Cytotoxicity evaluation in cancer cells and bactericidal activity of nanoencapsulated extracts from *Myracrodruon urundeuva*

Alessandra Cury Machado, Marcelo Milanda MR Lopes, Luis Guillermo C Durango, Silvia Castro Coelho, Carla Andreotti Damante, Silvia Helena C Sales-Peres, Manuel AN Coelho and Moacir Rossi Formí

**Introduction**

Nanoparticles are inorganic, organic or polymeric particles that have size dimension in nanoscale (1-1000 nm) (Yuan *et al.*, 2019) [1]. Polymeric nanoparticles are prepared using biocompatible, biodegradable polymers such as poly (lactic-co-glycolic acid) (PLGA), polylactic acid (PLA), useful to drug delivery system (Nascimento *et al.*, 2016) [2]. Drug (botanical extract) nanoencapsulated can’t reduce its toxicity, but improving the drug performance through the vectorization to target cells reducing its dose and side effects (Simonetti *et al.*, 2019) [3] besides increase the stability, and dispersion in aqueous medium of active compounds, before reaching the goals (Bonifacio *et al.*, 2014) [4]. A botanical extract is a complex mixture of different organic compounds unstable (Hajialyani *et al.*, 2018) [5]. These features highlight the efficiency of liposomal encapsulated coconut extract enhanced antibacterial and antioxidant properties (Olatunde *et al.*, 2019) [6] besides soy lecithin-derived applications liposome on cancer treatment (Le *et al.*, 2019) [7]. *E. coli* is considered a foodborne pathogenic, as toxin causing gastrointestinal infections such as bloody diarrhea, hemorrhagic colitis, renal failure (Nadzirah *et al.*, 2015) [8]. In its turn, *S. aureus* is a pathogen of a variety of human infections ranging from skin infections to tissues (Kong *et al.*, 2016) [9]. Moreover, *S. mutans*, an etiological factor of dental caries, a microorganism that acquire new properties of expression for pathogenicity (Krzyściak *et al.*, 2014) [10]. In addition to nanomedicine development of bacterial control, its progress is evident in researches for cancer therapies. The literature highlights benefit of nanoparticle application during new pharmaceutical products. Pinto (2011) [11] demonstrates the efficacy of liposomal imatinib–mitoxantrone combination against cancer and emphasizes its potential as treatment. PLGA are attractive vehicles for protein antigen delivery, enhancement in antigen processing, immunogenicity, improved targeted delivery and slow release of antigens (Iranpour *et al.*, 2016) [12]. However, evidence supports the idea of antitumor activity of natural polyphenols, their antioxidant properties,
which help normal cells and increase the surveillance of cancerous cells against oxidative stress caused by anticancer agents (Davitgaran-Taghipour et al., 2017) [13]. Then, the nanoparticles loaded with natural polyphenols may overlap the surveillance system of cancerous cells. Myracrodruon urundeuva (Anacardiaceae) is popularly known as Aroeira, a medicinal plant widely used in Brazil and application includes activity against tumors, inflammations, infections, allergy, itching, bleeding, gastritis, and wound healing (Gomes et al., 2013) [14]. Studies highlight the antimicrobial activity of M. urundeuva and evaluated the extracts action bioassays as bactericide and/or bacteriostatic to S. mutans, S. mitis, S. sobrinus, S. sanguis and L. casei, and antifungal against C. albicans, C. tropicalis and C. kruasei (Alves et al., 2009) [15]. The evaluation of the antimicrobial activity of M. urundeuva, and its effectiveness to S. aureus were related the presence of phenolic compounds with antimicrobial activity, alkaloids flavonoids and tannins in antifungal activity, antioxidant, anti-inflammatory, and anticancer properties (Gomes et al., 2013) [14]. Bonifácio et al. (2015) [16] concluded M. urundeuva extract has increased antifungal activity in incorporation into a nanostructured system enhance. Ferreira (2011) [17] emphasize the antiproliferative power of M. urundeuva against HL-60 lines (leukemia) cancer, SF-295 (glioblastoma), HCT-8 (colon) and MDA/MB-435 (melanoma) cells assign the antitumor effects. Thus, the main aims of this work were to certify the bacterial activities from nonencapsulated M. urundeuva extract against E. coli, S. aureus, and S. mutans evaluating if nanoencapsulated extract are able to keep their activities, as well as to investigate the in vitro cytotoxicity activity against pancreatic ductal adenocarcinoma (S2-013).

Material and methods

Method of extraction to M. urundeuva

M. urundeuva leaves were collected in 2015, at Agudos, Sao Paulo, Brazil. The excissate was deposited in herbarium Rioclarens (HRCB) at Department of Botanic-IB-UNESP-Rio Claro (identification nº exsiccata HRCB59831). The leaves were ground using an industrial blender and analytical knife mill (IKA A11 basic, Wilmingtom). The powder material was macerate in ethanol: water (7:3, v/v) for 12 days. In each 72 h, we replaced the solvent mixture, stirred for 3 min before each maceration using a T25 disperse Ultra-turrax (IKA, Wilmingtom). After extraction, the solvent was filtered, removed using rotaevaporator (Büchi R-215) (Germany, Labortechnik GmbH) and stored in amber glass (Machado et al., 2016) [18].

Nano encapsulation of M. urundeuva extract

We obtained the nanocapsules (NC), and nanosphere (NS) in colloidal suspension by interfacial deposition/solvent displacement using preform polymer, according to Fessi et al. (1989) [19], and Forim et al. (2013) [20], with modifications. In organic phase the nanocapsules containing biopolymer poly(e-caprolactone) PLA (1,000 mg), a surfactant Span 60 (770 mg), isoeodeyl oleate (3100 mg), M. urundeuva extract (250/500 mg), acetone (267 mL). The aqueous phase was prepared with Tween 80 (770 mg) and ultrapure water (533 mL) under magnetic stirring (RT10P/IKA, Wilmingtom) at 40°C. For nanospheres, the process was carried out under similar conditions, except by presence of isodecyl oleate in organic phase. After synthesizing nanoparticles, the solvent was eliminated using rotaevaporator preparing the colloidal suspension, then was dried by a mini-Spray-Dryer B-290 (Buchi, Brasil), added one drying support (Aerosil 200, Labsynth) into a colloidal suspension (1.5 g/1 L), submitting it by nebulization in hot air obtaining microparticles in powder (Forim et al., 2013) [29]. The yield of 50%, whereas 100 ml to characterization analysis and 400 ml by Spray-Dryer. For each proportion 800 mL in each nanoparticle NC1, NC2, NS1 and NS2 yield powder total volume of 5890, 6140, 2790 and 3040 mg, respectively. Then, the compositions of organic and aqueous phases were previously investigated, the best stability, pH values, colloidal nanoparticle size, and nanoencapsulation efficiency are conditions to prepare the nanoparticles. The best-reached designs antibacterial and cytotoxicity activity were formulations prepared, changing the composition of nanocapsules and nanospheres respectively, previously prepared and achieved best results to nanoformulations will be select to future studies.

Characterization of colloidal suspensions and powder nanoparticles

Physicochemical analyses of nanoparticles were carried out immediately after their preparations, pH values to colloidal suspensions were used by potentiometer (B474; Micronal, Sao Paulo, Brazil). The particle size analysis of nanoparticles was performed by Photon Spectroscopy by measurements (nm). This analysis yields the mean particle hydrodynamic diameter and polydispersity index (Pd), which is a dimension less measure of the broadness of the particle size distribution. The zeta potential values were obtained by dynamic light scattering technique on Zetacatr (Microtrac Inc, Montgomeryville, PA) which was controlled by Flex version 10.5.0 software (Microtrac Inc, Montgomeryville, PA). For particle size and zeta potential (mV) measurements, 0.1 mL of each colloidal suspension was diluted into 10 mL of ultrapure water and 10 mM sodium chloride. Like drying support in 1% m/V amounts to powder nanoparticle obtention. For statistical analysis in triplicate, average values and standard deviation of measurements were calculated (Kuma et al., 2017) [21]. Morphological analyses of nanoparticles were performed by Scanning Electron Microscopy (SEM) (Field Emission Gun) (Nanolab Technologies, CA, U.S.A.). The samples were photomicrographed by Magellan XHR 400L FE-SEM-FEG (Field Emission Gun) (Nanolab Technologies, CA, U.S.A.) using different magnifications (Santos et al., 2012) [22].

Microbial bioassays

The following microbial strains were evaluated: Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 13565, and Staphylococcus mutans ATCC 25175 since pathogens monitored by Brazilian Public Health System (Wang & Dacheng, 2017) [23]. The strains were provided by Research Intensive Center, Bauru Dentistry Faculty. The bacterial suspensions were standardized during 24 h in Mueller Hinton broth. The growth media were centrifuged at 600 xg, and supernatants were discarded. The bacterial pellets were resuspended in tubes using PBS at pH 7.2, until reach a turbidity similar to one tube suspension in 0.5 scale McFarland (1.5x108 UFC/mL-1), homogenized and washing was repeated. The microbial dilution was detected in spectrophotometric readings at 590 nm (Spectrophotometer; Beckman Coulter; Du 730; Life Science UV-VIS, CA, U.S.A). The suspensions were diluted to 10 µg-mL-1 in BHI, resuspended 1.0x10⁶ UFC-mL-1. The bioassays were carried out in 96 wells plate, incubated 37°C, for 24 h, and the sample
microdilution until 10^6 UFC·mL^{-1}, and transferred to other 96 wells plate. Afterward, added NC1, NC2, NS1, NS2 nanoparticles and extract in 1000 to 0.1×10^{-1} µg·mL^{-1} dilutions, and negative control and well dilutions were placed in 20 µL dilution in 4 quadrants in each Petri dish (3 Petri dish/dilution). The assay was triplicate (n = 3), the scattering for seeding offered the growth and bacterial colonies score. Both E. coli, and S. aureus strains were cultivated in Sabouraud Dextrose Agar (BHI) (Brain Heart Infusion Agar, Acumedia, Neogen Corporation, Michigan, USA). But S. mutans was cultivated in Mitis salivarius Agar (Difco, Becton Dickinson and Company, USA), incubated for 24 h. We reported all results as mean ± standard error of three independent biological replicates and employed ANOVA followed by Turkeys HSD test (p ≤ 0.05) to analyze the antibacterial activity data.

**In vitro cytotoxicity assay**

The Sulfurhodamine RB Method (Skehan et al., 1990) [24] evaluated nanoencapsulated extract mechanism on Human pancreatic ductal adenocarcinoma (S2-013) cell line growth, supplied from Laboratory of the Government Chemist (LGC Standards) (Barcelona, Spain). Briefly, the S2-013 cells were seeded in 96-well dishes at cell density of 1000 cells/well, under normal conditions of 5% CO₂, at 37°C for 24 h and treated for 48 h with the samples NC1, NC2, NS1 and NS2 in 0.3 to 300 µg·mL^{-1} dilutions, fixed using trichloroacetic acid 10% (TCA) (w/v) for 60 min in ice bath, washed with ultrapure water and stained with 50 µL of SRB solution. The unbound dye was removed by washing with acetic acid solution 1% (v/v). The dried cells and protein-bound stain solubilized with 10 mM Tris solution. The SRB absorbance was measured at 560 nm in Synergy HT Multi-Mode Microplate Reader (Biotec Instruments Inc., Winooski, VT, USA). The IC₅₀ (concentration for 50% of cell survival) values determined. The wells absorbance containing nanospheres or nanocapsules and untreated cells following 48 h incubation compared with wells containing cells fixed at time zero.

**Results and discussion**

**Polymeric nano/microparticles and their characterization**

Nanoparticles have created opportunities for development of new therapeutic applications (Khan & Gurav, 2018) [25], especially phytotherapies. Polymeric nanoencapsulated with natural compound bioactivities proven successful increasing the bioavailability, permeability, bioavailability, prolonged half-life, tissue targeting, and minimal side effects (Aljuffali et al., 2016) [26]. The nanocapsules and nanospheres was chosen due to simple operation without applied targeted delivery, efficiency reduces stability and dispersion of natural products (Deng et al., 2020) [27]. After syntheses of nanoparticle, suspensions presented a milky and blue feature resulted from the Brownian movement (Tyndall effect), which occurs due to scattering of light in individual nanoparticles in Random motion in dispersion medium (Uma et al., 2011) [28]. This characteristic confirms the nanoparticle synthesis. The Spray-Dryer process are observed control with minimal increase in temperature (Oliveira & Petrovick, 2009) [29]. The calculated yields of powder for NC1, NC2, NS1, and NS2 were 69.7, 55.7, 77.1, and 77.0%, respectively. For spray dryers, typical process yields are usually in 50-70% range. Gallo et al., (2015) [30] did a comparative study by spray dryer medicinal plant extract of R. puschiana, H. virginiana, V. officinalis, H. perforatum and C. scolymus and the yield was 65 to 95%, being similar to our study.

The preparation of nanoparticles in powders can’t only increase the stability but also simplified their final as capsules or pills for oral administration, besides biological effects, as complete oral drug absorption from powders dispersed in water, reducing gastrointestinal irritant (Guteres et al., 2009) [31]. Nanoparticle characteristics (size, shape and surface properties) for biological interactions at molecular level created development of nanoparticles for therapeutic (Khan & Gurav, 2018) [25]. Nanoparticles have been used as drug delivery vehicles due to better encapsulation, control release and less toxic (Kumari et al., 2010) [32]. To available the nanoparticle stability in colloid suspension, did not observe precipitate formation, only a variation in the pH according to time of formulation preparation. In Figure 1 the pH suffered a slight variation in time function kept constant after 30 days, and variations were linked to hydrolysis of polymer chain, changing the pH of colloid suspension (DiDonato et al., 2017) [33].

![Fig 1: pH variation as in time function in each nanoparticle](http://www.plantsjournal.com)

The table 2 described the physicochemical properties of NC1, NC2, NS1 and NS2 nanoparticles characterized according zeta potential (ZP), particle diameter (PD) and polydispersity index (PdI) values.

| Nano formulation | Particle size (nm) | Polydispersity Index (PdI) | Zeta Potential (mV) |
|------------------|-------------------|---------------------------|---------------------|
| NC1              | 169.0 ± 5.72      | 0.185 ± 0.940             | -28.0 ± 5.07        |
| NC2              | 258.3 ± 4.31      | 1.000 ± 0.383             | -40.6 ± 7.31        |
| NS1              | 255.9 ± 6.53      | 0.512 ± 0.512             | -26.1 ± 7.20        |
| NS2              | 432.1 ± 6.94      | 0.912 ± 0.912             | -38.6 ± 5.34        |

Values express the average result ± standard deviation (n = 3)

The nanoparticles showed a particle size ranging from 169.0 to 432.1 nm. Usually, the nanoparticles prepared by preform polymer deposition present average diameter between 100 to 300 nm. Particle sizes are mainly influenced by the oil core
and rate between the quantity of active compounds and polymers. The nanoparticles prepared with twice, the extract presented a larger particle size in nanocapsules and nanospheres. While the PdI was identified 0.6 in nanoparticles prepared extract in the lower level (250 mg) and 1.0 to formulas with an extract in the higher (500 mg) (Table 2) indicating narrow and broad particle size distributions, respectively. The nanoparticles (NC and NS) showed a homogenous population when prepared a composition PCL and extract of 1001,2 g /250 mg range. A lower dosage compared our studies was realized by Busari et al. (2017) [34] in cytotoxicity assay with curcumin (5 mg drug/50 mg PLGA).

The zeta potential of colloidal suspensions showed negative values to prepared nanof ormulations using polymer/extract rate of 1001,2 g/500 mg. The values -28.0 and -26.1 mV obtained to NC1 and NS1, respectively and nanoparticles prepared with polymer/extract rate of 1001,2 g/500 mg with values of -40.6 and -38.6 mV to NC2 and NS2, respectively. This result is compatible with a system where compounds in extract may be adsorbed in nanoparticle surfaces. Independently of zeta potential, the precipitate in colloidal suspension after 180 days by Spray-dryer was uniform materials. Nanocapsules and nanospheres (Fig 2.) showed compact and spherical structures homogeneous in colloidal suspension and powder.

Bacterial bioassays with nanoencapsulated *M. urundeuva* extract
The antibacterial effects of nanoparticles were evaluated in powder loaded with extract and control against pathogenic bacterial strains like *S. aureus*, *S. mutans* and *E. coli*. At first NC1 presented smaller cells inhibition against *E. coli* in all evaluated dilutions, while NC2 inhibited 50 to 100%, but NC1 presented a high inhibition in all dilution against *S. aureus*. Then, NC2 was able to inhibit against *S. aureus* around 30 % to 100 and 1.0 µg·mL⁻¹ dilution, and 60% to 1,000 and 10 µg·mL⁻¹, respectively and the control was similar inhibition to 0.1 µg·mL⁻¹ dilution. NC1 and NC2 inhibited the most dilutions against *S. mutans*. The NS2 have slightly lower inhibition compared NS1 against *E. coli* in the most dilution. While NS2 presented better inhibition than NS1 against *S. aureus* and the NS2 was slightly better than NS1 against *S. mutans*. The extract was efficiently active to *E. coli* but to *S. aureus* showed 70% inhibition only to 1,000 and 10 µg·mL⁻¹ dilution. *S. mutans* presented lower inhibition in all evaluated dilution.
Therefore, the extract gained efficiency when nanoencapsulated, especially NC1, NC2 and NS1, because optimized the bacterial activities against the investigated strains. Previously, Bonifácio et al. (2015) [16] described the fungicide activities from *M. urundeuva* extract in a nanostructured lipid system. Similar to our results, they observed gains to eliminate the infectious agent to encapsulated extract.

**In vitro cytotoxicity activity of *M. urundeuva* nanoparticles on pancreatic ductal adenocarcinoma cells**

The NS1, NS2, NC1, NC2 nanoparticles and extract was evaluated cytotoxicity activity using in Fig. 4, the pancreatic adenocarcinoma cells exposed to the samples between 0.3 to 300.0 µg·mL\(^{-1}\) dilutions. According Machado et al. (2016) [18] dilution range is compatible with cytotoxicity bioassay. The cell growth was monitored by SRB method, applied in studies chemotherapeutic drugs or small molecules in adherent cells (Orellana & Kasinski, 2016) [35].

In 0.3 µg·mL\(^{-1}\) dilution, the NS1, NS2 and NC1 nanoparticles present 70% cell growth inhibition and 100% in NC2. The same is observed for inhibition of cell growth in response to the NC1 compared with NC2 only for extract in 0.3 µg·mL\(^{-1}\) dilution. At 3 µg·mL\(^{-1}\) dilution, the toxicity NS2, NC1 and NC2 reduced the cell growth around 45% to 50%, but the NS1 present around 70%. At 30 µg·mL\(^{-1}\) dilution, the toxicity on pancreatic cancer cells S2-013 were with a growth of 32%, 38% and around 42% for NS1, NS2 and NC1, respectively. At 300 µg·mL\(^{-1}\) dilution all nanoparticle presents only 10% cell growth. The results were similar except for NC2 and NS1 at 0.3 and 3 µg·mL\(^{-1}\), respectively. According to ANOVA statistical analysis, there was general best results the nanoparticles 300 µg·mL\(^{-1}\) dilutions. The similar results to nanoformulations may be explained by natural products with therapeutic, bioavailability limited and nanoparticle-delivered, naturally synthesized for treatment and prevention of cancers (Bharali, et al., 2011) [36]. In both assays we observed gains and efficiency in biological activity of extract nanoencapsulated than extract alone. *M. urundeuva* is a medicinal plant very well-known for its biological properties rich in polyphenols and nanoparticles vectored the Aroeira bioactive compounds (Zhang et al., 2010) [37].

We conclude using PCL is possible to prepare nanocapsules and nanospheres loaded with *M. urundeuva* extract by interfacial deposition of a preformed polymer and the nanoencapsulated extract was obtained in powder by Spray-drying. We found different physicochemical features according to rate between polymer/*M. urundeuva* extract mainly to particle size and zeta potential. All nanoparticles showed similar morphology. The nanoencapsulated extract showed better biological activities against pathogenic bacteria and pancreatic adenocarcinoma cells than the one non-encapsulated. The biological efficiency of nanoparticles changed according to their particle size.

**Acknowledgments**

The authors are grateful for the financial support received from National Council for Scientific and Technological Development-CNPq (403302/2013-7; 306109/2018-2; 429404/2018-2), and São Paulo Research Foundation–FAPESP (2012/25266-9; 2018/21201-8; 2014/50918-7). This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) - Finance Code 001.

**References**

1. Yuan D, He H, Wu Y, Fan J, Cao Y. Phisiologically based Pharmacokinetic modeling of nanoparticles. J Pharm Sci 2019;108:58-72.
2. Nascimento TG, Silva PF, Azvedo LF, Rocha LG, Porto ICCM, Lima e Moura TFA et al. Polymetric Nanoparticles of Brazilian Red Propolis Extract: Preparation, Characterization, Antioxidant and Leishmanicial Activity. Nanoscale Res Lett 2016;11:301.
3. Simonetti G, Palocci C, Valletta A, Kolesova O, Chronopoulou L, Donati L et al. Anti-Candida Biofilm
Activity of Pterostilbene or Crude Extract from Non-Fermented Grape Pomace Entrapped in Biopolymorphic Nanoparticles. Molecules 2019;24(11):2070.

4. Bonifácio BV, Silva PB, Ramos MAS, Negri KMS, Bauab TM, Chorillli M. Nanotechnology-based drug delivery systems and herbal medicines: a review. Int J Nanomedicine 2014;9:1-15.

5. Hajialyani M, Tewari D, Sobarzo-Sánchez E, Nabavi SM, Farzaei MH, Abdollahi M. Natural product-based nanomedicines for wound healing purposes: therapeutic targets and drug delivery systems. Int J Nanomedicine 2018;13:5023-5043.

6. Olatunde OO, Benjakal S, Vongkamjan K, Amnuaiakt T. Liposomal Encapsulated Ethanolic Coconut Husk Extract: Antioxidant and Antibacterial Properties. Int J Food Sci Tech 2019; 0: 0.

7. Le NTT, Cao VD, Nguyen TNQ, Le TTH, Tran TT, Thi TTH. Soy Lecithin-Derived Liposomal Delivery Systems: Surface Modification and Current Applications. Int. J Mol. Sci. 2019;20:4706. doi:10.3390/ijms20194706.

8. Nadzirah SH, Azizah N, Hashim U, Gopinath SCB, Kashif M. Titanium Dioxide Nanoparticle-Based Interdigitated Electrodes: A Novel Current to Voltage DNA Biosensor Recognizes E. coli O157:H7. PLoS One 2015, 10(10).

9. Kong C, Neoh H-M, Nathan S. Targeting S. aureus Toxins: A Potential form of Anti-Virulence Therapy. Toxins 2016;8:72.

10. Krzyściak W, Jureczak A, Kościeniak D, Bystrowska B, Skalniak A. The Virulence of S. mutans and the Ability to Form Biofilms. Eur J Clin Microbiol Infect Dis 2014;33(4):499-515.

11. Pinto AC, Moreira JN, Simões S. Liposomal Imatinib Mitoxantrone Combination: Formulation Development and Therapeutic Evaluation in an Animal Model of Prostate Cancer. The Prostate 2011;71:81-90.

12. Irpanour S, Nejati V, Delirezh N, Biparva P, Shirian S. Enhanced stimulation of anti-breast cancer T cells responses by dendritic cells loaded with poly lactic-co-glycolic acid (PLGA) nanoparticle encapsulated tumor antigens. J Exp Clin Cancer Res 2016;35:168.

13. Davatgaran-Taghipour Y, Masoomzadeh S, Farzaei MH, Bahramisoltani R, Karimi-Soureh Z, Rahimi R et al. Polyphenol nanoformulations for cancer therapy: experimental evidence and clinical perspective. Int J Nanomedicine 2017;12:2689-2702.

14. Gomes BVTL, Chaves TP, Alencar LCB, Dantas IC, Medeiros ACD, Felismino DC. Actividade antimicrobiana de produtos naturais de M. urundeuva All. Rev Cub de Plant Med 2013;18(4):529-533.

15. Alves PM, Queiroz LMG, Pereira JV, Pereira MSV. Atividade antimicrobiana, antiadherent, antifúngica in vitro de plantas medicinais brasileiras sobre microorganismos do biofilme dental e cepas do género Cándida. Rev Bras Soc Bras Med Trop 2009;42(2):222-224.

16. Bonifácio BV, Ramos MAS, Silva PB, Negri KMS, Lopes EO, Souza LP et al. Nanostructured lipid system as a strategy to improve the anti-C. albicans activity of Astronium sp. Int J Nanomedicine 2015;10:5081-5092.

17. Ferreira PMP, Farias DV, Viana MP, Souza TM, Vasconcelos IMV. Study of the antiproliferative potential of secon extracts from Northeastern Brazilian plants. Anais da Academia Brasileira de Ciências 2011;83(3):1045-1058.

18. Machado AC, Souza LP, Saldanha LL, Pieroni LG, Matos AA, Oliveira FA et al. Aroeira (M. urundeuva) methanol extract: the relationship between chemical compounds and cellular effects. Pharm Biol 2016;26:1-5.

19. Fessi H, Puisieux F, Devissaguet J, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 1989;113:1-14.

20. Forim MR, Costa ES, da Silva MFGF, Fernandes JB, Mondego JM, Boiça Junior AL. Development of a new method to prepare nano-/microparticles loaded with extracts of A. indica, their characterization and use in controlling P. xylostella. J Agric Food Chem 2013;61(38):9131-9139.

21. Kuma A, Dixit CK. Methods for characterization of nanoparticles. Advances in Nanomedicine for the Delivery of Therapeutic. Nucleic Acids Res 2017:43-43.

22. Santos CGM, Lane PMPB, Silva AAS, Barbato CN, Sampaio JA, Garrido FMS, et al. Síntese da Fase Sodalita com o Uso do Caulim da Região Borborema-Seridó (RN/PA) Ativado Termicamente. HOLOS 2012:28(5):52-59.

23. Wang H, Dacheng R. Controlling S. mutans and S. aureus biofilms with direct current and chlorhexidine. AMB Express 2017;7:204.

24. Skaheh P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D et al. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. J Nat Cancer Inst 1990;4;82(13):1107-1112.

25. Khan T, Gurav P. PhytoNanotechnology: Enhancing Delivery of Plant Based Anti-cancer Drugs. Front Pharmacol 09 February 2018 | https://doi.org/10.3389/fphar.2017.01002.

26. Aljuffali IA, Fang C-L, Chen C-H, Fang J-Y. Nanomedicine as a Strategy for Natural Compound Delivery to Prevent and Treat Cancers. Curr Pharm Des 2016;22(27):4219-4231.

27. Deng S, Gigliobianco MR, Censi R, Di Martino P. Polymeric Nanocapsules as Nanotechnological Alternative for Drug Delivery System: Current Status, Challenges and Opportunities. Nanomater 2020;1

28. Uma B, Swaminathan TN, Radhakrishnan R, Eckmann DM, Ayyaswamy PS et al. Nanoparticle Brownian motion and hydrodynamic interactions in the presence of flow fields. Phys Fluids 2011;23:073602.

29. Oliveira OW, Petrovick PR. Spray drying of plant extracts: basic remarks and application. Rev Bras Farmacogn 2009, 20(4).

30. Gallo L, Ramirez-Rigo MV, Piña J, Bucalá V. Comparative study of spray-dried medicinal plant aqueous extracts. Drying performance and product quality. Chem Eng Res Des 2015;104:681-694.

31. Gutierrez SS, Beck RCR, Pohlmann AR. Spray-drying technique to prepare innovative nanoparticulated formulations for drug administration: a brief overview. Braz J Phys 2009, 39(1A).

32. Kumari RU, Usharani KS, Suguna R, Anandakumar CR. Relationship between the yield contributing characters in cowpea for grain purpose V. unguiculata (L). Wapl. Electron. J Plant Breed 2010;1:882-884.

33. Di Donato N, Hatcher PG. Alicyclic carboxylic acids in soil humid acid as detected with ultrahigh resolution mass spectrometry and multi-dimensional NMR. Org Geochem 2017;112:33-46.

34. Busari ZA, Dauda KA, Morenikje OA, Afafayan F, Oyeyemi OT, Meena J et al. Antiplasmodial Activity and
Toxicological Assessment of Curcumin PLGA-Encapsulated Nanoparticles. Front Pharmacol 2017;8:622.

35. Orellana EA, Kasinski AL. Sulforhodamine B (SRB) Assay in Cell Culture to Investigate Cell Proliferation. Bio Protoc 2016;6(21):e1984.

36. Bharali DJ, Siddiqui IA, Adhami VM, Chamcheu JC, Aldahmash AM, Mukhtar H et al. Nanoparticle Delivery of Natural Products in the Prevention and Treatment of Cancers: Current Status and Future Prospects. Cancers 2011;3(4):4024-4045.

37. Zhang L, Pornpattananangku D, Hu C-MJ, Huang C-M. Development of Nanoparticles for Antimicrobial Drug Delivery. Curr Med Chem 2010;17:585-594.