mexicana de Ingeniería Química*. 10(1) : 343–352.

Orozco, F. 2009. *Efecto de la oferta de oxigeno sobre el crecimiento y la producción de terpenoides con células de Azadirachta indica en un biorreactor*. Instituto Politecnico Nacional.

Park, S. U. 2012. Anticancer compounds from plants. *EXCLI Journal*. 11 : 386–389.

Ramachandra Rao, S. and Ravishankar, G. A. 2002. Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnology Advances*. 20(2) : 101–153.

Revankar, H. M., Bukhari, S. N. A., Kumar, G. B. and Qin, H. L. 2017. Coumarins scaffolds as COX inhibitors. *Bioorganic Chemistry*. 71 : 146–159.

Riveiro, M., De Kimpe, N., Moglioni, A., Vazquez, R., Monczor, F., Shayo, C. and Davio, C. 2010. Coumarins: Old Compounds with Novel Promising Therapeutic Perspectives. *Current Medicinal Chemistry*. 17(13) : 1325–1338.

Rodríguez-Monroy, M. and Galindo, E. 1999. Broth rheology, growth and metabolite production of *Beta vulgaris* suspension culture: A comparative study between cultures grown in shake flasks and in a stirred tank. *Enzyme and Microbial Technology*. 24(10): 687–693.

Romero, M., Cabrera, E. and Ortiz, N. 2008. *Informe sobre el estado de la biodiversidad en Colombia 2006-2007*. Bogotá, Colombia.

Shi, H., Dong, L., Jiang, J., Zhao, J., Zhao, G., Dang, X. and Jia, M. 2013. Chlorogenic acid reduces liver inflammation and fibrosis through inhibition of toll-like receptor 4 signaling pathway. *Toxicology*. 303 : 107–114.

Singh, N. p, McCoy, M. T., Tice, R. R. and Schneider, E.L. 1988. Technique for Quantitation Damage in Individual of Low Levels of DNA Cells. *Experimental Cell Research*. 175(1) : 184–191.

Sotelo, I., Casas, N. and Camelo, G. 2010. Borojó (*Borojoa patinoi*): Fuente de polifenoles con actividad antimicrobiana. *Revista de La Facultad de Química Farmacéutica Universidad de Antioquia*. 17 : 329–336.

Torres-Domínguez, A., Toro-García, G., Valdés-Rodríguez,

Y. C., León, J. L. and Merchán, F. 2005. Efecto in vitro de los 1-O-alquilgliceroles sintéticos en la falciformación y en la hemólisis de los eritrocitos SS. *Bioquímica*. 30(4) : 101–109.

Trejo-Tapia, G. and Rodríguez-Monroy, M. 2007. La agregación celular en la producción de metabolitos secundarios en cultivos vegetales *in vitro*. *Interciencia*. 32(10) : 669–674.

Vázquez, R., Riveiro, M. E., Vermeulen, M., Alonso, E., Mondillo, C., Facorro, G. and Davio, C. 2012. Structure-anti-leukemic activity relationship study of ortho-dihydroxycoumarins in U-937 cells: Key role of the λ-lactone ring in determining differentiation-inducing potency and selective pro-apoptotic action. *Bioorganic & Medicinal Chemistry*. 20(18) : 5537–5549.

White, P. R. 1951. Nutritional requirements of isolated plant tissues and organs. *Annual Review of Plant Physiology*. 2 (1) : 231–244.

Willimott, S., Barker, J., Jones, L. A. and Opara, E. I. 2007. Apoptotic effect of Oldenlandia diffusa on the leukaemic cell line HL60 and human lymphocytes. *Journal of Ethnopharmacology*. 114 (3) : 290–299.

Yan, Y., Liu, N., Hou, N., Dong, L. and Li, J. 2017. Chlorogenic acid inhibits hepatocellular carcinoma in vitro and in vivo. *The Journal of Nutritional Biochemistry*. 46 : 68–73.

Yun, N., Kang, J. W. and Lee, S. M. 2012. Protective effects of chlorogenic acid against ischemia/reperfusion injury in rat liver: molecular evidence of its antioxidant and anti-inflammatory properties. *The Journal of Nutritional Biochemistry*. 23(10) : 1249–1255.

Zhou, J. and Zhang, Y. 2008. Cancer stem cells: Models, mechanisms and implications for improved treatment. *Cell Cycle*. 7(10) : 1360–1370.