doi.org/10.36721/PJPS.2021.34.2.REG.599-606.1

Alkaloid and phenolic compounds of *Xylopia aromatica* inhibits tumor growth by down-regulating matrix metalloproteinase-2 (MMP-2) expression

Izabela Natalia Faria Gomes[1], Ana Gabriela Silva[1], Gustavo Fernando de Frazao Lima[1], Tamara Ribeiro Longatti[1], Lucas Fernandes Do Carmo[2], Jose Augusto Ferreira Perez Villar[2], Arali Aparecida Da Costa Araujo[3], Ralph Gruppi Tome[4], Helio Batista Dos Santos[4] and Rosy Iara Maciel De Azambuja Ribeiro[1]*

[1]Experimental Pathology Laboratory - Federal University of Sao Joao del Rei - Centro-Oeste D. Lindu Campus (UFSJ/CCO) -400, Sebastiao Goncalves Coelho, Chanadour, Divinopolis MG, Brazil
[2]Laboratory of Organic Synthesis and Nano Structures, Federal University of Sao Joao del Rei – Centro-Oeste D. Lindu Campus (UFSJ/CCO) -400, Sebastiao Goncalves Coelho, Chanadour, Divinopolis MG, Brazil
[3]University of the State of Minas Gerais (UEMG), Rua Vereador Geraldo Moises da Silva s/nº - Campus Universitario, Setor Universitario, Ituiutaba, MG, Brazil
[4]Tissue Processing Laboratory, Federal University of Sao Joao del Rei - Centro-Oeste D. Lindu Campus (UFSJ/CCO) -400, Sebastiao Gonçalves Coelho, Chanadour, Divinopolis MG, Brazil

Abstract: *Annonacea* species have been reported to possess antitumor properties. However, the i*n vitro* and *in vivo* antitumor activities of *Xylopia aromatica* (*Annonace*a) have not yet been elucidated. This study aimed to investigate the effects of *Xylopia aromatica* leaves hexane fraction (XaHF) on Ehrlich ascites carcinoma cells lines (EAC), both *in vitro* and *in vivo*. *In vitro* assays revealed a significant cytotoxic effect with the two lower XaHF concentrations (62.5 and 32.3mg/mL). EAC ($2.5x10^6$ cells) were inoculated in the right flank of Swiss mice, and the animals were treated intraperitoneally with 32.3mg kg^{-1} of XaHF daily, for 20 days. Our findings indicate that XaHF suppressed the growth of EAC *in vivo*, with a significant decrease (46%) in tumor volume. There was also a decrease in the necrosis area (71%), inflammatory infiltrate, and MMP-2 expression. High-Performance Liquid Chromatography with Diode Array Detector (HPLC- DAD) identified secondary metabolites possibly related to phenolic acids, flavonoids, and alkaloids. Thus, the results confirmed the antitumoral activity that may be related to the presence of the identified metabolites in XaHF extract

Keywords: Ehrlich tumor, matrix metalloproteinase, secondary metabolites, medicinal plants, antitumoral activity.

INTRODUCTION

Cancer mortality corresponds to the second cause of deaths by disease worldwide, and 19 million new cases are expected until 2025 (Adusumilli *et al*., 2017). Despite this high incidence, major pathways involved in the progression of the disease are not yet fully understood (Jin and Mu, 2015). Cytotoxic chemotherapies are the principal modalities of treatment for cancer patients, and indeed can increase their survival, if diagnosed at early stages (Gamucci *et al*., 2017). Despite the relative effectiveness of such approach, discovery and development of new chemotherapeutics drugs with less side effects, selective cytotoxicity and low resistance are priority (Calderon *et al*., 2009).

Some chemotherapeutic agents used in clinical practice for cancer treatment are from natural origin, that include vinblastin, etoposide, topotecan, irinotecan, and taxol (Newman *et al*., 2002). The anticancer activity of these compounds is related to their high phytochemical diversity, all of them being secondary metabolites, such as alkaloids, acetogenins, and flavonoids (Seca and Pinto, 2018). Moreover, the antitumor properties of these compounds are related with different mechanisms, such as induction of apoptosis, cell cycle arrest, inhibition of DNA repair enzymes and Reactive Oxygen Species (ROS) generation (Alam, Hossain *et al*., 2016, Cragg and Pezzuto, 2016).

Brazil is the largest reservoir of plant-related genetic resources in biosphere, with more than 45,000 known species, corresponding to approximately 20%-22% worldwide (Dutra, Campos *et al*., 2016). *Xylopia aromatica* is a species belongs to *Annonaceae* family distributed throughout Brazilian Cerrado. Its leaves, bark, fruits and seeds have been demonstrated some therapeutic applications as anti-parasitic, anti-inflammatory, hypoglycemic, sedative, and hypotensive (Caxton-Martins 2 and 2006, Islam, Nasrin *et al*., 2013, MISHRA, 2013). Recently, the ethanolic bark extract of *X. aromatica* have demonstrated *in vitro* cytotoxic properties in several tumor types (breast, prostate, colon, leukemia and mouse mammary cancer cell lines)(Taylor, Arsenak *et al*., 2013).

**Corresponding author:* e-mail: rosy@ufsj.edu.br

However, more studies are needed to elucidate the molecular basis for its therapeutic antitumor potential.

In this study, we investigated the anticancer activity of X. *aromatica* leaves hexane fraction (XaHF) in Ehrlich ascites carcinoma cell line (EAC), a transplantable murine mammary adenocarcinoma model that induces solid and ascitic tumors in mice, *in vitro* and *in vivo*. In addition, the meanly compounds in XaHF was chemically characterized by HPLC-DAD.

MATERIALS AND METHODS

Plant samples

Xylopia aromatica leaves were collected on Cerrado area (S 18º 58' 08" and W 49º 27' 54"), identified by Dr. Arali Aparecida Costa Araújo and deposited in the Herbarium of the Botany Department of Federal University of Minas Gerais (BHCB: 143397), (SisGen register - A421DA3).

Chemicals, cell culture reagents and antibodies

DMEM culture medium, Dimethyl sulfoxide, Trypan-blue, L-glutamine, ethanol, hexane, penicillin G and streptomycin were purchased from Sigma Aldrich (St. Louis, Missouri, USA). Fetal bovine serum was purchased from Becton Dickinson (BD) (Bedford, MA, USA). Antibodies were purchased from Cell Signaling Technology (Danvers, Massachusetts, USA).

XaHF extraction

The extracts were obtained as previously described by our group (Silva-Oliveira *et al*., 2016). Briefly, *X. aromatica* leaves were washed, cut and dried for 5 days at room temperature. After drying, samples were submitted to extraction with aqueous ethanol (70%V.V^{-1}) in plant/solvent proportion of 1:5, for 5 days. The crude extract (CE) obtained was filtrated, frozen and lyophilized. Dried CE was re-solubilized in aqueous ethanol (70%V.V^{-1}) and subjected to extraction in a separation funnel with hexane. The obtained fraction was lyophilized, and the dry extract obtained was identified as XaHF. The XaHF was initially dissolved in dimethyl sulfoxide (DMSO) at concentration of 50 mg/mL and stored at -20°C.

Chromatographic profile by High-Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD)

Liquid chromatography was performed using the liquid chromatographer Shimadzu (model SPD-M20A Diode Array Detector, Shimadzu, Kyoto, Japan), Bomb: model LC-20 AD, automatic injector: SIL-20AHT, Communicator: Model CBM-20ª, column: Shim-pack UP - ODS C18 (5µm) 150 x 4.6 mm, software Lab Solution with flux0.8mL/min with detection at 350 nm. XaHF was diluted in pure methanol to obtain a standard solution with 1mg.mL^{-1}. This standard was centrifuged for 10 minutes at room temperature and filtered in a 0.45µm-pore filter before injection. The standard injection volume was 45 µL, following the running time gradient method (table 1). The chromatogram was digitally recorded.

The qualitative profile of chemical constituents present in the XaHF was obtained by comparison of retetion times or UV spectrum. The standards used were gallic acid, chlorogenic acid, catechin, quercetin, rutin, and boldine (fig. 1).

Cell culture

EAC primary cultures were established from an intraperitoneally inoculated female Swiss mouse as previously described (de Carvalho Maroni *et al*., 2012, Mishra *et al*., 2018). Seven days after inoculation, 3.0mL of ascites liquid were collected, and viable cells were counted using a Neubauer haemocytometer (Bright-line - 0.025mm^2; L-Optik,) using Trypan blue positive cells as an exclusion method. Cells were re-suspended and seeded in a 24-well plate (1x10^5 cells/well). Serial dilutions were used as treatment, ranging from 1000µg/mL to 32.2µg/mL of XaHF diluted in culture medium (DMEM 10%, containing 100 mg.mL^{-1} streptomycin, 100U.mL^{-1} penicillin G and 2mM L-glutamine). Cells were counted every 6h during 24h, using Trypan blue method. The experiment was performed in triplicate.

In vivo assays guidelines and ethical statement

Female Swiss mice, aged 2-3 months, weighing about 30g were obtained from Federal University of São Joao del Rei (UFSJ). For acclimation and experiments, animals were maintained properly caged, allocated in the animal house at UFSJ - *Campus Centro-Oeste,* with constant 12:12 light/dark cycle and free access to food and fresh water. All animal procedures were conducted in accordance with the "International Guidelines for Animal Experiment", adopted by UFSJ and were approved by the "Ethics Committee for Animal Experiments" of the Institution (protocol UFSJ 27/2013). All other applicable international, national, and/or institutional guidelines for the care and use of animals were also observed.

In vivo assays

Seven- day-old Erlich carcinoma cells was collected from a female mouse using a syringe (1mL). Viable tumor cells were counted in a Neubauer haemocytometer by the trypan blue exclusion method (Meireles *et al*., 2016, Mishra *et al*., 2018)

To verify *in vivo* effects of XaHF on Ehrlich tumor growth, 2.5x10^6 EAC suspended in PBS (pH 7.2) were inoculated in the right flank of 10 animals, divided in two groups (n=5): vehicle control (NaCl 0.9% m.V^{-1} + DMSO 1%V.V^{-1}) and XaHF treatment (32.3mg.kg^{-1}), both administered intraperitoneally. Tumor sizes were measured with manual pachymeter daily for 20 days to estimate tumor growth curves.

Histological analysis

After 20 days of treatment, mice were euthanized and tumor collected, fixed in 10% neutral buffered formalin, dehydrated in an ascending concentration series of aqueous ethanol, cleared 3 times using xylene and embedded in paraffin. Sections of 4,0µm were obtained from specimens and stained with hematoxylin and eosin (H&E).

Morphometric evaluation was carried out using an optical microscope with 40x magnification, and 10 fields per slide were examined. Representative images were captured with a digital camera (Zeiss microscope, Carl Zeiss, Germany) and analyzed using the Axion Vision 4.8 software (Carl Zeiss, Germany). Quantification of the inflammatory infiltrate was qualitatively performed and was classified as weak, moderate or strong according to the relative number of leukocytes. The percentage of necrosis area was calculated using the Axion Vision 4.8 software (Oliveira *et al*., 2018).

Immunohistochemical analysis (IHC) of Metalloproetinase-2 (MMP-2), Tissue inhibitor of metalloproteinases 2 (TIMP-2), and Proliferative cell

Table 1: Gradient program used to analyse the chromatographic profile of XaHF.

Time (min)	Mobile phase A (ultra-pure water + 0,1% formic acid (%)	Mobile phase B (acetonitrile) (%)
0.01	95	5
10.00	70	30
15.00	10	90
25.00	95	5

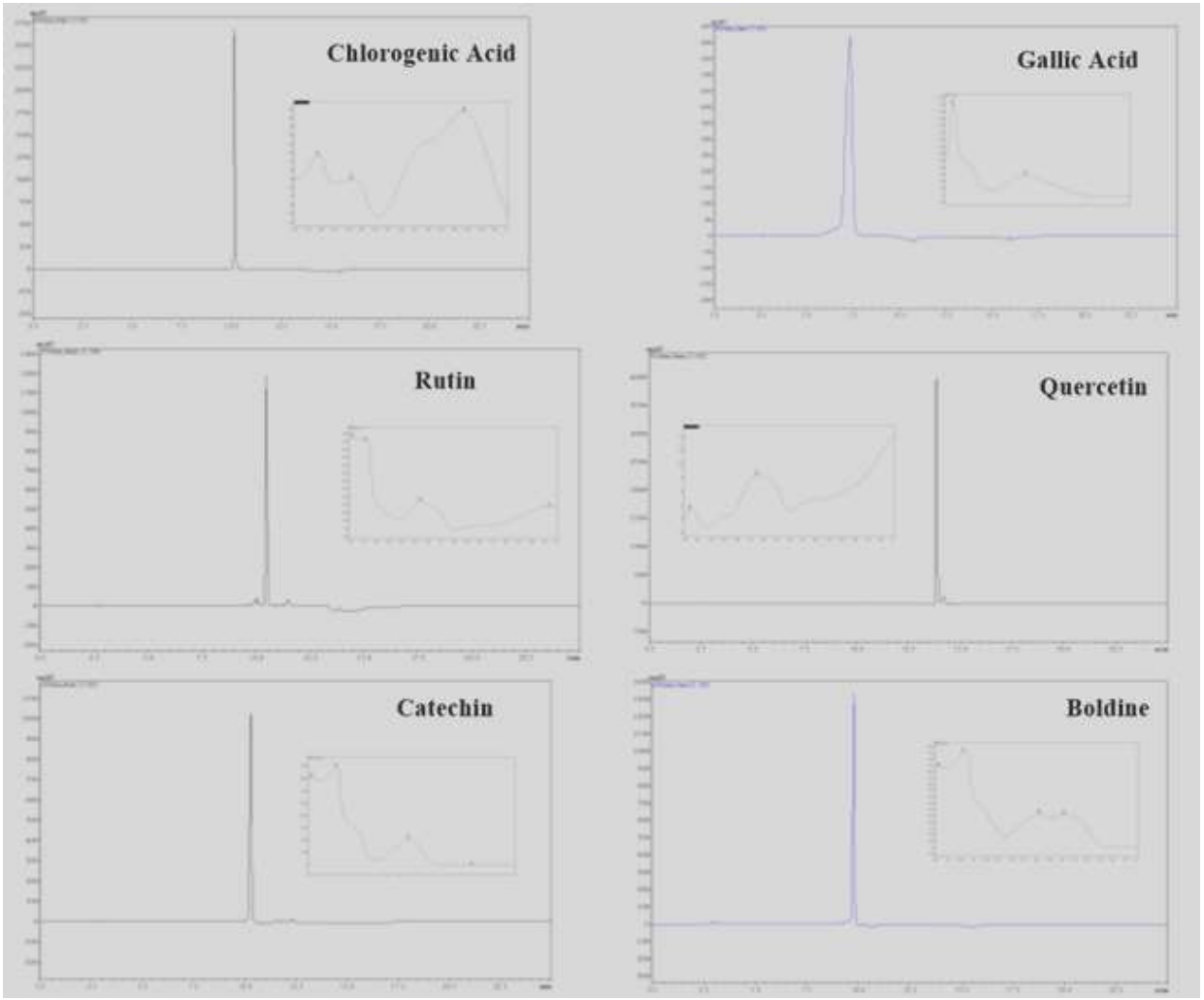

Fig. 1: Profile chromatographic standards by HPLC-DAD in the region of 200-800nm (C), Mobile phase ACN:. H2O + 0.1% c. Formic (90:10), flow 0.8mL/min.

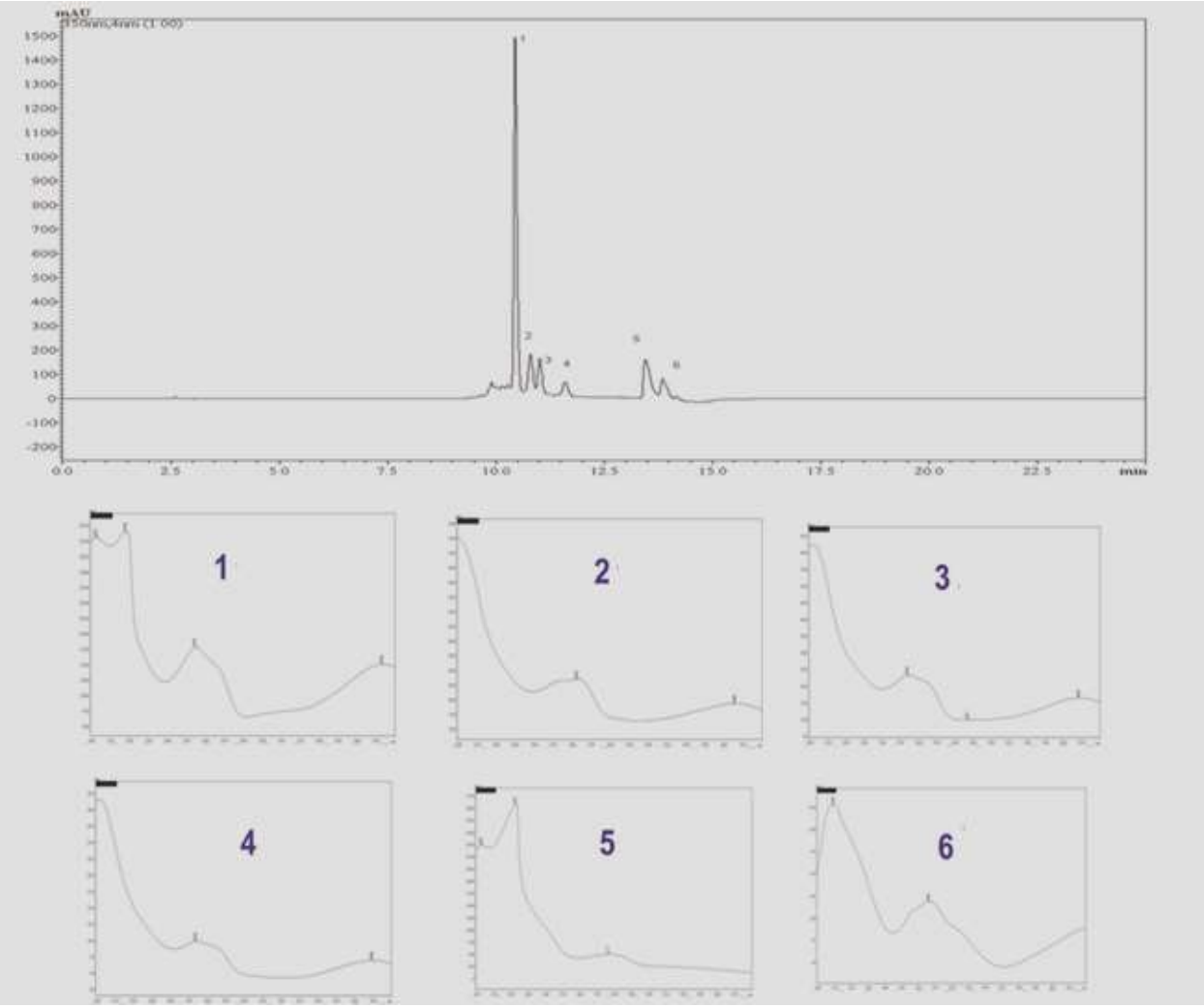

Fig. 2: Profile chromatogram of the XaHF (hexanic fraction) of the leaves of *Xylopia aromatica*. Analysis by HPLC-DAD detector at 270 nm UV spectrum peaks.

nuclear antigen (PCNA)

IHC analyses were performed using 4,0µm sections. Inactivation of endogenous peroxidase was performed treating sections with methanol + H_2O_2 3%V.V^{-1} for 15 min at room temperature, followed by incubation with anti-MMP-2 (1:100, ab76003, Abcam), anti-TIMP-2 (1:100, AF971, BD Systems) and anti-PCNA (1:100, sc-56, Santa Cruz Biotechnology) antibodies overnight, at 4°C. In order to prevent nonspecific binding and permeate the tissue, samples were incubated with blocking buffer for 45 min, followed by incubation with DAKO kit LINK and HRP-streptavidin. 3,3'-diaminobenzidine (DAB) chromogen (Sigma) was used to visualize the peroxidase activity. Sections were counterstained with hematoxylin. Primary antibodies were absent in negative controls. Quantification of staining was performed using Zeiss software to compare groups (Carl Zeiss, Germany). The immunostained were identified by a strong color using Adobe Photoshop CC 2018 (Oliveira *et al*., 2018).

STATISTICAL ANALYSIS

Groups were compared using Student´s t test, using GraphPad Prism version 5. Statistical significance was assumed for $p<0.05$.

RESULTS

Compounds Chemical characterization

Standards of gallic acid, chlorogenic acid, catechin, rutin, quercetin and boldine were eluted for peak comparisons with the liquid-liquid fractionation chromatogram of XaHF. The spectral patterns are shown in fig. 1. The obtained spectra were compared with those available in databases, and the main peaks revealed the presence of phenolic acids, flavonoids, and alkaloids on XaHF phytochemical composition (fig. 2).

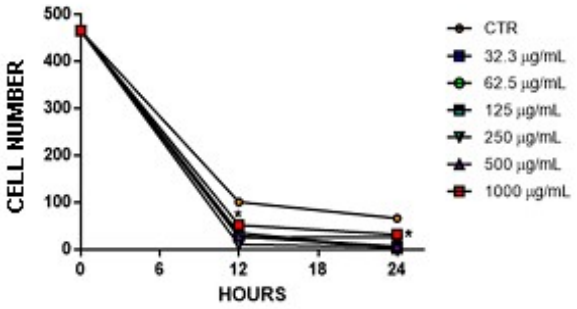

Fig. 3: The EAC was treated with a serial dilution (1000µg/mL to 32.2µg/mL) of XaHF. The cell counting was performed four times, every 6 hours after treatment application, using a Trypan blue dye. The procedure was performed in triplicate.

XaHF chromatogram presented six peaks, with retention times between 10 and 15 min. The UV spectral bands revealed compounds related to phenolic acids (263 nm), flavonoids (255 and 354 nm), and alkaloids (282 and 302 nm).

In vitro cytoxic effects of XaHF on EAC

In vitro treatment of EAC with XaHF revealed a significant cytotoxic effect of the fraction, with the two lowest concentrations (62.5 and 32.3mg/mL) being the most efficient in reducing viable cells (fig. 3).

In vivo effects of XaHF on Ehrlich's tumor growth, necrosis and inflammatory infiltrate

Treatment with XaHF was associated with a significant decrease (46%) in tumor volume compared to control (fig. 4; p=0.0017). Histopathological examination of sections revealed typical malignant features, including cellular infiltrate in adjacent muscular tissue and necrotic areas. Cells showed mitotic activity, pleomorphism, and hyperchromatism (fig. 5a, b). XaHF-treated (5b) group showed significant reduced inflammatory infiltrate when compared to control (5a). Necrotic areas (5c, d) were also reduced (71%) in XaHF treated group in comparison with control (5e).

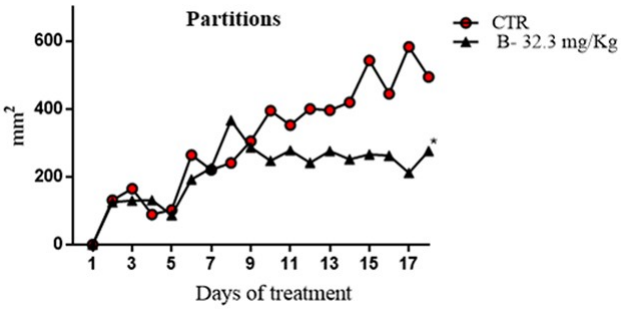

Fig. 4: Tumor size after 20 days of treatment with the XaHF (hexanic partition, 32.3mg kg^{-1}) in mice inoculated with Ehrlich tumor. *Shows statistical difference between groups.

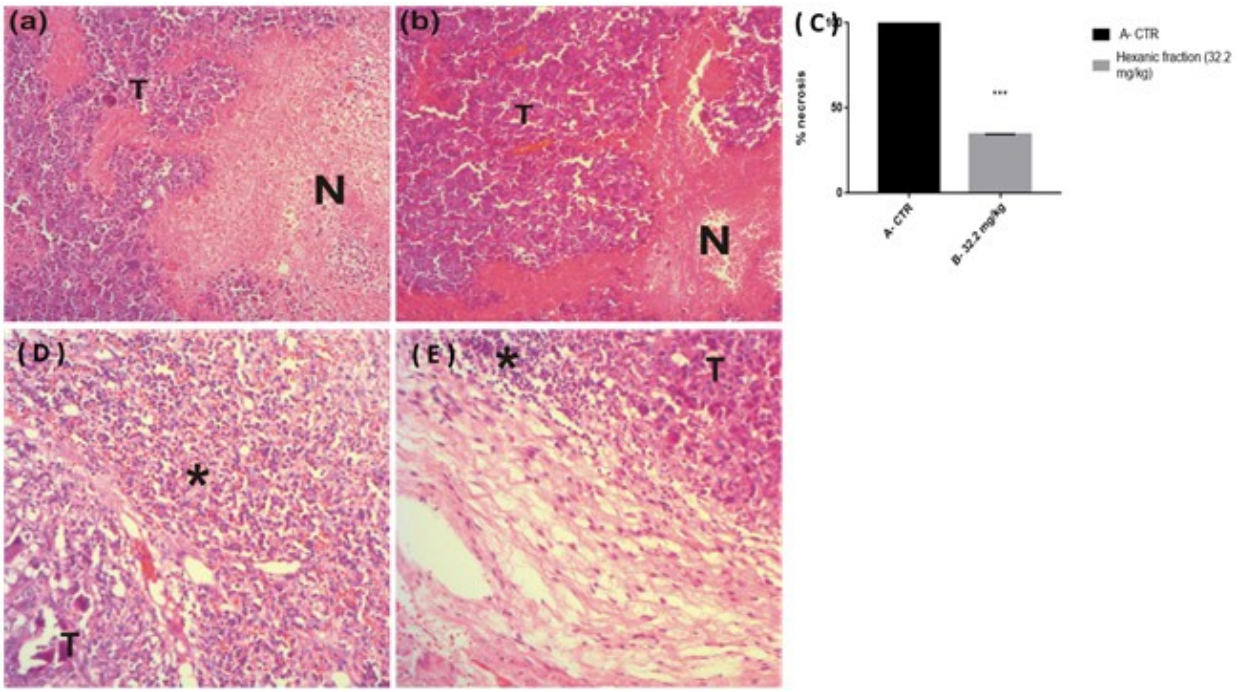

Fig. 5: Necrotic Area (a, b) and infiltrate inflammatory (c, d, e) from mice treated with hexanic fraction. Necrotic Area: (a) Control; (b) Lower necrosis area in the XaHF group (hexanic fraction; 32.2 mg kg^{-1}); (c) Necrotic area percentage. Inflamatory infiltrate (*): (d) Control; (e) Weaker inflammatory infiltrate in the XaHF group (hexanic fraction; 32.2 mg kg^{-1}) The XaHF promoted smaller areas of necrosis then control ($p< 0.05$). In this picture is possible to see the connective tissue high infiltrated by leukocytes (*). T= tumor area N= necrotic area. Photos were captured at 20x magnification.

MMP-2, TIMP-2 and PCNA expression in Ehrlich Tumor

Immunohistochemical analyses of the expression levels of MMP-2 revealed reduced immunostaining in tumor samples of animals treated with the XaHF (6b), compared to control group (6a) (p=0.0220). Statistically significant differences in immunostaining were not observed for TIMP-2 (6c and d) and PCNA (6e and f) (fig. 6).

DISCUSSION

In this work, in order to better understand the role of XaHF as a promising chemotherapeutical, the antiproliferative activity of this fraction against EAC was assessed both *in vitro* and *in vivo.* XaHF treatment decreased the cell viability after 24 hours of exposure and also reduced tumor volume, inflammatory infiltrate, necrosis area and MMP-2 expression *in vivo*.

Besides, analysis of XaFH chromatographic profiles revealed the presence of phenolic acids, flavonoids, and alkaloids as phytochemical constituents. These secondary metabolites are characterized by diverse biological activities, such as antioxidants, cardioprevention, hepatoprotection, anti-inflammatory and antiviral effects, in addition to antitumor activity (Formagio *et al*., 2015, Kumar and Pandey, 2013, Lucio *et al*., 2015). This work presents strong evidence of the anticancer effects of XaHF upon EAC tumor model.

The anticancer effects of the secondary metabolites found in XaHF are described as involving diverse mechanisms, such as inhibition of protective enzymes, stimulation of apoptosis, inhibition of cell proliferation and angiogenesis. (Batra and Sharma, 2013, Ghasemzadeh and Jaafar, 2013, Moudi *et al*., 2013). These compounds are also known for their protective role in carcinogens, by interaction with phase I metabolizing enzymes such as cytochrome P450 (Salminen *et al*., 2011). In several species of *Xylopia,* alkaloids have already been identified in leaves, bark, and fruits (Moreira *et al*., 2013, Silva, Reis *et al*., 2015). *Xylopia aromatica* leaves also contain essentials oils mainly composed of sabene, β-pinene, myrcene, limonene, ocimene, germacrene D and B, bicyclogermacrene, espatulenol, globulol (Lago, de Avila *et al*., 2003). This is the first study that characterized the composition of secondary metabolites on XaHF.

Chandrashekar *et al*. (2012) showed that baicalein, a flavonoid present on *Scutellaria baicalensis* Georgi, has antitumoral activity, decreasing expression levels of MMP-2 and MMP-9, leading to reduced *in vitro* cell invasiveness. The *in vivo* findings presented herein showed an inhibitory effect on tumor development. XaHF treatment reduced tumor size, necrotic areas and inflammatory infiltrate. Additionally, expression levels of MMP-2 were lower in the treated tumors. These effects could be attributed to flavonoids, but also to alkaloids and phenolic acids found (Kim *et al*., 2018, Santos *et al*., 2018).

Alkaloids are highly diverse nitrogenated compounds present in Metaphyta kingdom, and their antitumor activity is attributed to multiple mechanisms, such as inhibition of topoisomerases, cell cycle arrest, activation of apoptosis, and inhibition of adhesion junctions (Isah, 2016, Liu, Liu *et al*., 2013). In addition, studies have also shown that some alkaloids inhibited metastasis by downregulation of the EMT- related protein (Zhang *et al*., 2018; Santos *et al*., 2018).

Phenolic acids and flavonoids are the most abundant polyphenols that occur in plants (Falcone Ferreyra *et al*., 2012). Phenolic compounds, including quercetin-type flavonoids, were also identified in leaves and fruits and the presence of these may be associated with factors favoring some pharmacological activities (Ezuruike and Prieto, 2014). In fact, there is evidence for the anticancer activity of these compounds. Studies reported the downregulation of several oncogenes (PI3K and AKT), cell proliferation factors (CDK and ERK½) and transcriptional factors (NRF2 and STATs) associated with treatments with phenolic compounds, along with antiangiogenic effects linked to inhibition of VEGF and MIC-1 (Anantharaju *et al*., 2016, Batra and Sharma, 2013).

The present study shows, for the first time, the anticancer activity of XaFH against EAC both *in vitro* and *in vivo*. Taken together our results demonstrated that XaHF reduce the cell viability of EAC *in vitro*. Moreover, XaFH has an antitumor and antimetastatic potential against EAC also *in vivo* with MMP-2 expression decreased. This effect can be attributed to the alkaloids, phenolic acids, and flavonoids present its the fraction. These results provide evidences for further studies of *X. aromatica* as antineoplasic agent.

ACKNOWLEDGEMENTS

We would like to thank the Minas Gerais State Research Foundation (FAPEMIG) and National Council for Scientific and Technological Development (CNPq) for their financial support.

REFERENCES

Adewole and Caxton-Martins (2006). Morphological Changes and Hypoglycemic Effects of A*nnona Muricata* Linn. (Annonaceae) Leaf Aqueous Extract on Pancreatic B-Cells of Streptozotocin-Treated Diabetic Rats. *Afr J Biomed Res,* **9**: 173-187.

Adusumilli P, Konatam ML, Gundeti S, Bala S and Maddali LS (2017). Treatment challenges and survival analysis of human epidermal growth factor receptor 2-

positive breast cancer in real world. *Indian J. Med. Paediatr Oncol.,* **38**(1): 22-27.

Alam AK, Hossain AS, Khan MA, Kabir SR, Reza MA, Rahman MM, Islam MS, Rahman MA, Rashid M and Sadik MG (2016). The antioxidative fraction of white mulberry induces apoptosis through regulation of p53 and NFkappaB in EAC Cells. *PLoS One,* **11**(12): e0167536.

Anantharaju PG, Gowda PC, Vimalambike MG and Madhunapantula SV (2016). An overview on the role of dietary phenolics for the treatment of cancers. *Nutr. J.,* **15**(1): 99.

Batra P and Sharma AK (2013). Anti-cancer potential of flavonoids: Recent trends and future perspectives. *3 Biotech,* **3**(6): 439-459.

Calderon LdA, Silva-Jardim I, Zuliani JP, Silva AdAe, Ciancaglini P, Silva LHPd and Stabeli RG (2009). Amazonian biodiversity: A view of drug development for Leishmaniasis and malaria. *J. Braz. Chem. Soc.,* **20**(6): 1011-1023.

Cragg GM and Pezzuto JM (2016). Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. *Med. Princ. Pract.,* **25**(Suppl 2): 41-59.

De Carvalho Maroni L, de Oliveira Silveira AC, Leite EA, Melo MM, de Carvalho Ribeiro AF, Cassali GD, de Souza CM, Souza-Fagundes EM, Caldas IR, Araujo MS, Martins-Filho OA, de Oliveira MC. Teixeira-Carvalho A (2012). Antitumor effectiveness and toxicity of cisplatin-loaded long-circulating and pH-sensitive liposomes against Ehrlich ascitic tumor. *Exp. Biol. Med. (Maywood).,* **237**(8): 973-984.

Dutra RC, Campos MM, Santos AR and Calixto JB (2016). Medicinal plants in Brazil: Pharmacological studies, drug discovery, challenges and perspectives. *Pharmacol. Res.,* **112**: 4-29.

Ezuruike UF and Prieto JM (2014). The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. *J. Ethnopharmacol.,* **155**(2): 857-924.

Falcone Ferreyra ML, Rius SP and Casati P (2012). Flavonoids: biosynthesis, biological functions and biotechnological applications. *Front Plant Sci.,* **3**: 222.

Formagio ASN, Vieira MC, Volobuff CRF, Silva MS, Matos AI, Cardoso CAL, Foglio MA and Carvalho JE (2015). *In vitro* biological screening of the anticholinesterase and antiproliferative activities of medicinal plants belonging to Annonaceae. *Braz. J. Med Biol Res,* **48**(4): 308-315.

Gamucci T, Pizzuti L, Sperduti I, Mentuccia L, Vaccaro A, Moscetti L, Marchetti P, Carbognin L, Michelotti A, Iezzi L, Cassano A, Grassadonia A, Astone A, Botticelli A, Magnolfi E, Di Lauro L, Sergi D, Fuso P, Tinari N, Barba M, Maugeri-Sacca M, Landucci E, Conti F, Sanguineti G, De Tursi M, Iafrate G, Giordano A, Ciliberto G, Natoli C and Vici P (2017). Neoadjuvant chemotherapy in triple-negative breast cancer: A multicentric retrospective observational study in real-life setting. *J. Cell Physiol*., **233**(3): 2313-2323.

Ghasemzadeh A and Jaafar HZ (2013). Profiling of phenolic compounds and their antioxidant and anticancer activities in pandan (*Pandanus amaryllifolius* Roxb.) extracts from different locations of Malaysia. *BMC Complement. Altern. Med.,* **13**: 341.

Isah T (2016). Anticancer alkaloids from trees: Development into drugs. *Pharmacogn. Rev.,* **10**(20): 90-99.

Islam S, Nasrin S, Khan MA, Hossain AS, Islam F, Khandokhar P, Mollah MN, Rashid M, Sadik G, Rahman MA and Alam AH (2013). Evaluation of antioxidant and anticancer properties of the seed extracts of *Syzygium fruticosum* Roxb. growing in Rajshahi, Bangladesh. *BMC Complement. Altern. Med.,* **13**: 142.

Jin X. Mu P (2015). Targeting breast cancer metastasis. *Breast Cancer (Auckl),* **9**(Suppl 1): 23-34.

Kim S, Lee J, You D, Jeong Y, Jeon M, Yu J, Kim SW, Nam SJ and Lee JE (2018). Berberine suppresses cell motility through downregulation of tgf-beta1 in triple negative breast cancer cells. *Cell Physiol. Biochem.,* **45**(2): 795-807.

Kumar S and Pandey AK (2013). Chemistry and biological activities of flavonoids: An overview. *Sci. World J.,* p.162750.

Lago JHG, de Avila P, Moreno P, Limberger RP, Apel MA and Henriques A (2003). Analysis comparison and variation in the chemical composition from the leaf volatile oil of Xylopia Aromatica (Annonaceae) Biochem. Syst. Eco., **31**(6): 669-672

Liu Y, Liu J, Di D, Li M and Fen Y (2013). Structural and mechanistic bases of the anticancer activity of natural aporphinoid alkaloids. *Curr. Top. Med. Chem.,* **13**(17): 2116-2126.

Lucio AS, Almeida JR, Da-Cunha EV, Tavares JF, Barbosa Filho JM (2015). Alkaloids of the Annonaceae: Occurrence and a compilation of their biological activities. *Alkaloids Chem. Biol.,***74**:233-409.

Meireles DRP, Fernandes HMB, Rolim TL, Batista TM, Mangueira VM, Sousa TKGd, Pita JCLR, Xavier AL, Beltrão DM, Tavares JF, Silva MS, Medeiros KKP and Sobral MV (2016). Toxicity and antitumor efficacy of Croton polyandrus oil against Ehrlich ascites carcinoma cells. *Revista Brasileira de Farmacognosia* **26**(6): 751-758.

Mishra S, Tamta AK, Sarikhani M, Desingu PA, Kizkekra SM, Pandit AS, Kumar S, Khan D, Raghavan SC and Sundaresan NR (2018). Subcutaneous Ehrlich Ascites Carcinoma mice model for studying cancer-induced cardiomyopathy. *Sci. Rep.,* **8**(1): 5599.

Mishra Sa S, Kumar N and Sharma BK (2013). *Annona muricata* (the cancer killer): A review. *Glob. J. Pharm. Res.,* **2**(1): 1613-1618.

Moreira IC, Roque NF, Vilegas W, Zalewski CA, Lago JH and Funasaki M (2013). Genus xylopia (Annonaceae): Chemical and biological aspects. *Chem. Biodivers,* **10**(11): 1921-1943.

Moudi M, Go R, Yien CY and Nazre M (2013). Vinca alkaloids. *Int. J. Prev. Med.,* **4**(11): 1231-1235.

Newman DJ, Cragg GM, Holbeck S and Sausville EA (2002). Natural products and derivatives as leads to cell cycle pathway targets in cancer chemotherapy. *Curr. Cancer Drug Targets,* **2**(4): 279-308.

Oliveira SE, Costa PM, Nascimento SB, Castro WV, Ribeiro R, Santos HB and Thome RG (2018). Atrazine promotes immunomodulation by melanomacrophage centre alterations in spleen and vascular disorders in gills from *Oreochromis niloticus*. *Aquat Toxicol.,* **202**: 57-64.

Salminen KA, Meyer A, Jerabkova L, Korhonen LE, Rahnasto M, Juvonen RO, Imming P and Raunio H (2011). Inhibition of human drug metabolizing cytochrome P450 enzymes by plant isoquinoline alkaloids. *Phytomedicine,* **18**(6): 533-538.

Santos KM, Gomes INF, Silva-Oliveira RJ, Pinto FE, Oliveira BG, Chagas RCR, Romao W, Reis RMV and Ribeiro R (2018). *Bauhinia variegata* candida fraction induces tumor cell death by activation of Caspase-3, RIP, and TNF-R1 and inhibits cell migration and invasion *In Vitro*. *Biomed. Res. Int.,* p.4702481.

Seca AML. Pinto D (2018). Plant secondary metabolites as anticancer agents: Successes in clinical trials and therapeutic application. *Int. J. Mol. Sci.,* **19**(1): 263.

Silva-Oliveira RJ, Lopes GF, Camargos LF, Ribeiro AM, Santos FV, Severino RP, Severino VG, Terezan AP, Thome RG, Santos HB, Reis RM and Ribeiro RI (2016). *Tapirira guianensis* Aubl. extracts inhibit proliferation and migration of oral cancer cells lines. *Int. J. Mol. Sci.,* **17**(11): 1839.

Silva Le, Reis Ra, Moura Ea, Amaral W and Sousa Jr PT (2015). Plantas do Gênero Xylopia: Composição Química e Potencial Farmacologico. *Rev. Bras. Pl. Med.,* **17**(4): 814-826.

Taylor P, Arsenak M, Abad MJ, Fernandez A, Milano B, Gonto R, Ruiz MC, Fraile S, Taylor S, Estrada O and Michelangeli F (2013). Screening of Venezuelan medicinal plant extracts for cytostatic and cytotoxic activity against tumor cell lines. *Phytother. Res.,* **27**(4): 530-539.