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Ana Paula Costa Rodrigues Ferraz

Atividade bioativa do extrato dos frutos de Jurubeba (*Solanum paniculatum* L.) em linhagens celulares humanas de adenocarcinoma mamário

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu, para obtenção do título de Doutor(a) em Patologia.

Orientador (a): Prof(a). Dr(a). Camila Renata Corrêa
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Palavras-chave: Estresse oxidativo; Jurubeba; ROS; *Solanum paniculatum* L.

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Dedico este trabalho primeiramente ao Deus do meu coração pela oportunidade de fazer do meu ser, veículo de contribuição a humanidade.

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Epígrafe

“...é verdade que Deus faz o mundo calculando, mas seus cálculos nunca estão corretos, e é mesmo esta injustiça no resultado, esta irredutível desigualdade, que forma a condição do mundo.”

— Gilles Deleuze

Resumo

Estimativas epidemiológicas mundiais consideram o câncer de mama como a segunda causa de morte na população mundial. Diversos são os fatores que contribuem para o desenvolvimento do câncer, este utiliza o estresse oxidativo para proliferação celular, metástase e angiogênese. *Solanum paniculatum* L. ou popularmente conhecida como Jurubeba, é uma planta medicinal reconhecida pela ANVISA onde suas folhas e raízes são popularmente utilizadas para tratamento gástrico, antibacteriana, antidiarreica e anti-inflamatória, entretanto, pouco se sabe sobre o efeito biológico no câncer bem como o seu perfil fitoquímico de antioxidantes. O objetivo geral desse trabalho foi avaliar a atividade bioativa do extrato dos frutos de Jurubeba (*Solanum paniculatum* L) em linhagens celulares de adenocarcinoma mamário. Objetivo específico 1: Investigar o perfil fitoquímico da formulação hidroetanólica dos frutos e o seus efeitos na produção de ROS e citocinas em células humanas de câncer de mama e células endoteliais. Objetivo específico 2: Avaliar o efeito biológico do extrato de Jurubeba no Estado Redox em células MCF-7 de câncer de mama. O extrato hidroetanólico foi formulado e caracterizado o perfil de antioxidantes. Células endotelias e de câncer de mama (HUVEC, MDA-MB-231 e MCF-7) foram utilizadas para estabelecer os parâmetros farmacológicos (doses 1.87, 3.75, 7.5, 15, 30 e 60 μg/mL) e avaliou-se por citometria de fluxo as Espécies Reativas (ROS) e citocinas (interleucina 6 (IL-6) e interleucina 1-β) por kit ELISA. Posteriormente, em células MCF-7 testou-se na expressão de enzimas antioxidantes endógenas e o estado redox por biomarcadores de proteína carbonilada (PCO), 4 – Hidroxinonenal (4-HNE) e Malonaldeído (MDA). A luteína foi o composto majoritário encontrado no extrato hidroetanólico de *Solanum paniculatum* L. A dose 3.75 μg/mL diminuiu significativamente a produção de IL-6 e não mostrou-se citotóxica, o estado redox aumentou com a dose de 3.75 μg/mL mostrando-se com um potencial pro-oxidante e a dose 7.5 μg/mL modulou positivamente a produção de ROS. O presente estudo são dados iniciais sobre o efeito do extrato em células de câncer de mama e mostram-se como importantes achados em futuras terapêuticas tumorais.

Palavras-chave: Jurubeba, *Solanácea*, estresse oxidativo, ROS.

Abstract

Worldwide epidemiological estimates consider breast cancer as the second leading cause of death in the world population. There are several factors that contribute to the development of cancer, which uses oxidative stress for cell proliferation, metastasis and angiogenesis. *Solanum paniculatum* L. or popularly known as Jurubeba is an ANVISA-recognized medicinal plant where its leaves and roots are popularly used for gastric, antibacterial, antidiarrheal and anti-inflammatory treatment, however, little is known about the biological effect on cancer as well as its phytochemical profile of antioxidants. The aim of this work was to evaluate the bioactive activity of Jurubeba (*Solanum paniculatum* L.) fruit extract in mammary adenocarcinoma cell lines. Specific Objective Article 1: To investigate the phytochemical profile of fruit hydroethanolic formulation and its effects on the production of ROS and cytokines in human breast cancer cells and endothelial cells. Specific objective Article 2: To evaluate the biological effect of Jurubeba extract in Redox state on MCF-7 breast cancer cells. The hydroethanolic extract was formulated and characterized the antioxidant profile. Endothelial and breast cancer cells (HUVEC, MDA-MB-231 and MCF-7) were used to establish pharmacological parameters (doses 1.87, 3.75, 7.5, 15, 30 and 60 μg / mL) and were evaluated by cytometry. Reactive Oxygen Species (ROS) and cytokines (interleukin 6 (IL-6) and interleukin 1-β) by ELISA kit. Subsequently, MCF-7 cells were tested for expression of endogenous antioxidant enzymes and redox status by biomarkers of carbonylated protein (PCO), 4-hydroxynonenal (4-HNE) and Malonaldehyde (MDA). Lutein was the major compound found in the hydroethanolic extract of *Solanum paniculatum* L. The dose 3.75 μg / mL significantly decreased IL-6 production and was not cytotoxic, the redox state increased with the dose of 3.75 μg / mL with a pro-oxidant potential and the 7.5 μg / mL dose positively modulated ROS production. The present study provides initial data on the effect of the extract on breast cancer cells and is an important finding in future tumor therapies.

Key-words: Jurubeba, *Solanácea,* oxidative stress, ROS

Lista de Ilustrações

Lista de abreviaturas e siglas

Classificação de Tumores Malignos	TNM
Instituto Nacional do Câncer	INCA
Agência Internacional de Pesquisa em Câncer	IARC
Marcadores Imunohistoquímicos	IHC
Espécies Reativas	ROS
Superóxido dismutase	SOD
Catalase	CAT
peroxidação lipídica de ácidos poli-insaturados	PUFA
radical lipoperoxil	LOO•
hidroperóxido lipídico	LOOH
Malonaldeído	MDA
4-Hidroxinonenal	4-HNE
Fator de Necrose Tumoral	TNF
Interleucina 6	IL-6
International Union of Pure and Applied Chemistry	IUPAC
Agência Nacional de Vigilância Sanitária	ANVISA
Hydroethanolic Extract of *Solanum Paniculatum* L.	HESPL
Entrance Potential	EP
Collision Energy	CE
Collision Cell Exit Potential	CXP
Curtain Gas	API
Medium Collision Gas	CAD
Selective Reaction Monitoring	SRM
Radical Sequestration Method	DPPH •
Fetal Bovine Serum	FBS
Dimethyl Sulfoxide	DMSO
Interleukin 1β	IL- 1β
Aperfeiçoamento de Pessoal de Nível Superior	CAPES
Protein Carbonyl	PCO
Manganese Superoxide Dismutase	MnSOD
Malondialdehyde	MDA
Antioxidant Capacity	AC
Phosphatidylcholine	PC
Standart Deviation	SD
Total Antioxidant Performance	TAP
Chemotherapy-Induced Nausea And Vomiting	CINV
Chemotherapy-Induced Peripheral Neuropathy	CIPN

Sumário

Capítulo II

1. INTRODUÇÃO

1.1. O estudo do câncer de mama : Histórico e definição

Desde a época Mendeliana (1860), as alterações genéticas podiam ser estudadas e analisadas por diversos pesquisadores. Devido as inúmeras alterações no gene, o desenvolvimento de técnicas de seqüenciamento do DNA permitiu os estudos das mutações genéticas, de importante interesse nas pesquisas iniciais de câncer bem como na evolução das espécies, contribuindo com os estudos darwinianos (1). Modificações na expressão gênica e epigenéticas como alterações histonais, e controle de fatores de transcrição mostraram-se como principais áreas em estudos iniciais na investigação fenotípica do câncer conferindo a hereditariedade como primeiro principal fator contribuinte para o desenvolvimento tumoral(2).

> "...Quando publiquei os resultados de meus experimentos sobre o desenvolvimento de ovos de ouriço-do-mar com fertilização dupla em 1902, acrescentei a sugestão de que tumores malignos podem ser o resultado de uma determinada condição anormal dos cromossomos, que podem surgir da mitose multipolar. … Assim Eu tenho continuado por muito tempo o tipo de experimentos que eu sugeri, que são até agora sem sucesso, mas minha convicção permanece inabalável."
> Theodor Boveri, patologista, 1914 (Boveri T apud Weinberg, Robert et al. 1942).

A habilidade da natureza do câncer de desenvolvimento somático e alterações morfológicas atraíram diversos pesquisadores em relação às primeiras classificações morfológicas do câncer (Figura 1). A morfologia dos tecidos tumorais mostra-se com desarranjos das suas células e nos espaços intercelulares com características de anormalidades do núcleo, aumento do espaço tecidual e invasividade em outros tecidos (3).

> "O câncer é "um grupo de doenças, que têm em comum o crescimento desordenado de células", as quais proporcionam uma desequilíbrio na homeostasia biológica e ao formar nódulos ou massas, são chamadas de tumores e posteriormente, são nomeados individualmente após acometer uma parte específica do organismo" (Adaptado de Breast cancer: Facts & Figures 2017-2018. In: American Cancer Society. 2018.)

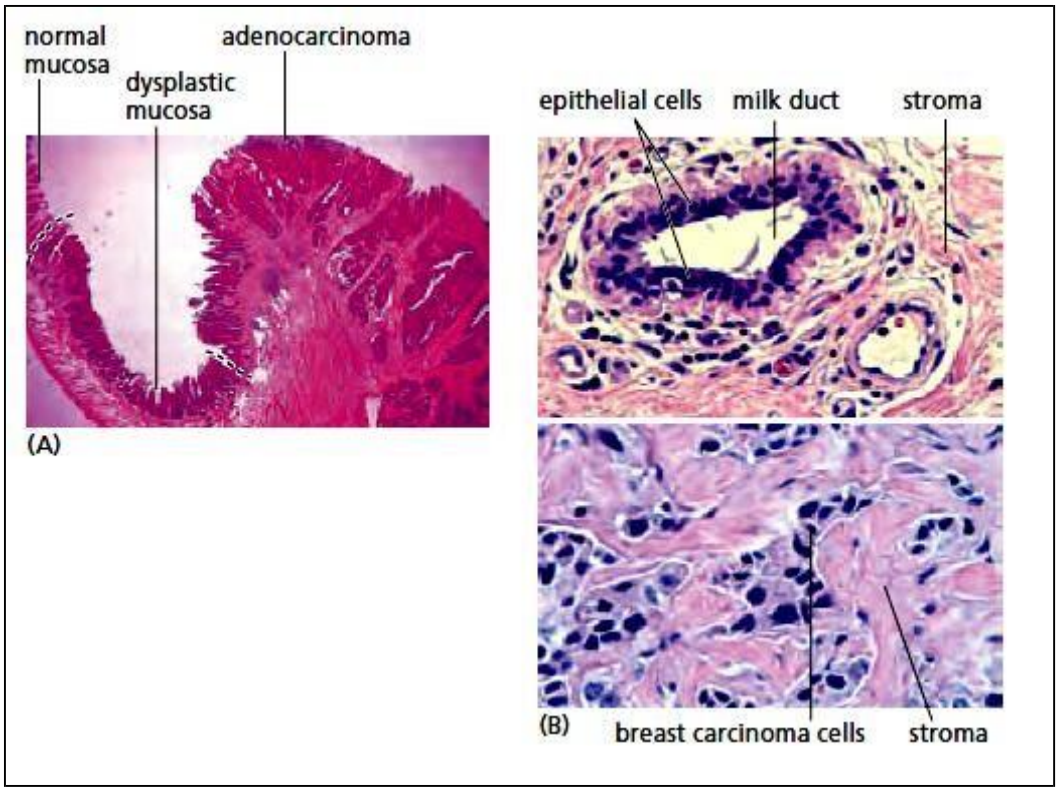

Figura 1. Representação de estudos morfológicos teciduais em câncer de mama. Adaptação de Weinberg et al. (1942).

O estudo histológico do câncer contribuiu para evoluções na prática clínica por meio das suas classificações comumente utilizadas. Dentre as diversas classificações dos cânceres, desenvolveu-se no Brasil, um método de Classificação de Tumores Malignos (TNM) pelo Instituto Nacional do Câncer (INCA) (4). Em situações de invasividade em outros tecidos, o câncer se classifica como maligno e quando há apenas um crescimento local, é um tumor benigno. Em relação à natureza tecidual, quando é de natureza epitelial é classificado como "carcinoma", e de natureza glandular, possui a abreviação "Adenoma"(4).

Ainda de acordo com o INCA (2004), existem duas terminologias para o câncer de mama: Adenocarcinoma mamário, quando maligno ou "Adenoma" quando benigno. Estes possuem um tipo epitelial de tecido que contém células especializadas em secretar substâncias nos ductos ou cavidades (Lumina) (Figura 2)

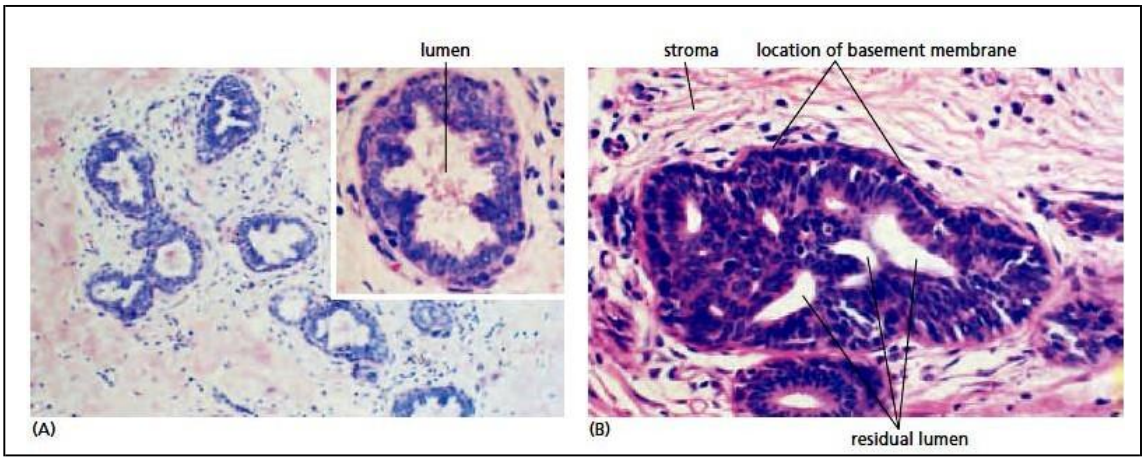

Figura 2. Representação de (A) ductos hiperplásicos mamários (B) invasividade das cavidades luminais. Adaptado de Weinberg et al. (1942).

Células especializadas no estudo do câncer de mama são comumente utilizadas em pesquisas científicas e estas estão classificadas de acordo com a sua distribuição heterogênea e seus biomarcadores associados. Os distintos subtipos funcionais de câncer de mama são conhecidos como Luminal, células MCF-7, de tumor primário entram nessa subclassificação. Basal A ou B, a MDA-MB-231, por exemplo, é uma célula potencialmente invasiva classificada em Basal B, entre outros tipos celulares (5).

1.2. Epidemiologia e formação do câncer de mama

Estimativas epidemiológicas mundiais consideram o câncer de mama como a segunda causa de morte na população, atingindo-a de uma forma significativa (6) existindo mais de 18,1 milhões de novos casos de acordo com a IARC and New Global Cancer Data (2018), com 21% nas Américas, 23,4% na Europa, 48,4% na Ásia e 5,8% nas regiões da África, mostrando um perfil heterogêneo de distribuição entre os diversos países que são acometidos (7,8). Essa heterogeneidade se dá principalmente ao acesso ao diagnóstico precoce e a sobrevivência relacionada aos acessos a tratamentos quando detectado (8).

No ano de 2018 foi estimado em torno de 2.088.849 (11.4%) novos casos de câncer de mama (9), acometendo as mulheres nos países desenvolvidos e nos em desenvolvimento (10). Entretanto, quando diagnosticado em seus primeiros estágios, por meio de marcadores imunohistoquímicos (IHC) e variáveis clínico

patológicas como tamanho e grau do tumor, envolvimento nodal, tipos histológicos, margens cirúrgicas e entre outros (11), é um tipo de câncer que possui um bom prognóstico para tratamentos e/ou métodos profiláxicos (12).

A formação do câncer, ou o processo de carcinogênese podem ser influenciadas por agentes cancerígenos conhecidos como fatores instrínsecos e extrínsecos (13). As mutações ao DNA relacionados com a susceptibilidade genética que podem ser associados com a hereditariedade e fatores ambientais como radiações (UV, ionizante, eletromagnética), consumo de álcool, uso de tabaco, microorganismos (vírus, bactérias, e parasitas) e agentes químicos são conhecidos como fatores intrínsecos e extrínsecos, respectivamente (14).

O efeito cumulativo dos agentes cancerígenos proporcionam ações genotóxicas no microambiente celular e o processo de formação do câncer é subdivido em iniciação, a qual envolve a formação de mutação genética a partir da genotoxicidade; promoção, com expansão clonal seletiva dose-dependente das células iniciadas para a células malignas e por fim, a progressão caracterizado por irreversibilidade, instabilidade genômica com perturbação da integridade cromossomal e alterações moleculares bem estabelecidas desde o processo inicial da carcinogênese (iniciação) (15,16).

A célula neoplásica possui características biológicas individuais como resistência à morte, ativação de crescimento e metástase, sustentação da sinalização da proliferação celular, imortalidade e indução de angiogênese (11,17,18), as quais sustentam a malignidade do câncer, concomitantemente com fatores como a inflamação crônica e o estresse oxidativo, que auxiliam no aumento da sobrevivência e proliferação celular, ativação de sinalização pré-tumorigênica e conduz a instabilidade genômica em diversos tipos de cânceres (19–21).

Dentre as diversas maneiras de atenuar o desenvolvimento de células tumorais, a prevenção primária se destaca por ser de baixo custo e que possa promover alguma repercussão para diversos tipos de câncer que possuem causas em comum, como a redução do tabagismo, alimentação saudável, práticas de atividades físicas e terapia molecular individualizada (22). Adicionalmente, o desenvolvimento de estratégias terapêuticas para atenuação e/ou profilaxia aos diversos tipos de cânceres têm se evidenciado em pesquisas científicas na atualidade (23–28).

1.3. Estresse Oxidativo, Inflamação e Câncer

Durante o metabolismo normal do organismo, substâncias oxidantes conhecidas como radicais livres ou espécies reativas (ROS) são produzidas naturalmente em todos os tipos celulares principalmente por meio da fosforilação mitocondrial (29). As espécies reativas possuem um importante papel fisiológico na biologia celular como defesa imunológica e sinalização (30). Dentro desta condição é estimado que aproximadamente 4 - 5% do oxigênio molecular é convertido em espécies reativas, conjuntamente estas também são produzidas durante o metabolismo da enzima P450, na ativação de processos inflamatórios e na ação de peroxisomos a níveis celulares (31).

O grupo do ROS é contemplado por variedades de espécies reativas como o superóxido ($O_2 \bullet^-$), peróxido de hidrogênio (H_2O_2) e hidroxila (OH•) como os mais comuns encontrados na biologia celular. Dentro desta família o peróxido de hidrogênio (H_2O_2) destaca-se como um importante sinalizador tumorigênico (32).

Para a homeostase celular, existe um sistema de defesa antioxidante constituído por enzimas na função de neutralizar essas espécies oxidantes. Para a manutenção e integridade de membranas e biomoléculas são necessárias redes de defesa antioxidantes que podem ser realizadas por qualquer substância que previna o processo de oxidação, através da adição de um oxigênio ou a remoção de um hidrogênio ou elétron das espécies reativas (33,34) presentes no microambiente celular. A enzima superóxido dismutase (SOD) atua na dismutação do superóxido $O_2 \bullet^-$ produzindo peróxido de hidrogênio (H_2O_2), o qual é tolerável pelo organismo biológico, passível à sua degradação por peroxissomos e a enzima Catalase (CAT) (21). Adicionalmente, existem antioxidantes exógenos provenientes da alimentação que podem atuar em sinergismo à SOD e a Catalase (35)

Dentro do contexto do câncer, estas espécies são produzidas em maior quantidade em relação a uma célula normal uma vez que a proliferação celular demanda maior atividade da mitocôndria que confere em maior produção de espécies reativas. Em contrapartida, a célula tumoral possui mecanismos compensatórios para que não ocorra um estresse oxidativo por meio do aumento da atividade de enzimas antioxidantes, mantendo assim o equilíbrio redox para a

manutenção da proliferação celular(36).

O uso de quimioterápicos, ativação do sistema imunológico e depleção do sistema antioxidante leva ao aumento da produção de ROS. Este aumento sinaliza apoptose e morte celular (necrose). (32,36).

O mecanismo de ação no ROS na sinalização celular no câncer é bem estabelecido por meio da ativação de vias como a proteína quinase ativada por mitógeno (MAPK)/ERK ½, a via fosfoinositido-3-quinase (PI3K/Akt) e proteína quinase D (PDK), por sua vez, ativando fator nuclear kappa B (NF-kB) e aumentando a proliferação celular. Já na morte celular, os principais mecanismos são envolvidos na translocação do citocromo C para o citoplasma conferindo ações na caspase -3 e -7 por meio da atuação da caspase 9 e 8 (21) (Figura 3).

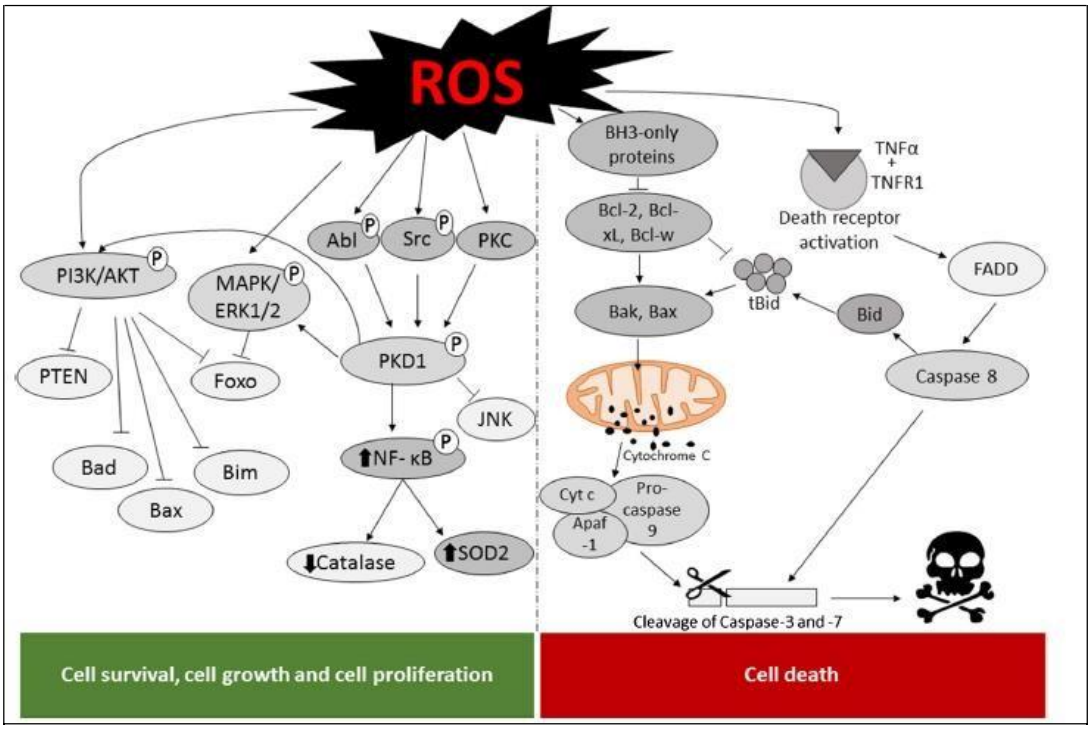

Figura 3. Sinalização do ROS no câncer. ROS ativa vias como PI3K/AKT, MAPK e PKD1 na ativação de diversos mecanismos. Já na morte celular, ativa mecanismos como Bcl-2, Bax e caspase 8. O aumento exarcebado de ROS danifica a membrana mitocondrial, deslocando o citocromo C que atua na clivagem da caspase 3 e 7. Adaptado de Moloney et al. 2018.

As espécies reativas são capazes de quebrar cadeias duplas e ligações

transversais de DNA e modificar as bases nitrogenadas do DNA (37). Ao oxidar as bases do DNA, por exemplo, estas são capazes de induzir mutações comumente observadas nos estágios iniciais de formação do câncer (20). O estresse oxidativo pode produzir produtos intermediários por meio de alterações na homeostase de membranas biológicas via peroxidação lipídica de ácidos poli-insaturados (PUFA), os quais por sua vez, promovem a formação de radical lipoperoxil (LOO•) que reage com hidroperóxido lipídico (LOOH) e visto sua característica molecular susceptível à instabilidade, forma novos produtos derivados de aldeídos, como o MDA, Hexanal, HNE ou Acroleínas (38), uma vez que são biomarcadores de estresse oxidativo utilizados comumente utilizáveis na ciência devido à sua facilidade em detecção e abundância em células e diversos tecidos em organismos celulares à matriz biológica humana (39–41). Dentro deste espectro, o HNE é um importante marcador na carcinogênese regulando o crescimento tumoral (42,43). ROS, por sua vez, pode ativar kinases como a proteína kinase C via metabolização enzimática da fosfolipase C e atuar na regulação da proliferação celular induzindo a carcinogênese conjuntamente com a ativação de diversos fatores de transcrição como NF-kB e entre outros (32).

Ao estimular NF-kB, ocorre a ativação da cascata inflamatória. A inflamação crônica, é outra condição encontrada no câncer que participa principalmente no processo de crescimento neoplásico estimulando a expressão de COX 2 (43). A malignidade da célula tumoral proporciona diversos tipos de condições de inflamação, e em uma situação de proliferação celular descontrolada, alguns oncogenes da família RAS e MYC induzem programas transcricionais que expressam quimiocinas e citocinas pró-inflamatórias como fator de necrose tumoral (TNF), IL-6, as quais auxiliam na sobrevivência, angiogênese e proliferação tumoral (45,46) (Figura 4).

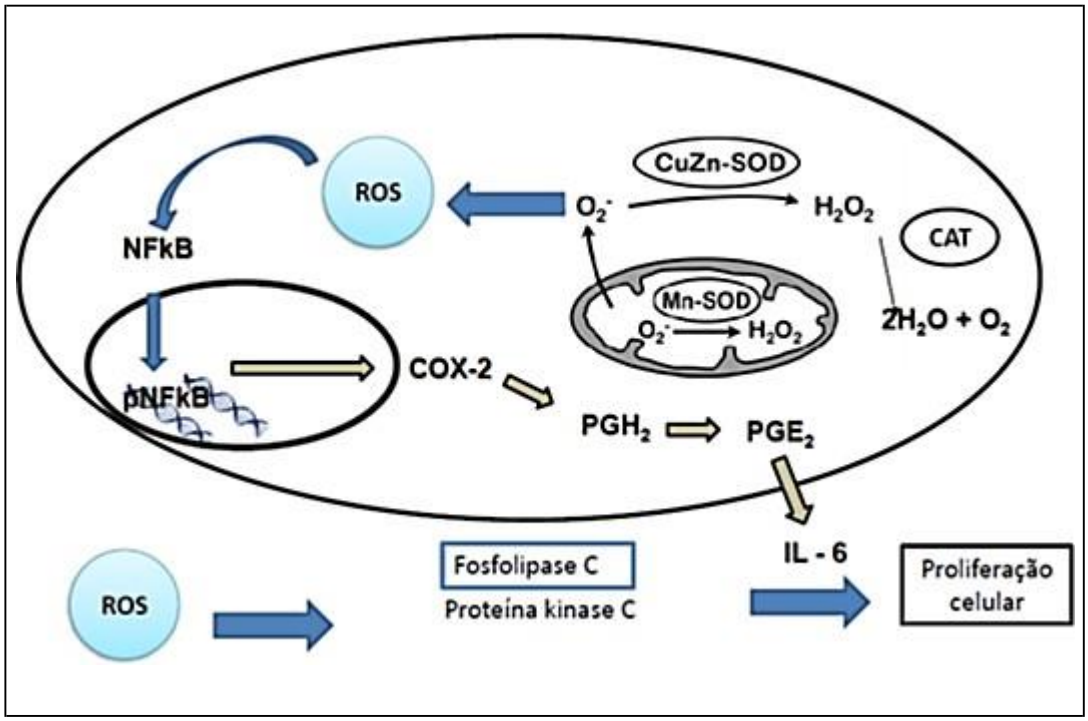

Figura 4. Relação do mecanismo de estresse oxidativo, inflamação e câncer. O aumento da produção de ROS, ativa NF-kB, este fosforilado dentro do núcleo da célula, ativa a via de inflamação COX-2, que ativa prostaglandinas e consequentemente, a produção de citocinas. Para conter o ROS, SOD transforma o superóxido (O_2^-) em peróxido de hidrogênio (H_20_2) e estes são transformados em água e oxigênio. Elaborada pela autora.

1.4. A Fitoterapia e sua aplicabilidade biológica

Em uma situação de estresse oxidativo faz-se necessário a complementação de substâncias antioxidantes provenientes da alimentação com afinidades lipo e hidrossolúveis presentes em principalmente frutas, hortaliças e plantas medicinais com evidente atuação na medicina translacional (47,48).

Dietas ricas em frutas e vegetais, assim como o emprego da fitoterapia, têm sido cada vez mais estudados, em função das atividades anti-inflamatórias e antioxidantes de compostos bioativos como polifenóis e carotenoides em diversas doenças e suas adversidades clinico patológicas (49–57) atuando em vias metabólicas favorecendo a prevenção ou atenuação em terapias como fator

complementar e/ou coadjuvante (58–60).

O grupo dos carotenoides possuem afinidades lipofílicas e são adquiridos a partir de alimentos com pigmentações amarelas, laranja e vermelhas (61). A sua estrutura química é composta por 40 cadeias de carbono com destaque para Zeaxantina, β-Criptoxantina, α e β-Caroteno, Licopeno e Vitaminas como A e como os compostos com níveis elevados presentes na matriz biológica humana (62).

Os polifenóis classificados em grupos dos ácidos fenólicos, ácidos hidroxicinâmicos, cumarinas, xantonas e flavonóides possuem propriedades hidrofílicas e lipofílicas, os quais proporcionam uma alta performance antioxidante principalmente porque possuem pelo menos dois anéis fenil com um ou mais substituintes hidroxila e derivados funcionais, tais como ésteres e glicosídeos (63,64) e devido ao seu menor tamanho molecular quando comparado com os carotenoides, interagem mais facilmente e com ação sinérgica nos eventos de desequilíbrio redox em uma matriz biológica (65)

O uso de etanol como solvente em métodos de extrações efetivos foi estabelecido pela Farmacopéia Homeopática Brasileira (66,67) poderia ser uma boa fonte de pesquisa científica com aplicabilidades biológicas e industriais (68,69) pois, é bem estabelecido na literatura os efeitos benéficos de extratos hidroetanólicos formulados de plantas para estudos biológicos em diversas doenças (70–79). Adicionalmente, o uso de etanol proporciona a minimização de depósitos de resíduos químicos no extrato de interesse, abordando os princípios ecológicos da “Química verde”, definida pela União Internacional de Química Pura e Aplicada (IUPAC) como "A invenção, desenvolvimento e aplicação de produtos e processos químicos para reduzir ou eliminar o uso e a geração de substâncias perigosas" (68,69,80). Visto que o termo “perigosas” deve ser entendido como substâncias nocivas de algum modo à saúde humana ou ao meio ambiente, os quais são comumente encontrados em solventes orgânicos (clorofórmio, tetraidrofurano (THF), hexano) utilizados em laboratórios científicos, portanto, o uso do etanol como solvente extrator é evidenciado em estudos com sustentabilidade na atualidade (81–83).

1.4.1. Solanum paniculatum L. ou “Jurubeba”

Solanum paniculatum L. [Roem. & Schult.], é planta arbustiva, perene e uma hortaliça-fruto não convencional (84), originária da América Tropical, pertencente à família Solanaceae, nativa das regiões Norte e Nordeste do Brasil e encontrada nas regiões do Norte (Pará), Nordeste (Alagoas, Bahia, Ceará, Maranhão, Paraíba, Pernambuco, entre outros), Centro-oeste (Distrito Federal, Goiás, Mato Grosso do Sul, Mato Grosso), Sudeste (Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo) e Sul (Paraná, Rio Grande do Sul e Santa Catarina) (85). É conhecida popularmente como Jurubeba, Jurupeba, Juripeba, Juvena, Juina ou Juna (86).

Possui em torno de 1 – 1,5 m de altura, tricomatosa revestida de indumento alvo-tomentoso e raiz ramificada em crescimento secundário inicial, com xilema em estrutura hexarca. A raiz é composta por pedaços tortuosos, com diâmetro de até 3 cm, de cor cizento-pardecente clara, levemente sulcada no sentido longitudinal com fendas transversais e várias radículas deixadas em sua queda e, na sua secção transversal é visto uma casca estreita e um lenho bem desenvolvido. O caule, semelhante à raiz, pode atingir até 8 cm, a sua superfície é menos sulcada no sentido longitudinal, não mostrando raízes e com inúmeras lenticelas em forma de pequenas verrugas achatadas. As flores monoclinas e estaminadas, com cálice campanulado, triangular-acuminados com coloração lilás ou alva (85).

Folhas largo-ovaladas a lanceoladas com margens lobadas e acúleos cônicos evidentes e frutos solanídios globulosos de coloração esverdeada a amarela presos em um pendúnculo de acordo com as descrições de Nurit et al. (2007) (87).

Figura 5. *Solanum paniculatum* L. Adaptado de Ministério da Saúde, 2015.

Geralmente é consumida pela população com fins medicinais, sendo suas folhas, caules e raízes preparados por meio de infusões, sucos, chás e extratos alcoólicos que atribuem à Jurubeba o reconhecimento como uma planta medicinal reconhecida pela ANVISA (88) e é um representante de *Solanum* reconhecido como fitoterápico pela Farmacopéia Brasileira, segundo a Farmacopeia dos Estados Unidos do Brasil (1959) (89).

A composição quimica da Jurubeba é contemplada pela presença de substâncias como neotigogenin, sapogenina nitrogenada esteriodal paniculidina, saponina jurubina e alcalóides como solamargina, solanina e solanidina e nas folhas foram isolados dois glicosídeos espirostânicos denominados paniculonina A e B, geninas neoclorogenina, paniculogenina. Já nas folhas e flores foi encontrado o alcaloide solanina e esteróides como B-sitosterol, estigmasterol, β-D-xylopyranosyl-(1”” → 3””)-β-D-quinovopyranosy e diogesnin (85).

Os frutos da Jurubeba, são consumidos popularmente na forma processada, conservas ou em preparações como o arroz com jurubeba e açafrão (*Curcuma longa*

(86) e recentemente, encontramos apenas um estudo da atividade anti-inflamatória dos frutos por meio da ação das propriedades provenientes de fitoesteróis (sigmasterol e β-sitosterol) (91). E diferentemente dos frutos, os extratos aquosos de flores e raízes e folhas da jurubeba já possuem propriedades medicinais bem descritas na atividade inibitória da secreção de ácido gástrico, atuando no tratamento de dispepsia (92,93), antidiarreica (94), ação antibacteriana (95), antiviral (96) e antioxidante (97).

A proteção gástrica das partes das folhas, caules, frutos e raízes desta planta é descrita em diversos trabalhos como o estudo de Mesia-Vela et al. (2002), o qual utilizou camundongos híbridos (C57BL e Balb) para avaliar a atividade antisecretora gástrica e antiúlcera nas concentrações de 0,5 e 2g/kg do extrato aquoso de todas as partes de *S. paniculatum*. O extrato formulado pela raiz mostrou-se com atividade anti-secretora do ácido gástrico mais elevada do que caules e flores, para as folhas, nenhum efeito foi observado e os frutos por sua vez, estimularam a secreção do ácido gástrico. Já no estudo de Botion et al. (2005) com intervenção da Ierobina, a qual é um medicamento formulado com diversas plantas incluindo a S. paniculatum e comercializada comumente nas redes de drogarias, esta melhorou a absorção de lipídeos.

No câncer, alguns trabalhos mostram atividade antitumoral de quimioprevenção de *Solanum paniculatum* L. como o estudo de Endringer et al. (2010) com frações clorofórmio e acetato de etila e em células de câncer de fígado (HepG2) (98). De Souza et al. (2019) por sua vez, com um extrato rico em polifenóis, é promissor para efeitos analgésicos da planta (99). A espécie *Solanum paniculatum* também apresentou atividade contra *Staphylococcus aureus* (ATCC 12692), *Escherichia coli* (ATCC 25922) e *Pseudomonas aeruginosa* (ATCC 15442) no estudo de Lôbo et al.(2010) mostrando-se como um potente anti-bacteriano por meio de inibição de crescimento de *E.coli* devido a presença de alcaloides e taninos na composição de *Solanum paniculatum* L. (100).

Com relação à atividade antioxidante, no estudo de Ribeiro et al. (2007) o extrato aquoso bruto das folhas por meio da avaliação do DPPH mostrou-se comparável ao hidroxitolueno butilado (BHT), um antioxidante sintético. Já para atividade anti-inflamatória no estudo de Rios et al. (2017) diminuiu os níveis de

citocinas inflamatórias por meio da redução da expressão de NF-kB e de genes TBET e GATA3.

JUSTIFICATIVA

Considerando a escassez literária sobre os potenciais terapêutico, perfil fitoquímico e potencial antioxidante com enfoque nos compostos bioativos provenientes dos frutos de jurubeba, é de suma importância estabelecer e avaliar estes parâmetros iniciais na ciência básica a fim de contribuir para futuros estudos científicos e possíveis desenvolvimentos de terapias complementares na saúde humana.

HIPÓTESE

A hipótese deste trabalho consiste em que o extrato hidroetanólico de Jurubeba pode melhorar o estado redox de células de câncer de mama por meio do aumento dos marcadores de estresse oxidativo e da produção de ROS bem como diminuir os parâmetros de inflamação.

1. OBJETIVO

O objetivo desse trabalho foi avaliar a atividade bioativa do extrato dos frutos de Jurubeba (*Solanum paniculatum* L) em linhagens celulares de adenocarcinoma mamário.

Objetivos específicos

- Investigar o perfil fitoquímico da formulação hidroetanólica dos frutos e o seus efeitos na produção de ROS e citocinas em células humanas de câncer de mama e células endoteliais.

- Avaliar o efeito biológico do extrato de Jurubeba no Estado Redox em células MCF-7 de câncer de mama.

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Capítulo II

Hydroethanolic extract of *Solanum paniculatum* L fruits modulates ROS and cytokine in human cell lines

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Research Article

Hydroethanolic Extract of Solanum paniculatum L. Fruits Modulates ROS and Cytokine in Human Cell Lines

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Solanum paniculatum L. or popularly known as "jurubeba" is an herbal medicinal plant. A few studies have investigated its biological effects; however, research aimed at elucidating the redox balance effects from its fruits has not been reported so far. ROS interplays in various fields of medicine such as chemotherapy. Here, we evaluated antioxidant and inflammatory activities of the hydroethanolic extract of *Solanum Paniculatum* L. (HESPL) fruits in breast cancer cells, as well as its phytochemical profile. The antioxidant profile (carotenoids and phenolic compounds) was obtained by HPLC-DAD-UV and HPLC-APCI-MS. Cancer cell lines and human vein endothelial cells (HUVECs) were cultivated and treated with 1.87-30 μg/mL of HESPL for 24 hrs. Cytotoxicity, oxidative, and inflammation biomarkers were evaluated. The dose of 30 μg/mL of the HESPL extract presented cytotoxicity in the MCF-7 cell line. However, for MDA-MB-231, the cytotoxicity was observed in the dose of 1.87 g/mL. The 1.87 μg/mL and 3.75 μg/mL doses decreased the concentration of IL-6 in MCF-7 cells. In the MDA-MB-231 cells, the HESPL did not decrease the IL-6 concentration; however, in the doses of 15 and 30 μg/mL, an increase in this parameter was observed. The HESPL increased IL-1β concentration in HUVECs. The ROS level in MCF-7 was elevated only at the 30 μg/ml dose. Regarding MDA-MB-231, HESPL promoted increased ROS levels at all doses tested. HUVEC showed no increase in ROS under any dose. HESPL treatment may modulate cytotoxicity, ROS, and cytokine levels due to its phytochemical profile, and it has shown an antioxidant or anti-inflammatory effect.

1. Introduction

Cancer is a multidimensional and complex onset of diseases with deregulated mechanisms and biochemical signaling, leading to pathology progress in a biological system [1]. Among all cancers, breast cancer is the main cause of worldwide women's death; therefore, it is a target for research studies involving early diagnosis detection and therapies [2].

ROS, a type of unstable molecules that contain oxygen, which are rapidly transformed into other species can induce oxidation of free amino acids, residues, and proteins. On the other hand, they can be a target of multiresistant drugs, enhancing cell death [3]. These species play a role in various fields of biology and medicine of cancer on protumorigenic signaling, cell proliferation, and tumorigenesis and transcription factor activation, which in turn can promote cytokines and chemokines such as IL-6 production in inflammation pathways as well as regulating and inducing apoptosis [4]. These reactive species were counteracted or stimulated by substances known as antioxidants, acting on the endogenous cellular and exogenous environments, or by interactions of low molecular mass antioxidants such as carotenoids and phenolic compounds [5].

Natural products from medicinal and nonconventional plants and functional food intake have also been reported by data, which prevent the onset of several diseases or are used as a treatment for several diseases, providing ethnopharmacological knowledge on nutrition therapeutic formulations containing antioxidants on their phytochemical profile [6]. Several studies show their effectiveness in modulating ROS, inflammation, and chemotherapeutic resistance [7, 8].

Solanum paniculatum L. (*Roem. and Schult.*), *Solanaceae* family, popularly known as "jurubeba," "jurupeba," "jubeba," or "juna" is an unconventional fruit-vegetable native to Tropical America. Its leaves and roots are widely used in traditional Brazilian medicine as tonic eupeptic agents to treat gastric and liver dysfunctions [9]. Its fruits are consumed by decoctions in culinary preparations and with oil or vinegar on pickled jurubeba [10]. Evidence has reported the presence of many steroidal compounds such as glycoalkaloids and saponins [11] and β-sitosterol [12] used in folk medicine. Endringer and colleagues showed chemoprevention in liver cells via NF-κB inhibitory activity after *Solanum paniculatum* L. treatment [13]. On the other hand, the study of Rios et al. (2017) showed its potential treatment of inflammatory conditions, reducing cell proliferation, IL-4, NO production, and other inflammatory markers; however, no chemical investigation of the antioxidant profile of *Solanum paniculatum* L. fruits as an hydroethanolic formulation and its biological mechanisms in breast cancer has been reported so far. These evaluations provided by HESPL response could be important on ROS and cytokine pathway knowledge. The present study was undertaken to investigate the phytochemical profile of HESPL fruit formulation and its effects on ROS and cytokine production in human breast cancer and endothelial cells.

2. Materials and Methods

2.1. Plant Material. Solanum paniculatum L. (*in natura*) fruits were collected on December 31, 2017, in the south of Mato Grosso State (Campo Grande region), with the coordinates 20°47′27″S latitude and 54°56′86″S longitude, by Ana Paula Costa Rodrigues Ferraz; these were deposited to the herbarium at the Institute of Biosciences, São Paulo State University (UNESP), Botucatu, SP, Brazil, under the voucher number 33072. Fruiting ratios for jurubeba were established according to Nurit et al. [14] and Forni-Martins et al. [15] through its globulous greenish to yellow appearance releasing from a peduncle; after this, 392g of fruits was separated according to their appearance, washed in water, and stored at -80°C until the extraction process.

2.2. Preparation of the HESPL Extract. Jurubeba fruits were macerated in a cryogenic mill (6775 Freezer/Mill® Cryogenic Grinder), and lyophilized in a lyophilizer (Liotop L108®), obtaining a dry weight of 129g of the powdered extract. The powdered extract (109 g) was percolated by exhaustion according to the Brazilian Homeopathic Pharmacopoeia (2011) with a slow rate of 3mL/min/kg using 70% ethanol. After twenty-four days of percolation, solvents were evaporated in a vacuum rotating evaporator with a horizontal condenser (Marconi MA-122) under low pressure (45°) and the remaining liquid was lyophilized to obtain the HESPL crude extract (24.12 g). The yield of the extract was calculated by $x = \frac{\text{finalweight g}}{\text{dry weight g}} \times 100$.

2.3. Extraction of Carotenoids and Vitamin E via

HPLCDAD-UV and HPLC-APCI-MS. The hydroethanolic extract (100 mg) was subjected to basic hydrolysis to separate the liposoluble components using 30% KOH in ethanol and solubilized with ether/hexane (2 :1), using the saponification method adapted for plant samples from Qin et al. (1997). For HPLC-DAD-UV analysis, aliquots of 20 μL were injected into the Waters Alliance 2695e Separation Module (Waters Corporation, Milford, MA, USA) coupled with the Waters PDA 2998 detector and analytical C_{30} column (Thermo Scientific—3μm (4.6 × 150mm)). The mobile phases were composed of methanol/methyl tert-butyl ether/water: (A) 85/12/3 (*v*/*v*/*v*) and (B) 8/90/2 (*v*/*v*/*v*), with 6mmol/L of ammonium acetate. The gradient was 2min at 5% B, 3min at 10% B, 6min at 15% B, 10 min at 25% B, 12min at 40% B, 16 min at 83%B, 20 min at 95% B, 24min at 95% B, 26 min at 40% B, 30min at 5% B, and 32min at 5% B with a flow rate of 1.0 mL/min. Complementary to these data and with the same method for sample extraction (2.5 mg), HPLC-APCI-MS analysis of carotenoids was determined. Aliquots of 10 μL were injected into an Agilent 1290 Infinity High-Performance Liquid Chromatography system (Agilent Technologies, United States) adapted by Etzbach et al. for total carotenoids, performed on 40-minute run and at 0.5 mL/min flow rate. The eluent B proportion was modified for 30/60/10 (*v*/*v*/*v*) using the same HPLC-DAD-UV column previously described [16].

2.3.1. HPLC-APCI-Mass Spectrometry (MS) Instrumentation and Carotenoid Identification. Mass spectrometry was performed in an AB Sciex Triple Quad™ QTRAP® 5500 Mass Spectrometer equipped with an Atmospheric-Pressure Chemical Ionization (APCI) source on a positive mode for carotenoid analysis. Also, 5mmol/L of ammonium acetate was added to the mobile phases for improving compound ionization, as well as the column temperature (25°C). The conditions for mass spectrometry were adapted from Etzbach et al. (2018) with the following modifications: entrance potential (EP), 10 V; collision energy (CE), 30V; collision cell exit potential (CXP), 8V; time, 50 ms; curtain gas, 10 (API); medium collision gas (CAD); ion spray voltage, 5500 V; temperature, 450°C; and arbitrary units for ion source gas 1 (GS1), 30.0.

A selective reaction monitoring (SRM) experiment was performed to identify the analytes, the first mass transition following two or three mass transitions was used to confirm the compound profile, and these transitions were determined by the literature [16] (see Table 1).

Table 1: SRM transitions for total carotenoids.

Compound name	[M+H]+ (*m*/*z*)	Fragment ion	Declustering potential (V)
Lutein	569.0	476.0 175.0 551.0 533.0	80
Zeaxanthin	569.0	551.1 533.1 395.0 93.0 135.1	50
β-Cryptoxanthin	553.5	119.0	50
β-Cryptoxanthin	553.0	135.0 495.0 461.0	50
α-Carotene	537.0	123.0 481.0 444.1 177.2 445.4	50
β-Carotene	537.0	413.3 269.2	50

The major carotenoids (see Figure S1 in Supplementary Materials) constituent from HPLC-DAD-UV were focused on these evaluations, and the SRM identification transition selected was 569.00/551.00. The Analyst® 1.5.1 software (AB Sciex®) and MultiQuant™ 3.0.3 software (AB Sciex®) were used for data analysis.

See Figure S1 in Supplementary Materials for comprehensive carotenoid image analysis.

2.4. Extraction and Identification of Phenolic Compounds via HPLC-DAD-UV. The HESPL extraction (100 mg) was determined by modifications of the method described by PalafoxCarlos et al. [17]. The extract was dried in N_2, resuspended in HPLC-grade methanol, and filtered on a 0.22μm Analytical® nylon membrane. Aliquots (20μL) were injected into a UHPLC Thermo Scientific Dionex UltiMate 3000 system (Thermo Fisher Scientific Inc., MA, USA), coupled with a quaternary pump, an Ultimate 3000RS autosampler, and a diode array detector (DAD-3000RS) using a C_{18} column, and the flow rate was

0.8mL/min. The mobile phase was composed of phosphoric acid/water (A) (0.85/99.15, *v*/*v*) and acetonitrile (C) (100, *v*). The gradient was 2 min at 7% B, 3min at 9% B, 5min at 10% B, 7min at 12% B, 8.5 min at 13% B, 11 min at 15% B, 12.5 min at 20% B, 13 min at 21% B, 14 min at 23% B, 15 min at 25% B, 17min at 30% B, 19 min at 45% B, 22min at 65% B, 23 min at 75% B, and 24 min at 30% B. See Figure S2 in Supplementary Materials for comprehensive phenolic compound image analysis.

2.5. Antioxidant Capacity

2.5.1. Radical Sequestration Method (DPPH˙) and FRAP Assay. A methanolic solution was prepared for the extraction with 0.100g of jurubeba for both methods. The DPPH method was performed according to Brand-Williams et al. [18], and the FRAP assay was adapted according to the methodology proposed by Benzie and Strain [19], which is efficient in the determination of antioxidant activity by iron reduction.

2.6. Cell Culture Experimental Design. Breast cancer cell lines were MCF-7 (ATCC® HTB22™), luminal, HER2+, and estrogen- and progesterone-positive receptors and MDA-MB-231 (ATCC® HTB26™), basal and with invasive potential. MCF-7 were cultivated in RPMI Medium 1640 supplemented with 10% FBS, 1% NEAA, 1% sodium pyruvate, and 1% antibiotic and MDA-MB-231 were cultivated with 10% FBS and 1% antibiotic (anti-anti). HUVECs (ATCC® CRL-1730) were cultivated in F-12K Medium (Thermo Fisher Scientific® Gibco DMEM/F12 DULBEC) with 10% FBS and 1% antibiotic. All cell lines were maintained in a humidified incubator (5% CO_2 at 37°C). Cells were plated onto 175cm^2 tissue culture flasks, and treatments proceeded when the cells reached 80% of confluence between passages 6 and 8. A stock solution for HESPL induction (10mg/mL) was made in DMSO as a vehicle according to recommendations by Jamalzadeh et al. (2016) for an experimental application. Different doses (30, 15, 7.5, 3.75, and 1.87 μg/mL) were established by Rios et al. (2017) and were diluted in medium without FBS for further *in vitro* experimental research.

The cell viability was performed by the Trypan Blue assay. After 24 hours of treatment, cells were harvested and submitted to trypsinization, the pellet was resuspended in appropriate concentration of medium, and 10μl was collected and diluited in 10μl of Trypan Blue. The cells were counted on an improved Neubauer Haemocytometer (Weber Scientific International Ltd., UK). Viable and nonviable cells were counted under light microscopy, and the viable cells are considered above 80%.

2.7. Cytotoxic Activity. Approximately 1 × 10^6 cells were incubated on six-well plates for 12 hours on RPMI 1640 culture medium with 10% FBS and 24 hrs with 0.1% FBS and maintained at 37°C and an atmosphere of 5% CO_2. Different doses were applied for 24hrs aimed at providing a nonlethal dose for further research. Subsequently, cytotoxicity was determined using the rapid colorimetric assay based on the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl tetrazolium bromide) (0.5 mg/mL in HBSS) which can measure metabolic living cells and proliferation [20]. The cells were solubilized in DMSO and read at 570nm using a scanning multiwell spectrophotometer (SpectraMax 190, Molecular Devices).

2.8. Analysis of ROS Production. The cells were cultivated onto 75cm^2 flasks for each dose, and following the treatments, these were collected using trypsin and resuspended on Muse™ Oxidative Stress Assay Buffer (4700-1330, Merck Millipore) with a minimum of 1 × 10^6 cells for each replicate; these were performed through flow cytometry using Muse® Cell Analyzer (Merck, Darmstadt, Germany) with Muse™ Oxidative Stress Kit (MCH100111, Merck Millipore). The results were shown positively ROS (ROS (+)) which has the cell exhibiting ROS significance.

2.9. Inflammation Measurement. IL-6 and IL-1β evaluations were performed in a commercial immunoassay ELISA kit using 100 μL of cell supernatant according to the manufacturers' instructions (Linco Research Inc., R&D Systems®, Millipore, and B-Bridge International Inc.) and were determined by absorbance using a microplate reader (SpectraMax 190, Molecular Devices).

3. Statistics

Results are expressed as mean ± SD. One-way ANOVA followed by the Tukey test was performed by normal distribution in SigmaPlot using Windows 10. Statistical significance was considered when $p < 0{:}05$.

4. Results

4.1. HESPL Extract and Bioactive Compounds. The results for a phytochemical profile of HESPL in HPLC-DAD-UV presented with four carotenoids (lutein, zeaxanthin, β-cryptoxanthin, β-carotene), one vitamin E (γ-tocopherol), two phenolic compounds (chlorogenic and caffeic acids), and one flavonoid (quercetin) (see Table 2).

The major active compound in HESPL is lutein, identified for the retention time of 17.52min based on similar [M+H]+ (m/z) (569.0) and fragments (175/135/551) on a sample (Figure 1(a)) compared with the lutein standard (Figure 1(b)).

HESPL has a yield of 22.19%. The FRAP procedure measures the antioxidant capacity through the interaction between the reductants (antioxidants) and Fe^{II}-TPTZ creating a blue color showing 423:32 ± 1:70μmol of quercetin 100g^{-1} DW (CV = 0:40). Additionally, the DPPH method has an affinity of reducing a free radical by a hydrophilic affinity, and HESPL shows 89:13 ± 1:20 (mg of quercetin 100g^{-1}) (CV = 1:34) of % DPPH reduction.

4.2. Cell Viability of the Cell Lines Submitted to Different Doses. The dose of 30 μg/mL (82:29 ± 19:11, CV = 23:22) shows a significant toxicity (p < 0:05) when compared with control in MCF-7 cells (see Figure 2). However, for MDAMB-231, the cytotoxicity was observed in the dose of 1.87 μg/mL (97:22 ± 2:04, CV = 2:10). Regarding the HUVEC, the extract did not show cytotoxicity.

4.3. Concentration of Interleukin in the Cell Lines in Different Doses of HESPL. In this study, the 1.87 μg/mL (0:04 ± 0:02, p < 0:005) and 3.75 μg/mL (0:03 ± 0:00, p < 0:005) concentrations decreased with significance in MCF-7 cells (Figure 3(a)). In the MDA-MB-231 cells, the HESPL did not decrease the IL-6 concentration; however, in the doses of 15 and 30 μg/mL, an increase in this parameter was observed (130:88 ± 1:52 /125:46 ± 4:88, p < 0:005) (Figure 3(b)). The HESPL increased IL-1β concentration in HUVECs (dose of
3.75 μg/mL (4:513 ± 0:280, p < 0:05) vs. control (2:965 ±
1:108); Figure 3(c)).

4.4. The Levels of ROS Were Increased by the Dose of 7.5µg/mL of HESPL vs. Control. The higher dose of 7.5 μg/mL of HESPL improves ROS levels (see Figure 4(a)) in MDA-MB-231 (15:47 ± 4:88, p < :0001) vs. control (2:87 ± 0:57) and also improved increased levels in all tested doses. HUVECs did not present higher levels of ROS. See Figure 5 for a comprehensive example of cell flux analysis.

5. Discussion

Dietary phytochemicals can act as an antioxidant or prooxidant and may participate in the development of new anticancer drugs [21]. Elevated levels of ROS and deregulated redox signaling are common hallmarks of cancer progression and resistance to treatment [22]. ROS production is involved in two faces of the cancer environment: in basal levels, these species are involved in PI3K/Akt-mediated cell survival and proliferation, or when excessive intracellular ROS accumulation occurs, these are involved in the cleavage of caspase-3 and caspase-7 also damaging nucleic acid bases and other compounds [4].

It is known that MDA-MB-231 are malignant cells, and in this study, they could be a great model to investigate the effects of natural products on ROS modulation since this kind of cell is classified with invasive potential [23]. Our dose-response study demonstrated that the dose ranging from 1.87 to 7.5μg/mL of HESPL enhanced ROS production (Figure 4). These results suggest that the phytochemical profile of plant- and food-based diets may act on the redox balance. Phytochemicals present in plants can act as a prooxidant via the Fenton reaction whose mechanism occurs via iron-dependent ROS production [24]. It can also inhibit NADPH expression and the blockade of the Nrf-2 signaling pathway [25]. This transcription factor was commonly elevated in various types of cancer and consequently as a result of the activation of oncogenes such as K-Ras, B-Raf, and cMyc which in turn are involved in survival and cell proliferation [21].

The mechanism of action of plant-based diets may possess intervention capacity acting at specific pathways such as reduction of NF-κB DNA-binding activity, TNF-α inhibition, increased caspase-3 and caspase-7, bax/bcl-2 ratio, and fraction with sub-G0/G1 DNA content in apoptosis [26]. Additionally, Sinha et al. demonstrated that phytoconstituents of tea (*Camellia sinensis*) modulate epidermal growth factor receptor, B-cell lymphoma 2 (Bcl-2), and Bcl-2associated X protein in the breast carcinoma [27].

The phytochemical profile of the jurubeba fruit extract consists of phenolic compounds, vitamin E, and carotenoids. Polyphenols can act via noncovalent interaction with cellular proteins promoting the inhibition of prooxidant enzymes and diminishing DNA damage and lipid peroxidation as well as inhibition of ROS-dependent signal transduction [28]. In our phytochemical profile, caffeic acid is the major phenolic compound, but we also identified chlorogenic acid and

Table 2: Phytochemical profile of HESPL determined by HPLC-DAD-UV.

Analyte	RT	Linearity range (ng/μL)	LOD/LOQ (ng/μL)	r_2	*Solanum paniculatum* L. (pg/mL) in 100 mg of extract	SD (%)	CV (%)
			Carotenoids				
Lutein	3.449	5-100	1.45/5	0.9988	103.70	2.3049	2.222719
Zeaxanthin	4.133	2.28-36.5	0.80/2.28	0.9972	8.9	0.48608	5.461573
β-Cryptoxanthin	9.017	5.30-84.9	0.97/5.30	0.9963	8.8	0.5913	6.719318
β-Carotene	15.666	15.6-250	0.89/15.6	0.9821	8.7	1.1533	0.131655
			Vitamin E				
γ-Tocopherol	5.333	0.075-2.42	1.09/0.075	0.9999	1.6	0.00206	0.12875
			Phenols				
Chlorogenic acid	3.517	10-500	1.40/10	0.9956	17.5	0.7415	4.237143
			Flavonoids				
Caffeic acid	6.617	10-500	1.30/10	0.9991	23.9	0.9641	4.033891
Quercetin	19.12	10-500	1.33/10	0.9999	2.9	0.1306	4.503448

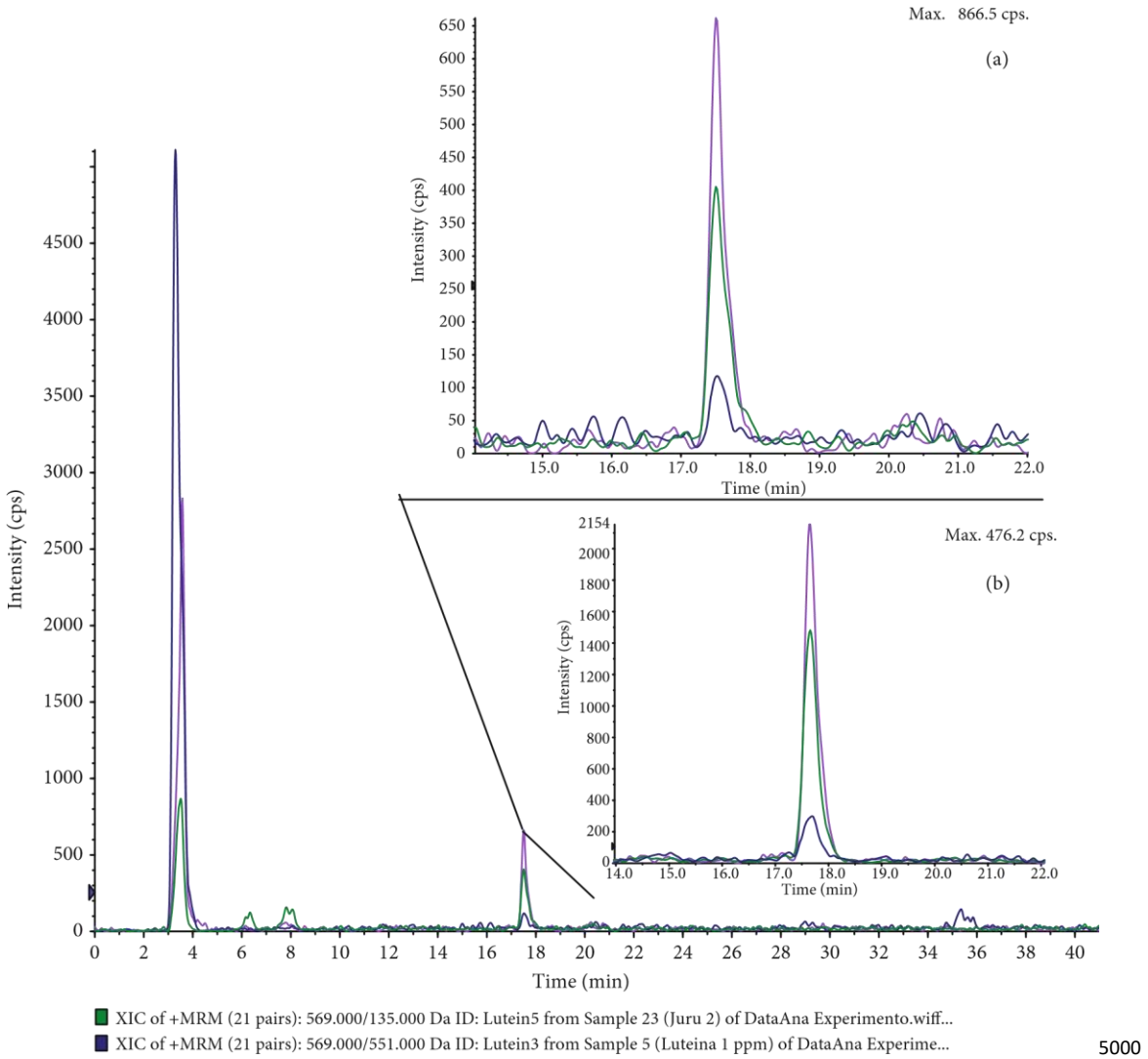


Figure 1: Carotenoid profile of *Solanum paniculatum* L. by HPLC-APCI-MS: (a) lutein compound in the sample; (b) lutein standard.

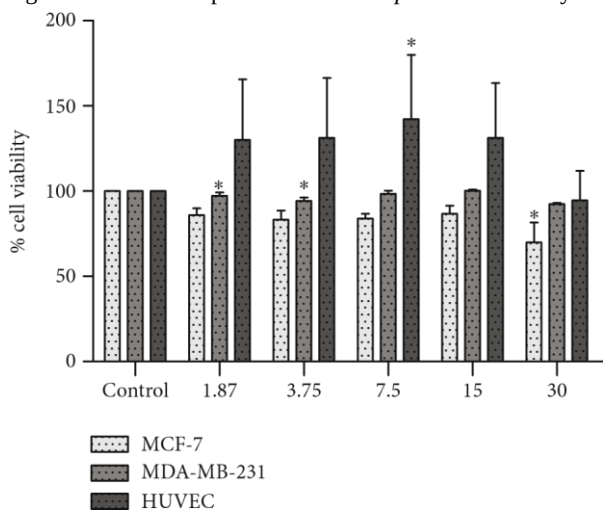


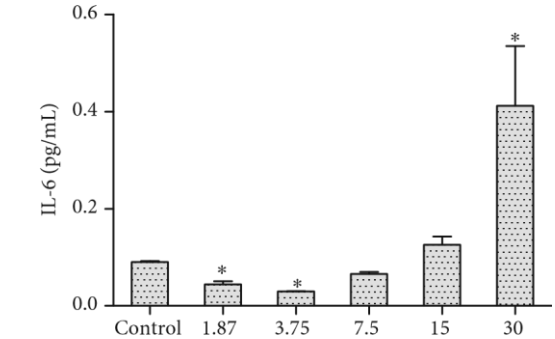
Figure 2: % cell viability from MCF-7, MDA-MB-231, and HUVECs. *Versus control when p<0:05. The viability was determined by the MTT assay. The dose of 30 μg/mL presented cytotoxicity.

quercetin. Yu et al. demonstrated that caffeic acid can lead to apoptosis in YD-15 (human mucoepidermoid carcinoma), HSC-4, and HN22 (human oral squamous cell carcinoma). Moreover, the authors have shown the apoptotic effect by cleavages of caspase-3 and poly (ADP-ribose) polymerase and activation of Bax protein [29]. In parallel, AbouHashem et al. showed the apoptotic effect of chlorogenic acid via cell cycle arrest at the sub-G0 phase and DNA fragmentation [30], whereas quercetin can induce apoptosis through proteasome inhibition such as the 20S and 26S proteasome in Jurkat T cells and accumulation of polyubiquitinated proteins [28].

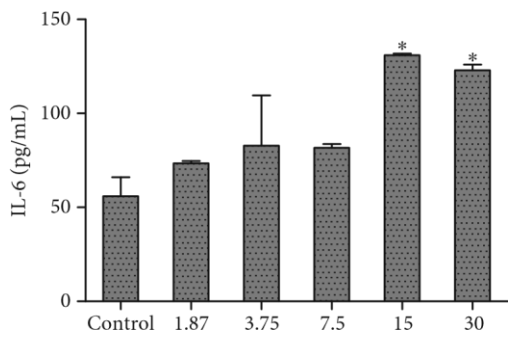
Evidence suggests that dietary carotenoids may

help in reducing the risk of breast cancer [31]. Lutein is the major carotenoid identified in our extract. Lutein acts by suppressing inflammation, and it was involved in the inhibition of NF-κB signaling [32]. In summary, benefits of lutein intake consist in eye health and antioxidant and anti-inflammatory activities. It is suggested that lutein mechanisms of action in cancer might be involved in cell growth inhibition by inducing cell cycle arrest and caspase-independent cell death also, activating p53 signaling [33]. Juin et al. showed the apoptotic effect of zeaxanthin through the expression of the BRAF V600E oncogene [34]. Additionally, Gao et al. demonstrated the antiproliferation effect of β-cryptoxanthin by G0/G1 cell cycle arrest and AMPK signal inactivation [35]. The effects of β-carotene on metastasis was evidenced by Kim et al. where it downregulated the expression of CSC markers, MMPs, and HIF-1α in cancer tissues [36]. Inhibition of the HMG-CoA reductase enzyme and inhibition of the NF-κB pathway are the main antiangiogenic mechanisms found for tocotrienols such as γ-tocopherol [37].

Cytokine levels are naturally enhanced in conditions such obesity and cancer [38]. A few reports related the reduction of IL-6 to a targeted therapy for cancer [39, 40]. In our study, the HESPL treatment diminished the levels of IL-6 in MCF-7 cells (Figures 3(a) and 3(b)), enhancing the importance of this cytokine acting as having multifaceted cellular displayed and physiological functions on biological systems [41]. Primary tumors such as MCF-7 cells may be an important tool on research focusing on chemopreventive actions [42]. The chemopreventive actions can attenuate cell cycle arrest [43] and other various tumor-promoting pathways in cancer [44]. IL-6 seems to be an important cytokine in breast cancer studies [39, 44, 45] acting in several pathways involved in



(a)



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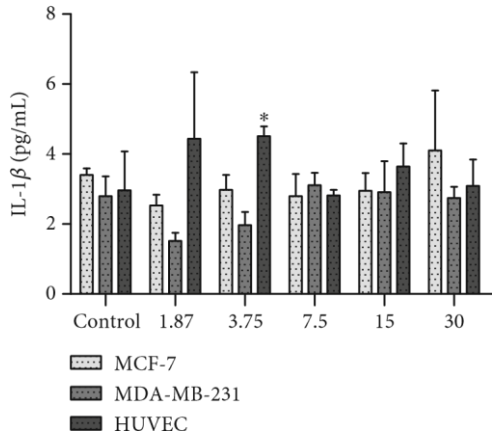

(c)

Figure 3: Different doses of HESPL effect in interleukin-6: (a) MCF-7 cells, (b) MDA-MB-231 cells, and (c) different doses of HESPL effect in IL-1β in the cell lines. *Versus control when $p<$ 0:05. The dose of 3.75 μg/mL diminished significantly compared with other doses. On the other hand, the doses of 15 and 30 μg/mL were increased in MDA-MB-231, and for IL-1β, the dose 3.75 μg/mL was enhanced.

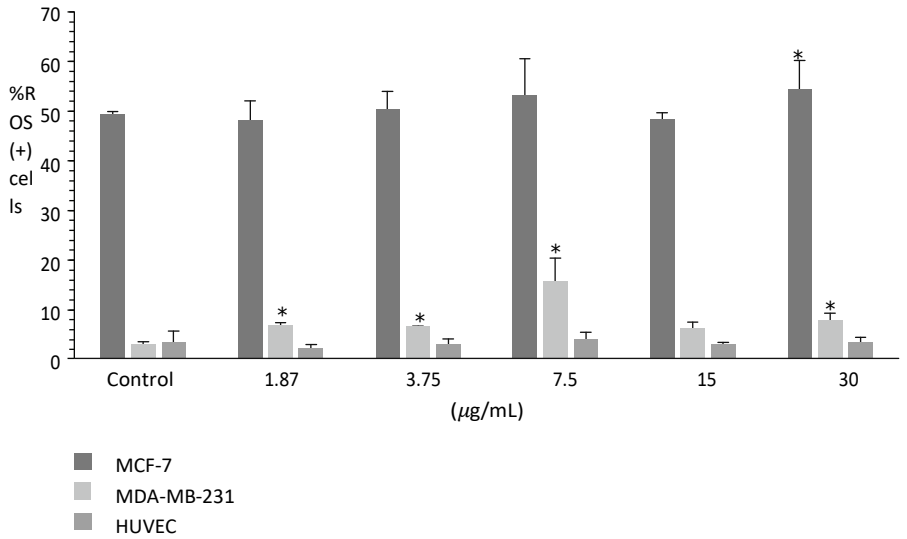

Figure 4: ROS parameter from MCF-7, MDA-MB-231, and HUVECs. *Versus control when p<0:05. The ROS was enhanced in all doses tested in invasive cells, and the dose 30 μg/mL was increased in MCF-7 cells.

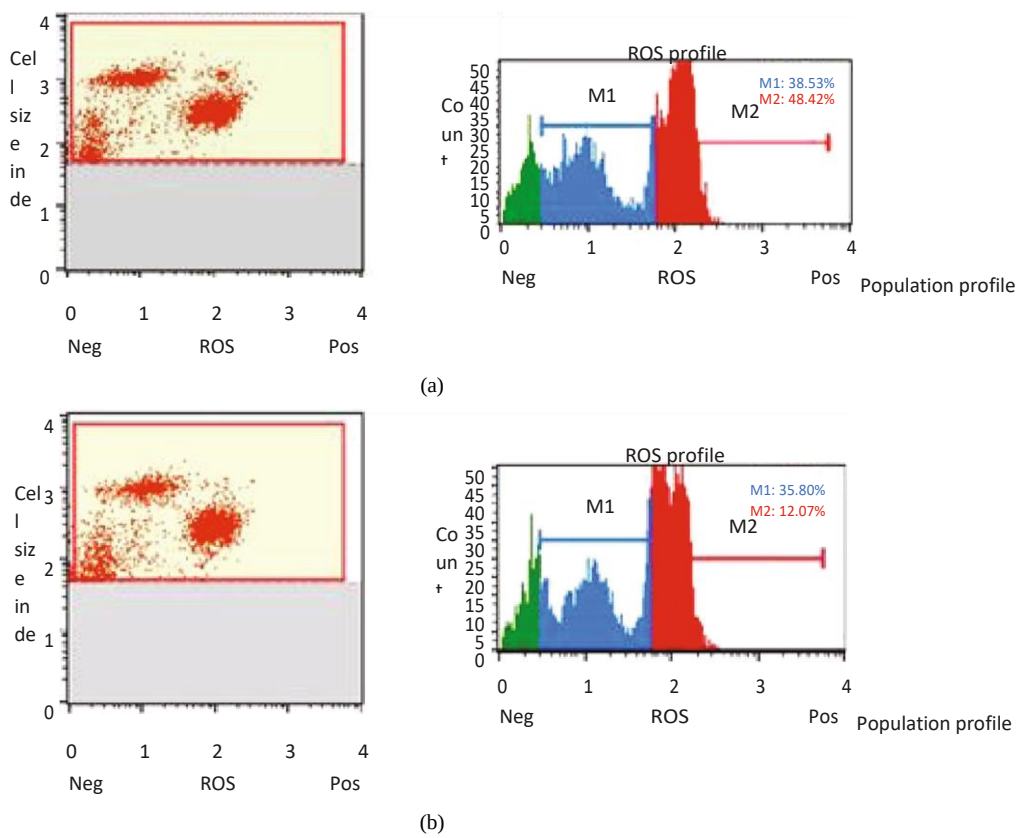

Figure 5: Cell flux analysis in MCF-7 cells: (a) 30 μg/mL treatment; (b) control.

cancer progression [46]. Moreover, IL-1β, another important cytokine in the chronic inflammation process, was enhanced in HUVECs after treatment with 3.75 μg/mL HESPL (Figure 3(c)).

The synergic effects of different compounds presented in the HESPL might provide an adjuvant strategy on cancer therapies since multiple phytochemicals may act on various biological microenvironments. We have shown in this study that phytochemicals presented in the crude extract of jurubeba fruits can act on the oxidative and inflammation balance. A positive control study may be

a scientific contribution for our current findings since the isolated phytochemicals contribute to antitumoral mechanisms such as apoptosis. However, its known that individual compounds have not shown benefits in some clinical trials since bioactivity might be affected or do not react in the same effective way when compared to crude extracts of plant-based foods [26]. Our data suggests a redox/inflammation modulation network of the *Solanum paniculatum* L. fruits, and its potential as a nutraceutical was shown to be enhanced as it successfully modulates ROS and cytokine production as well as inhibiting the growth of cancerous cells.

6. Conclusions

The molecular mechanisms of ROS and cytokine play an important role in various cancer models evidenced in both *in vitro* and *in vivo* studies. ROS and cytokine modulation seems to be a promising chemotherapeutic target for treatments. Here, we demonstrated that HEPSL can act by diminishing ROS production and modulate levels of IL-6 and IL-1β. Most importantly, our dose-response studies demonstrated that different concentrations of phytochemicals, acting synergically, might affect the outcomes either positively or negatively, raising the importance of ingestion-response ratio effects of diets. Our findings, even as a basic research model, provide information that can guide future studies aimed at elucidating new therapeutic alternatives for cancer. **Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

All authors participated in the study design, interpretation, analysis, and review of the final version of the manuscript. Ferraz APCR, dos Santos KC, and Correa CR elaborated the experimental design. Ferraz APCR, dos Santos KC, Garcia JL, Namba V, and Pereira BS conducted the experiment. Corrente JE performed the statistical analysis, and Ferraz APCR, dos Santos KC, Garcia JL, Costa MR, FrancisquetiFerron FV, Ferron AJT, Silva CCVA, Manfio VM, Minatel IO, and Correa CR analyzed the data and revised and wrote the manuscript. Klinsmann C. dos Santos is the joint last author.

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Supplementary Materials

Figure S1: molecular structures of carotenoids. Figure S2: molecular structures of phenolic compounds. *(Supplementary Materials)*

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Capítulo III

Hydroethanolic Extract of *Solanum paniculatum* L Increases Redox State in Breast Cancer Cell Line MCF-7

Artigo no formato para a revista **Asian Pacific Journal of Cancer Prevention**.

Hydroethanolic Extract of *Solanum paniculatum* L Increases Redox State in Breast Cancer Cell Line MCF-7

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Abstract

Objective: The aim of this study was to evaluate the biological effect of the Hydroethanolic Extract of *Solanum paniculatum L* (HESPL) extract on the redox state in breast cancer cell line MCF-7 cells.

Methods: HESPL was characterized using HPLC-APCI-MS. MCF-7 cells were treated of HESPL and were mainted for 24 hrs. Citotoxity was evaluated using MTT assay and redox state biomarkers (4-hydroxynonenal (4- HNE), Malondialdehyde (MDA) and protein carbonylation (PCO)) were analyzed by ELISA kit. Also, SOD and CAT were evaluated.

Result: The results showed that lutein is the main bioactive compound on HESPL. The HESPL treatment in the dose of the 3.75 (µg/mL) with lower toxicity led to oxidative stress represented by increased 4-HNE, MDA and PCO levels. Also the breast cancer cells showed increased of the antioxidant enzymes activity manganese superoxide dismutase (MnSOD) and catalase (CAT).

Conclusion: The HESPL treatment shows to modulate the redox state profile of MCF-7 cells increasing oxidative stress parameters.

Keywords: Jurubeba, *Solanum paniculatum L*, breast cancer, MCF-7, antioxidant, oxidant.

Introduction

The breast cancer is the most common cancer in women worldwide (1) representing about 25% of all cancer cases and being responsible for 15% of all cancer deaths among females (2). In 2018 it was estimated 2.088. 849 new cases of breast cancer (3). Important diagnostic and therapeutic advances have been achieved over the years, but breast cancer remains a major health problem being extensively studied (4).

The carcinogenesis process is complex and involves the interaction between several biological mechanisms characteristic of the neoplastic cell. Among these mechanisms are the support of cell proliferation signaling, immortality and angiogenesis induction (5–7), related to an inflammatory process and oxidative stress which contribute to pre-tumorigenic signaling and genomic instability (8–10). In particular, oxidative stress plays a key role in the development of cancer.

Oxidative stress is defined by an imbalance between the reactive species and the antioxidants (11). Reactive oxygen species (ROS) are physiologically produced by mitochondria during cellular respiration and by inflammatory activated cells. The antioxidants are capable of neutralizing these species and may be endogenous such as superoxide dismutase (SOD), catalase (CAT) and glutathione, or exogens as vitamins, polyphenols and carotenoids, present in natural products commonly consumed (12).

A healthy cell has a balance between ROS production and its antioxidant defense system ensuring cellular homeostasis. Tumours cells have a high basal redox state. The metabolic activity of these cells leads to high ROS concentrations leading to enhance cell survival and proliferation, genomic instability and decreased cellular repair. These cells also express increased antioxidant activity contributing to the redox balance. However, if ROS concentration increases substantially, the ROS-inducing chemotherapy will result in oxidative stress, irreparable cellular damage and cellular death (13). In this sense, therapeutic potential of some plants has been studied by being able to modulate the redox state in cancer cells Aditionally, some biomarkers such MDA, HNE and PCO are commonly used in clinical pratice (14).

Jurubeba (*Solanum paniculatum* L., Solanaceae family), is a shrub, perennial and an unconventional fruit vegetable originating in tropical America

(15). The fruits of Jurubeba, are consumed popularly in the processed form, pickled or in preparations as the rice with jurubeba and saffron (Curcuma longa) (16). Several studies shown the aqueous extracts of flowers and, roots and, leaves of jurubeba already have medicinal properties well described in the inhibitory activity of gastric acid secretion, acting in the treatment of dyspepsia (17,18), antidiarrheal (19), antibacterial action (20) , antiviral (21) and antioxidant (22). In this context, the present study aimed to evaluate the biological effect of the Jurubeba extract on the redox state in breast cancer cell line MCF-7 cells.

Considering that no evidence for fruits of Jurubeba and its effects of redox balance. This study is useful for determining the redox state profile of the MCF-7 breast cancer cell line following treatment with *jurubeba* extract

Material and Methods Cell Culture

Human breast cancer cell line MCF-7 was purchased from American Type Culture Collection (ATCC®, HTB22, Manassas, Virginia, USA). Cells were cultivated with RPMI medium 1640 supplemented with 10% FBS, 1% NEAA (non- essential amino acids, LGC Biotecnologia, Brazil) solution, 1% Sodium piruvate and 1% antibiotic-antimycotic (Gibco - Thermo Fisher Scientific, cat. number15240-062) in humidified incubator (5% CO2 at 37°C). To avoid cell passage-dependent effect and ensure reliable results, the cells culture and treatments were performed up to ten passages. Cells were plated onto 75cm² tissue culture flasks ($4x10^6$ cells) and treatments were performed when the cells reached 80% confluence between passages 6 and 8 (24h of treatment). A Stock Solution for hydroethanolic extract solanum paniculatum L (HESPL) induction (10mg/mL) was prepared in DMSO as a vehicle according with recommendations of Jamalzadeh et al. (2016) for establishes
3.75 µg/mL as a dose for further in vitro experimental research. After the experimental research, the supernatant was removed and collected for further analysis, MCF-7 cells were washed twice with PBS, collected with trypsin and submitted to lysis using RIPA buffer (Sigma-Aldrich ®) providing a cell lysate for analysis.

Hydroethanolic Extract of Solanum paniculatum L (HESPL)

Plant material

The fruits *in natura* of *Solanum paniculatum* L. were collected in December 31th, 2017 in the South of Mato Grosso State (Campo Grande region), with the coordinates: Latitude: 20º 47 '27 "S and Longitude: 54º 56 '86 "S by Ana Paula Costa Rodrigues Ferraz, these were deposited to the Herbarium at São Paulo State University (UNESP), Institute of Biosciences, Botucatu, SP, Brazil, under the voucher number 33072. Fruiting ratios for Jurubeba were established according to Nurit et al. (2007) (23) and Forni-Martins et al. (1998) (24) through its globulous greenish to yellow appearance releasing from a peduncle, after this, 392 g of fruits were separated according to their appearance and stored at -80ºC until the extraction process.

Preparation of HESPL

For the experiment, Jurubeba fruits were macerated in a cryogenic mill (6775 Freezer / Mill® Cryogenic Grinder), and lyophilized in Lyophilizer (Liotop L108®), obtaining a dry weight of 129g of powdered extract.

The powdered extract (109 g) was percolated by exhaustion according to the Brazilian Homeopathic Pharmacopoeia (2011) with a slow rate of 3 mL/ min/kg using 70% ethanol. After twenty-four days from percolation, solvents were evaporated in a Vacuum Rotating Evaporator with a horizontal condenser (Marconi MA-122) under low pressure (45º) and, the remaining liquid was lyophilized to obtain the HESPL crude extract (24.12 g). The yield of the extract was calculated by Equation (1).

$$x = \left(\frac{\text{Final Weight(g)}}{\text{Dry Weight (g)}}\right) \times 100 \quad (1)$$

Measurements of Lipophilic Bioactive Compounds (Carotenoids)

The hydroethanolic extract (100 mg) was subjected to basic hydrolysis toseparate the liposoluble components using 30% KOH in ethanol and solubilized with ether/hexane (2:1), using the saponification method adapted for plant samples from Qin et al. (1997). For HPLC-DAD-UV analysis, aliquots of 20 µL were injected into the Waters Alliance 2695e Separation Module (Waters Corporation, Milford, MA, USA)

coupled with Waters PDA 2998 detector and Analytical C_{30} column (Thermo Scientific – 3 µm (4.6 x 150mm). The mobile phase were composed by Methanol/Methyl Tert-Butyl Ether/Water (A) 85/12/3 (v/v/v) and (B) 8/90/2 (v/v/v) with 6 mmol/L of ammonium acetate. The gradient was 2 min at 5 % B; 3 min at 10 % B; 6 min at 15 % B; 10 min at 25 % B; 12 min at 40 % B; 16 min

at 83 %B; 20 min at 95 % B, 24 min at 95 % B; 26 min at 40 % B; 30 min at 5 % B; and 32 min at 5 % B with flow rate of 1.0 mL/min.

Complementary to these data and with the same method for sample extraction (2.5mg), HPLC-APCI-MS analysis of carotenoids was determined. Aliquots of 10 µL were injected in an Agilent 1290 infinity High-Performance Liquid Chromatography (Agilent Technologies, United States) adapted by Etzbach et al. (2018) for total carotenoids, performed on a 40 minutes run and 0.5 mL/min flow rate. The eluent B proportion was modified for 30/60/10 (v/v/v) using the same HPLC-DAD-UV column previously described (25).

Measurements of Hidrophilic Bioactive Compounds (Phenolic Compounds)

The HESPL extraction (100 mg) was determined by modifications of the method described by Palafox-Carlos et al. (2012) (26). The extract was dried in N_2, ressuspended in HPLC grade methanol and, filtered on 0.22 *µm* Analytical® nylon membrane. Aliquots (20 µL) were injected into a UHPLC Thermo Scientific Dionex UltiMate 3000 system (Thermo Fisher Scientific Inc., MA, USA), coupled to a quaternary pump an Ultimate 3000RS auto sampler and a diode array detector (DAD-3000RS) using a C_{18} column, the flow rate was of 0.8 mL / min. The mobile phase were composed by Phosphoric acid/Water (A) 0.85/99,15 (v/v) and Acetonitrile (C) 100 (v). The gradient was 2 min at 7 % B; 3 min at 9 % B; 5 min at 10 % B; 7 min at 12 % B; 8.5 min at 13 % B; 11 min at 15 %B; 12,5 min at 20 % B,

13 min at 21 % B; 14 min at 23 % B; 15 min at 25 % B; 17 min at 30 % B; 19 min at 45 % B; 22 min at 65 % B; 23 min at 75 % B and 24 min at 30 % B.

Cytotoxic activity

Cell viability and protective assay in MCF-7

Cell viability was assessed with the MTT staining assay as previously

described (27). In brief, approximately $1x10^6$ cells were incubated on six-well plates for 12 hours incubation on RPMI 1640 culture medium with 10% FBS and 24hrs with 0.1% FBS maintained at 37ºC and atmosphere of 5% CO_2. Different doses of HESPL were applied for 24 hours aimed to provide a non-lethal dose for further research. Doses ranging from 1.87 ug/mL to 15 ug/mL were tested based on Rios and colleagues (2017). (28). Subsequently, cytotoxicity was performed using the rapid colorimetric assay based on the tetrazolium salt MTT (3-(4,50 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma-Aldrich, Saint Louis, MO, USA) (0.5mg/mL in HBSS) which can measure metabolic living cells and proliferation (27). The cells were solubilized in DMSO and read at 570 *nm* using a scanning multiwell spectrophotometer (SpectraMax 190; Molecular Devices).

Evaluation of the Redox State

Measurement of Reactive Carbonyl Species and Protein Carbonyl (PCO)

To evaluate 4-Hydroxynonenal (4-HNE), 50µL were improved from the cell lysate and performed in an adduct competitive ELISA kit enzymatic immunoassay (Cell Biolabs, INC.). The amounts of HNE adducts were determined by absorbance using a microplate reader (Spectra Max 190; Molecular Devices). The results were corrected by the total protein of the samples measured before utilizing a Total Proteins Monoreagent (Bioclin®).

Malonaldehyde (MDA) and PCO were also evaluated. For that 200 µL of cell supernatant were added on Sodium Phosphate Buffer (Na2HPO4 143mmol/L, pH 7,5) with EDTA (1 mmol/L) (1:10 proportion of sample to buffer), these were centrifuged at 10.000xg for 15 minutes at 4ºC creating a homogenate. After these procedures, the homogenate (250µL) was added to 750µL of 10 % trichloroacetic acid, which was centrifuged again at 3000rpm for 5 minutes, and supernatant was transferred to new glass tube with 500µL of TBA 0.67%. This mixture was boiled at 100ºC for 15

minutes and read at 535nm using a microplate reader (Spectra Max 190; Molecular Devices). MDA was calculated based on quantities of protein sample. The same homogenate was used for quantifications of carbonyls in oxidized proteins (PCO) according to Mesquita et al. (2014) (29).

Measurement of Antioxidant Enzymes Activity

The activity of manganese superoxide dismutase (MnSOD) and catalase (CAT) was evaluated and the results were corrected by the total proteins. MnSOD activity was determined based on the inhibition of a superoxide radical reaction with pyrogallol and the absorbance values were measured at 420 nm (30), and the results are expressed as units per milligram of protein. CAT activity was evaluated by following the decrease in the levels of hydrogen peroxide measured at 240 nm (31). The CAT activity is expressed as picomol per minute per milligram of protein;

Measurement of hydrophilic antioxidant capacity (HAC)

The cells hydrophilic antioxidant capacity (HAC) was determined by the hydrophilic antioxidant performance test as described by Beretta et al. (2006) (32) using the VICTOR X2 reader (Perkin Elmer-Boston, MA, USA). The antioxidant capacity was quantified by the comparison between the areas on the curve relative to the oxidation kinetics of the phosphatidylcholine (PC) suspension, which was used as a reference for biological matrix. 2,2′-Azobis (2-aminopropano)-dihydrochloride (AAPH) was used as the peroxyl radical initiator. The results represent the percentage of inhibition of 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a- diaza-s-indacene-3-undecanoic acid) (BODIPY, 581/591) in plasma relative to that occurring in the control sample of BODIPY 581/591 in PC liposome. All analyses were performed in triplicate, and the results represent the percent protection.

Statistics

In this study, data are presented as mean ± standart deviation (SD). Differences between the groups (quadruplicate) were determined by normal distribuition on Student's t test. The software used were SigmaPlot version 3.5. (Systat Software, Inc.). A p value < 0.05 was considered statistically significant.

Results

Lutein is the main bioactive compound on HESPL

Lutein was the main bioactive compound present in HESPL. However, other compounds were found among them lutein, zeaxanthin, β-cryptoxanthin, β-carotene, γ-tocopherol, chlorogenic and caffeic acids, and quercentin (Table 1).

Table 1. Lipophilic and Hidrophilic Bioactive Compounds Concentration in HESPL

	Analyte	***Solanum paniculatum* L. [pg/mL] in 100 mg of extract**
Lipophilics	Lutein	103,70
	Zeaxanthin	8,9
	β-Cripytoxanthin	8,8
	β-Carotene	8,76
	γ-Tocopherol	1,6
Hidrophilics	Chlorogenic Acid	17,5
	Caffeic Acid	23,9
	Quercetin	2,9

The dose of 3.75 (µg/mL) lead to better relative cell viability

The figure 1 shows the relative cell viability. It is possible to verify that there was no toxicity after cells treatment with 1.87, 3.75 and 15 µg/mL concentrations of HESPL and the dose of 3.75 µg/mL showed the higher cell viability. Thus, this dose (3.75 µg/mL) were used for subsequent tests in the present study.

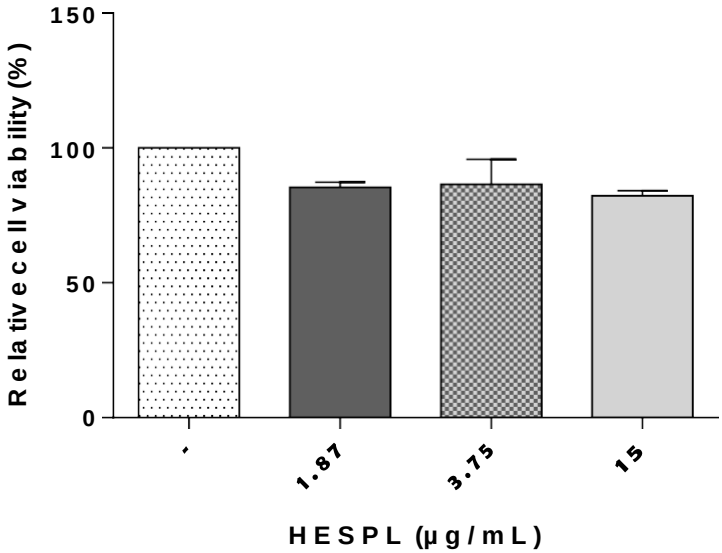

Figure 1. Cytotoxic effect of hydroethanolic extract of *Solanum paniculatum* L (HESPL). MCF-7 viability was assessed by the MTT-tetrazolium method. Cells were exposed to 1.87, 3.75, 15 µg/mL of *HESPL*.

HESPL treatment led to oxidative stress despite increasing antioxidant enzyme activity

The treatment with HESPL increased 4-HNE, MDA and PCO levels (Figure 2), also arised the antioxidant enzymes MnSOD and CAT (Figure 3). The HCA was increased in the treated cells (Figure 4).

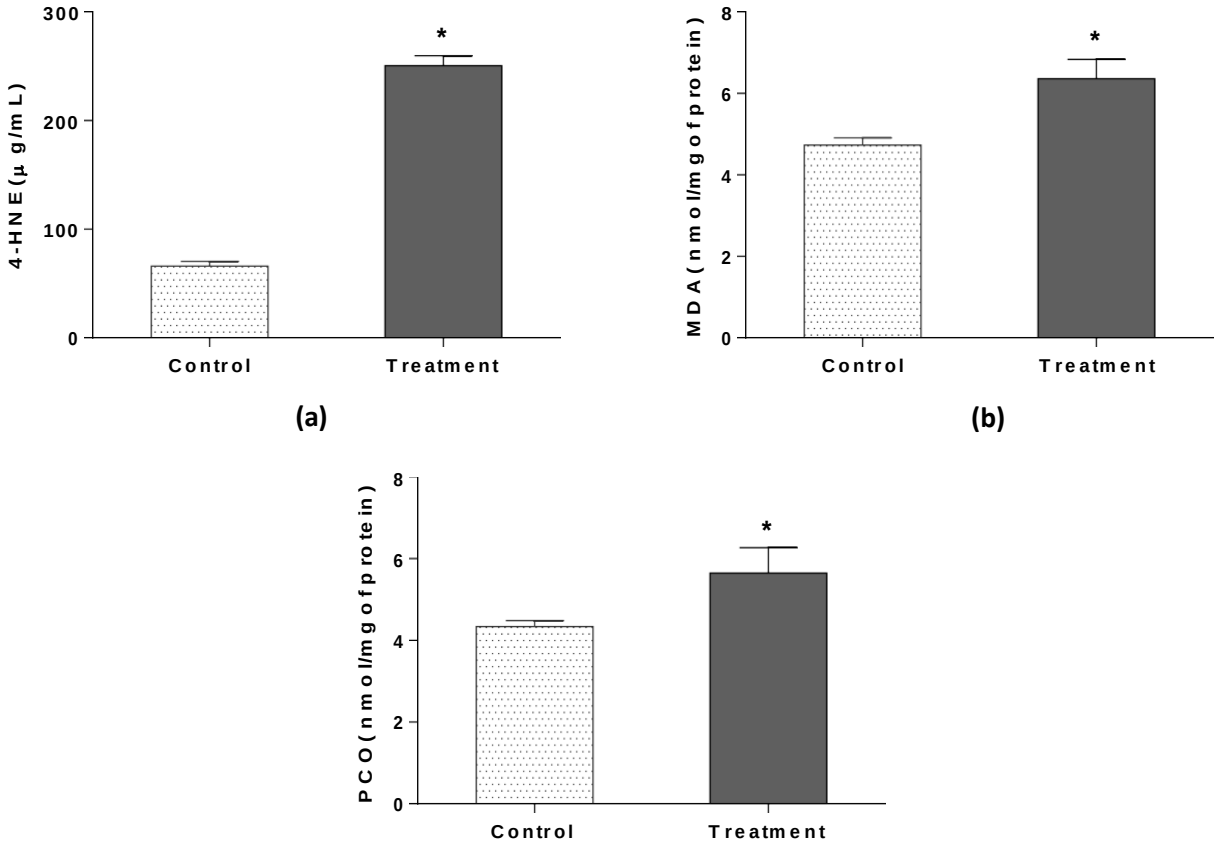

Figure 2. Reactive carbonyl species and PCO formation. **(a)** 4-HNE (µg/mL); **(b)** MDA (nmol/mg of protein); **(c)** PCO (nmol/mg of protein). Data are expressed in mean±standart deviation. Comparison by Student's t test. *Significant statistical difference ($p<0.05$). 4-HNE: 4-Hydroxynonenal; MDA: Malondialdehyde; PCO; carbonyl protein. MCF-7 cells for 24 h treatment.

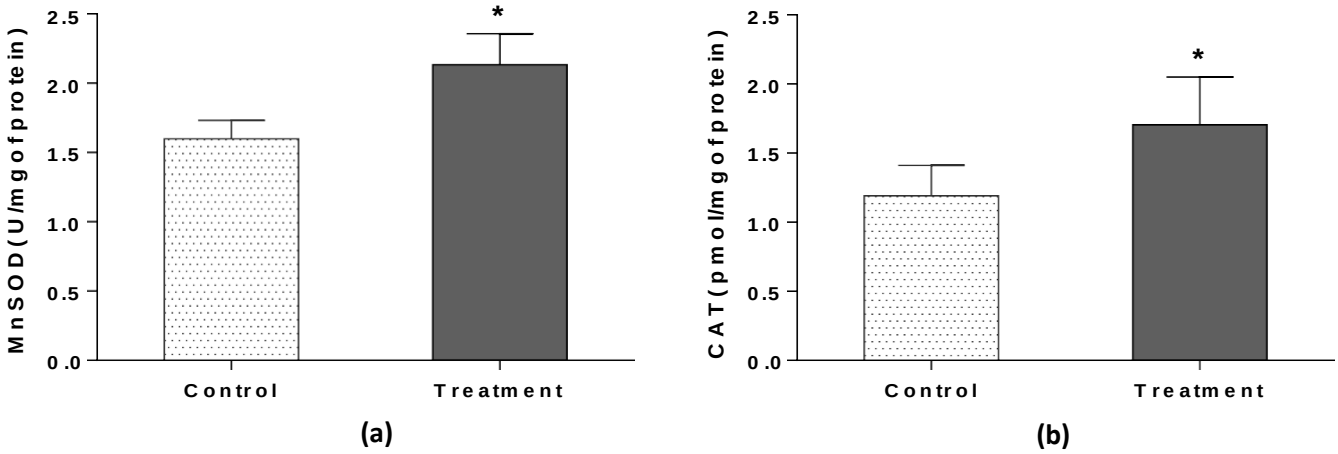

Figure 3. Antioxidants enzymes activity. **(a)** SOD (U/mg of protein); **(b)** CAT (pmol/mg of protein). Data are expressed in mean±standart deviation. Comparison by Student's t test.

*Significant statistical difference ($p<0.05$). SOD: Manganese Superoxide Dismutase; CAT:

Catalase.

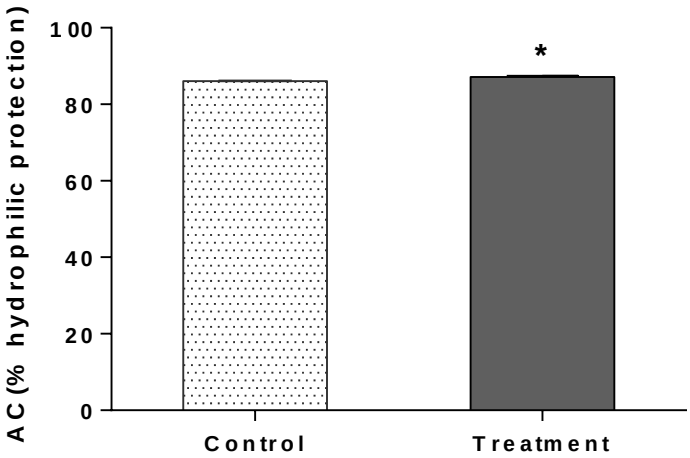

Figure 4. Cells Hydrophylic Antioxidant Capacity (HAC). Data are expressed in mean±standart deviation. Comparison by Student's t test. *Significant statistical difference ($p<0.05$).

Discussion

Fruits and vegetables are important components of the diet with components such carotenoids and phenolic compounds, which is related to preventing numerous diseases, including cancer. In this field, the bioactive compounds present in these foods arouse interest for their health protection effects associated with cancer treatment(33). The present study aimed to evaluate the biological effect of the HESPL on the redox state in breast cancer cell line MCF-7. In this research, we identified a wild range of bioactive compounds in the Jurubeba extract, including phenolic compounds and carotenoids, mainly the lutein which has been demonstrated to present an anti-inflammatory, anti-oxidant and antitumor effects (34). The synergism between these nutrients led to changes in the tumor cells redox status by increasing levels of 4-HNE, MDA and of PCO, as well as increasing the activity of MnSOD and CAT antioxidant enzymes. Although, there was an adjusted increase between oxidants and antioxidants enzymes the total antioxidant capacity of tumor cells was reduced compared to normal cells.

The cancerous cells present a high basal redox state and a delicate regulation between the production of ROS and antioxidants enzymes. Tumor cells produce a greater amount of ROS than normal counterpart cells, since the high mitochondrial activity

required by marked cell proliferation leads to an important formation of ROS (35). In turn, these ROS overproduction results in increasing levels of antioxidant enzymes as MnSOD and CAT responsible, respectively, for the neutralization of the reactive species superoxide and hydrogen peroxide. This tight regulation of the redox state is essential to prevent tumor cell death. As mentioned earlier, ROS are capable of increasing or inhibiting cell proliferation or inducing cell apoptosis, depending on their levels. Chemotherapy is based on this sensitivity of these cells to oxidative stress. Chemotherapy control the proliferation of tumor cells by inducing ROS overproduction, which is one of the mechanisms by which some chemotherapeutics lead to tumor regression(36,37). On the other hand, these drugs also act on non-tumor cells, leading to adverse effects such as gastrointestinal effects including chemotherapy-induced nausea and vomiting (CINV), both oral and gastrointestinal mucositis, which are associated with local ulceration and pain, and therefore , anorexia, weight loss and vulnerability to infections. Another consequence is central and peripheral neurotoxicity, such as chemotherapy-induced peripheral neuropathy (CIPN) (38). Thus, the use of bioactive compounds in cancer treatment, concomitant or not with chemotherapy, is an attractive field of study, as it may potentiate the anticancer effects of drugs and even mitigate the adverse effects of chemotherapy.

Interestingly, the Jurubeba extract was capable of altering the redox state of MCF-7 cells by inducing the oxidative stress, mimicking the action of chemotherapeutic agents. Such fact may have occurred because of the dual effect of antioxidants, since they have been reported to act as pro-oxidants(39). Although the activity of antioxidant enzymes MnSOD and CAT were higher than those of the control group, this increase was not sufficient to neutralize ROS production. ROS can integrate with glycidic and lipid molecules to form highly reactive compounds such as 4-HNE and MDA, known as reactive carbonyl species. These species can modify proteins to form carbonylated proteins, leading to changes in the structure and function of this molecule. Increased protein carbonylation in the MCF-7 cells, observed after HESPL treatment, is one of the best biomarkers of oxidative stress and oxidative damage (40). The decrease in the total antioxidant capacity of the cells confirms the onset of oxidative stress.

There is a lack of information in the literature about the effect of Jurubeba on breast cancer cells. In this sense the present findings are of great scientific contribution.

Although this work was limited to assessing the redox state of MCF-7 cells after treatment with Jurubeba, there are studies in the literature that point to lutein, one of the bioactive compounds present in the extract, as a carotenoid with anti-breast cancer effects (34). Gong and colleagues in their study of the effect of lutein on breast cancer cells showed that lutein significantly inhibits breast cancer growth by inducing cell-cycle arrest and caspase-independent cell death. Additionally, they found that lutein led to an inhibition of cell proliferation similar to that mediated by the taxanes, paclitaxel and docetaxel chemotherapies and yet the concomitant exposure of taxanes plus lutein additively inhibits breast cancer cell growth. One of the mechanisms associated with such effects was oxidative stress due to ROS overproduction (41). Obviously, the effects of Jurubeba extract on cancer cells are not limited to lutein, being undeniable the synergistic action of its components on the redox state. Such action may influence cellular proliferation as evidences support the contention that excessive ROS production and DNA damage promote apoptosis by common signaling pathways (35,42).

In conclusion, the HESPL treatment increased 4-HNE, MDA and PCO formation and MnSOD and CAT activity leading to cellular oxidative stress. These findings provide important insights for future research to investigate the consequences of changing the redox state of MCF-7 cells. Therefore, further studies involving *Solanum paniculatum L*. fruits may be a target for breast cancer research.

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Conclusão

Este estudo mostrou-se como inicial no campus do câncer. O câncer é um sistema multidimensional e complexo onde o estresse oxidativo pode contribuir para a sua formação. O extrato formulado é a primeira formulação estabelecida para os frutos bem como as doses iniciais estabelecidas nesse projeto podem auxiliar nos primeiros estudos do extrato do fruto. A dose de 3.75 μg/mL mostrou-se promissora na modulação na inflamação bem como aumentou o estresse oxidativo no câncer bem como no efeito das enzimas SOD e Catalase. A modulação positiva no estressse oxidativo pode ser uma medicina complementar como na atuação de quimioterápicos. O aumento do estresse oxidativo é uma das alternativas de atuação destes medicamentos e a sua ação como terapia adjuvante pode reduzir o uso de medicamentos bem como seus efeitos colaterais na saúde devido à sua formulação natural.

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ATIVIDADE BIOATIVA DE EXTRATOS DOS FRUTOS DE JURUBEBA (Solanum paniculatum L.) EM LINHAGENS CELULARES HUMANAS DE ADENOCARCINOMA MAMÁRIO

Pesquisador: Ana Paula Costa Rodrigues Ferraz

Área Temática:

Versão: 1

CAAE: 61878416.5.0000.5411

Instituição Proponente: Departamento de Patologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.851.266

Apresentação do Projeto:

O câncer de mama corresponde a principal neoplasia entre as mulheres no mundo, e no Brasil representa cerca de 25% dos casos novos a cada ano. Diante desta realidade faz-se necessária a busca por estratégias de prevenção e/ou auxílio no combate à proliferação de células mutagênicas. A modificação de hábitos alimentares e a inclusão de novos componentes na dieta têm sido associados a menor risco de desenvolvimento do câncer. Entre os alimentos utilizados na culinária regional brasileira, pode-se destacar a "Jurubeba" (Solanum paiculatum L.), uma hortaliça não convencional, que pode apresentar compostos bioativos protetores contra a carcinogênese. Suportados por dados prévios obtidos em nosso laboratório, o objetivo deste trabalho é avaliar a atividade biológica dos principais componentes bioativos de frutos de jurubeba in natura em linhagens de adenocarcinoma mamário. Inicialmente serão feitas coletas dos frutos de jurubeba da região de Minas Gerais ou Mato Grosso,quantificação e caracterização dos componentes fenólicos, carotenoides e a determinação do potencial antioxidante dos extratos de jurubeba. Estes extratos serão aplicados as linhagens MDA-MB-231 e MCF-7 de adenocarcinoma mamário. Doses ideais do extrato serão padronizadas através de testes de toxicidade e considerando concentrações que sejam passíveis de serem obtidas através de uma dieta comum. Também serão realizadas dosagens de Interleucina-6 (IL-6), Fator de necrose tumoral-alfa (TNF-) e Interleucina-8 (IL-8) no

sobrenadante celular. O fator nuclear kappa-B (NFB), marcadores de estresse oxidativo, multicaspases e dano de DNA serão avaliados através de kits comerciais específicos. Os dados serão apresentados por medidas descritivas de posição e variabilidade e analisados por testes específicos de comparação. Diferenças estatísticas serão consideradas para p<5%.

Objetivo da Pesquisa:

Objetivo Primário: O objetivo deste trabalho é avaliar a atividade biológica dos principais componentes bioativos de frutos de jurubeba in natura nas linhagens de
adenocarcinoma mamário.

Objetivo Secundário:

Objetivos específicos

• Quantificar e especificar os compostos bioativos nas amostras.

• Avaliação de espécies reativas presentes nas linhagens MCF-7 e MDA-MB-231

• Dosagem de marcadores pró-inflamatórios (NF-kB, IL-6, TNF- e IL-8)

• Avaliar o efeito dos extratos alcoólicos na proliferação celular.

Avaliação dos Riscos e Benefícios:

Riscos: O possível risco deste projeto é a atuação da planta de forma tóxica nas linhagens celulares avaliadas.

Benefícios: O presente projeto possui originalidade, pois não há estudos sobre os frutos da jurubeba e o seu possível potencial medicinal e este será o primeiro projeto a avaliar a atividade bioativa destes extratos no câncer de mama o qual possui grande relevância na saúde populacional.

A jurubeba já vem sendo estudada em outras abordagens pelo atual pesquisador responsável sendo assim este projeto considerado uma continuação de pesquisa, a qual pode estimular futuramente outros estudos relacionados com esta planta que ainda é pouco conhecida pela população e pode atuar de forma favorável para a saúde humana.

Comentários e Considerações sobre a Pesquisa:

O projeto de pesquisa é relevante, com metodologia adequada. O pesquisador informa que haverá um custo de R$ 20.000,00. Não informa financiamento externo do projeto.

Continuação do Parecer: 1.851.266

Considerações sobre os Termos de apresentação obrigatória:

O pesquisador solicitou a dispensa de aplicação de TCLE, tendo em vista que serão utilizadas células de linhagens celulares compradas comercialmente.

Foram apresentados também:

Folha de Rosto assinada pelo Diretor da Faculdade de Medicina

Anuência do EAP

Anuência da UNIPEX

Anuência do Departamento de Bioquímica Vegetal, colocando a disposição o laboratório para os pesquisadores.

Recomendações:

Ao final da execução do presente estudo apresentar Relatório Final do CEP.

Conclusões ou Pendências e Lista de Inadequações:

Sugiro aprovação, sem necessidade de envio à CONEP.

Considerações Finais a critério do CEP:

Projeto de Pesquisa APROVADO, deliberado em reunião ORDINÁRIA do CEP de 05 de Dezembro de 2.016, sem necessidade de envio à CONEP.

O CEP, no entanto, solicita aos pesquisadores que após a execução do projeto em questão, seja enviado para análise o respectivo “Relatório Final de Atividades”, o qual deverá ser enviado via Plataforma Brasil na forma de “NOTIFICAÇÃO”.

OBS: LEMBRAMOS QUE A PRESENTE PESQUISA SOMENTE PODERÁ SER INICIADA APÓS DIA 05/12/2016 – DATA DA APROVAÇÃO DO CEP.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_673583.pdf	09/11/2016 21:14:14		Aceito
Declaração de Instituição e Infraestrutura	declaracao_bioquimica.jpg	09/11/2016 21:13:39	Ana Paula Costa Rodrigues Ferraz	Aceito
Folha de Rosto	folha_rosto_nov.pdf	09/11/2016	Ana Paula Costa	Aceito

Endereço: Chácara Butignolli , s/n
Bairro: Rubião Junior **CEP:** 18.618-970
UF: SP **Município:** BOTUCATU
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Continuação do Parecer: 1.851.266

Folha de Rosto	folha_rosto_nov.pdf	21:12:43	Rodrigues Ferraz	Aceito
Declaração de Instituição e Infraestrutura	EAP.pdf	08/11/2016 10:46:46	Ana Paula Costa Rodrigues Ferraz	Aceito
Cronograma	cronograma_alterado.docx	17/10/2016 14:00:26	Ana Paula Costa Rodrigues Ferraz	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_comite.docx	17/10/2016 13:47:24	Ana Paula Costa Rodrigues Ferraz	Aceito
Declaração de Instituição e Infraestrutura	aceite.pdf	29/03/2016 11:46:52	Ana Paula Costa Rodrigues Ferraz	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BOTUCATU, 06 de Dezembro de 2016

Assinado por:
SILVANA ANDREA MOLINA LIMA
(Coordenador)

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