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Cnidoscolus sp DA CAATINGA: FITOQUÍMICA E ATIVIDADES BIOLÓGICAS

PÂMELLA GRASIELLE VITAL DIAS DE SOUZA

Recife - Brasil
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Tese apresentada ao Programa de Pós-Graduação em Bioquímica e Fisiologia do Centro de Biociências da Universidade Federal de Pernambuco como parte dos requisitos, para obtenção do título de Doutora em Bioquímica e Fisiologia.

ORIENTADOR: Prof. Dr. Nicácio Henrique da Silva

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RESUMO

Conhecida popularmente por "urtiga" ou "cansanção", *Cnidoscolus urens* é uma espécie pertencente a família Euphorbiaceae, endêmica da Caatinga. Bastante conhecida pela presença de pelos urticantes. Segundo conhecimentos populares, esta espécie é utilizada como fitorerápico, utilizando- se principalmente raiz e caule para o tratamento de muitas enfermidades, tais como inflamação da próstata e cistos ovarianos. Em estudos fitoquimicos, metabólitos secundários como flavonóides, antraquinonas, terpenos, taninos, antocianinas, esteróides e xantinas, foram detectados em extrados organicos de *C. urens*. O presente estudo objetivou investigar o perfil fitoquímico e avaliar o potencial antitumoral, antidiarreico e inseticida dos extratos aquoso e orgânico de *C. urens* coletadas em Caruaru/ área de Caatinga-PE. Para tanto, foi investigado por cromatografia de camada delgada (CCD), os metabólitos secundários produzidos por *C. urens* presente em extratos aquoso, acetato de etila e n-butanólico. A toxicidade foi verificada seguindo o guideline OECD. Para a atividade antitumoral, utilizou-se ensaios antiproliferativo *in vitro* sobre células de linhagem HELA e *in vivo*, em animais com carcinoma de Ehrlich induzido, sendo tratados com extrato aquoso e etanólico, administrados numa concentração igual a 200mg/kg. A atividade antidiarreica foi averiguada pelas metodologias sobre o efeito nas fezes normais, diarreia induzida por óleo de rícino, ensaio de enteropooling e transito intestinal por carvão ativado, para tal, os animais foram tratados com extrato etanólico de partes aéreas (folha, semente e flor) de *C. urens*. Quanto a atividade inseticida, o extrato metanólico e também suas frações: éter, clorofórmio, acetona e metanol, foram avaliados quanto a sua toxicidade por ingestão e contato, além do índice de deterrência alimentar. Os testes fitoquímicos demonstraram a presença de taninos, flavonoides, açúraces redutores, terpenos e cumarinas. No modelo de atividade *in vitro* o extrato aquoso e etanólico (Aq200 e EtOH200) demonstroram moderada ação citotóxica para as metodologias testadas para os modelos *in vitro* (MTT e Vemelho neutro). Para a atividade *in vivo*, os extratos Aq200 e EtOH200 produziram um percentual de redução do crescimento tumoral igual a 84.4% e 79.2%, além de causar uma melhora representativa do perfil bioquímico e hematológico. Todos os extratos de *C. urens* foram avaliados quanto a sua toxicidade e não mostrou ação toxica na dose máxima testada (2000mg/kg). Quanto a avaliação antidiarreica o extrato etanólico de partes aérea de *C. urens* nas doses de 200 e 400mg/kg, mostraram excelentes resultados. Para o ensaio de diarreia induzida por óleo de rícino foi observado uma redução no numero total de fezes (7.0 e 7.2), respectivamente, para o ensaio que avalia o acumulo de fluido,

a redução foi de 23.4 e 20.8 para as concentrações de 200 e 400 mg/kg, respectivamente. Já a avaliação do trânsito intestinal os resultados encontrados foram iguais a 36.3% para EtOH200 e 25.5% para EtOH400. Os extratos de *C. urens* também foram testados quanto sua ação inseticida e mostrou-se ser um potente agente. Para os testes inseticida, duas metodologias diferentes foram utilizadas, avaliando a ação inseticida por ingestão e contato. Quando espécies de *Sitophylos zeamais* foram expostos a uma dieta contendo MeOH (0.18; 0.25; 0.5 mg/g) e MeOHFrac (0.25- 0.5 mg/g) foi observado uma indução de 27 a 90% e 33 a 91% de mortalidade por ingestão, respectivamente. O percentual de mortalidade por contato foi avaliado para os mesmos extratos nas concentrações de 50 e 100 µg/mL e apresentou 45 a 75% e 77 a 87% de mortalidade respectivamente. A LC_{50} também foi determinada, tanto por ingestão para MeOH e MeOHFrac (LC_{50}= 0.241 mg/g e LC_{50}= 0.11 mg/g, respectivamente), quanto por contato para os mesmos extratos (LC_{50}=0,012 µg/inseto e LC_{50}= 0.026 µg/inseto), também respectivamente. Essas metodologias também nos permitiram avaliar que os extratos induziram mudanças significativas nos indices nutricional, além de induzir rejeição do alimento disponibilizado durante o ensaio. Todos estes resultados contribuem para confirmar a hipótese popular de que *C. urens* possui ação antitumoral, antidiarreica e inseticida. Estes resultados corroboram com o enriquecimento bibliográfico científico e contribuem para uma pespectiva na produção de novos fármacos e agentes inseticidas mais eficazes e com menor efeitos adversos.

Palavras-chave: *Cnidoscolus urens* (Euphorbiaceae). Antitumoral. Antidiarréia. Atividade inseticida.

ABSTRACT

Popularly known as "nettle" or "cansanção" Cnidoscolus urens is a species belonging to the family Euphorbiaceae, endemic to the Caatinga. Well known for the presence of stinging. According to popular knowledge, this species is applied as fitorerapico, using mainly the root and stalk for the treatment of many diseases, such as inflammation of the prostate and ovarian cysts. In phytochemical studies, secondary metabolites such as flavonoids, anthraquinones, terpenes, tannins, anthocyanins, steroids and xanthines were detected in extraneous organs of *C. urens*. The present study aimed to investigate the phytochemical profile and evaluation of the antitumor, antidiarrheal and insecticidal potential of the aqueous and organic extracts of *C. urens* collected Caatinga area in in Caruaru - PE area. Therefore, it was investigated by thin layer chromatography (TLC), secondary metabolites produced by *C.urens*. The toxicity was observed following the OECD guideline. or antitumor activity, in vitro antiproliferative assays on HELA lineage cells and in vivo in animals with induced Ehrlich carcinoma were treated with aqueous and ethanolic extract, administered in a concentration equal to 200mg / kg. The antidiarrheal activity was investigated by the methodologies on the effect on normal feces, castor oil-induced diarrhea, enteropooling test and activated carbon intestinal transit. The animals were treated with ethanolic extract of aerial parts (leaf, seed and flower) of *C. urens*. Regarding the insecticidal activity, the methanolic extract and also its fractions: ether, chloroform, acetone and methanol, were evaluated for their toxicity by ingestion and contact, besides the food deterrence index. Phytochemicals tests showed the presence of tannins, flavonoids, açúraces reducers, terpenes and coumarins. In the model of activity *in vitro* the aqueous extracts and ethanol (Aq200 and EtOH200) showed moderate cytotoxic action for the methodologies. For the *in vivo* activity, and Aq200 EtOH200 produced a reduction of percentage of tumor growth equal to 84.4 and 79.2%, and cause a representative improved biochemical and haematological profile. All *C. urens* extract was evaluated for toxicity and showed no toxic action at the maximum tested dose (2000mg / kg). The evaluation antidiarrheal ethanol extract of aerial parts of *C. urens* in doses of 200 and 400 mg / kg, showed excellent results. For diarrhea assay induced by castor oil has been observed a reduction in the total number of stools (7.0 and 7.2), respectively, to test that evaluates the accumulation of fluid, the reduction was 23.4 and 20.8 for concentrations of 200 and 400 mg / kg, respectively. Since the

evaluation of the intestinal transit the results were equal to 36.3% for EtOH200, and 25.5% for EtOH400. The extracts of *C. urens* were also tested for their insecticidal action and proved to be a potent agent. For the insecticide tests, two different methodologies were used, evaluating the insecticide action by ingestion and contact. When *S. zeamais* species were exposed to a diet containing MeOHC.urens (0.18, 0.25, 0.5 mg / g) and MeOHFrac (0.25- 0.5 mg / g) an induction of 27 to 90% and 33 to 91% of Mortality on ingestion, respectively. The percentage of contact mortality was evaluated for the same extracts at concentrations of 50 and 100 μg / mL and presented 45 to 75% and 77 to 87% of 006Dortality, respectively. LC_{50} was also determined, both by ingestion for MeOHC.urens and MeOHFrac (LC_{50} = 0.24 and LC_{50} = 0.11, respectively), and by contact for the same extracts (LC_{50} = 0.012 and LC_{50} = 0.026, respectively). Allowed to evaluate that the extracts induced significant changes in the nutricionail indices, in addition to inducing rejection of the food available during the test. All these results contribute to confirm the popular hypothesis that *C. urens* has antitumor, antidiarrheal and insecticide action. These results corroborate with the scientific bibliographic enrichment and contribute to a perspective on the production of new drugs and insecticides more effective and with less adverse effects.

Key words: Cnidoscolus urens (Euphorbiaceae). Antitumor. Antidiarrhea. Insecticidal activity.

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LISTA DE ABREVIATURAS

5-FU- 5- fluorouracil

ACh- acetilcolina

AMPc- 3,5 monofosfato cíclico de adenosina

Ca^{2+}- ion de cálcio

CaM- proteína ligadora de cálcio

Cav- canais de Ca^{+} dependentes de voltagem

Aq- Extrato aquoso

CFTR- regulador trnsmenbrana de fibrose cística

Cl^{-}- íon de cloro

DA- doença de Alzheimer

DAG- diacilglicerol

DDA- doença diarreica aguda

DMEM- dulbecco's modified Eagle

DPPH- 2,2-difenil-1-picrilhidrazil

EA- Extrato de acetato de etila

EC- Carcinoma de Herlich

GMPc- monofosfato cíclico de guanosina

GTP- trifosfato de guanosina

HCO^{3}- íon de bicarbonato

INCA- instituto nacional de câncer

IP3- 1,4,5- trifosfato de inositol

MLCK- cinase de cadeia leve de miosina

MLCP- fosfatase de cadeia leve de miosina

MTT- 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

Na+- íon de sódio

N- bu- butanol

OMS- organização mundial de saúde

PIP2- 4,5- difosfato de fosfatidil inositol

PKC- proteína cinase C

PLC β1- fosfolipase C β-1

PMCA- troca do sódio- cálcio da membrana plasmática

RN- Vermelho neutro

SBCO- sociedade brasileira de cirurgia oncológica

SERCA- Ca^{2+}- ATPase do retículo sarcoplasmático

SNC- sistema nervoso central

SNE- sistema nervoso entérico

TGI- tratogastrointestinal

SUMÁRIO

CAPÍTULO I

1 INTRODUÇÃO

Organização Mundial de Saúde (OMS), identificou que nos países em desenvolvimento, aproximadamente 80- 85% da população, utilizam práticas tradicionais para os cuidados com a saúde (OMS, 2002; CRUZ et al., 2015). Na atualidade, tem- se falado muito em produtos de origem natural, porém, sabemos que desde as mais antigas civilizações, as plantas são utilizadas para tratar diversas enfermidades, e esta prática, durante muito tempo foi a única alternativa de cuidados e prevenção que os indivíduos praticavam (BATTISTI et al., 2013; CRUZ et al., 2015).

Sendo o Brasil detentor da maior biodiversidade, cerca de 55 mil espécies já catalogada, sendo estimado um total de 550 mil espécies. Por isso, é tradição da população brasileira, utilizar recursos naturais como as plantas medicinais, sendo uma cultura transmitida entre as gerações (FONSECA, 2012). Outro fator que contribui para a continuidade desta cultura, é atribuído aos fatores socioeconômicos, principalmente pela falta de assistência médica (ROQUE; LOIOLA, 2013; MARREIROS et al., 2015). O quantitativo de novas pesquisas em torno das plantas medicinais tem crescido nos últimos anos, porém esse crescimento é muito pequeno, apenas 8% ao ano (FONSECA, 2012).

A Caatinga tem uma vegetação seca, e nem todas as partes das plantas (folhas, frutos e flores) estão disponíveis o ano inteiro, principalmente pela pequena duração do período chuvoso e por essas características, a maioria dos fitoquímicos retirados desta vegetação é produzido através da casca dos vegetais (PINTO; AMOROZO; FURLAN, 2006). A explicação para a ampla utilização da casca, pode ser justificada pelo fato de estarem disponíveis o ano inteiro independente da estação (ALBUQUERQUE; ANDRADE, 2002). Porém, essa prática pode colocar esse ecossistema em risco de extinção (ALBUQUERQUE et al., 2007).

Os jornais científicos revelam muitos exemplares de vegetais da Caatinga que são aplicados na medicina popular (MARQUES, 2008; ROQUE et al., 2010; PEDROSA et al., 2012; LUCENA et al., 2012). Como as plantas são amplamente utilizadas e muitas vezes de forma indiscriminada, é de grande valia que seja dada uma atenção maior a respeito desta causa.

A ciência revela e esclarece muitas informações, o semiárido brasileiro disponibiliza recursos fitoterápicos com ações e mecanismos diversificados: anti-inflamatória, tônico, analgésica, antidiabético, adstringente, entre outras ações (SANTOS; ARAÚJO; ALBUQUERQUE, 2008; MACENA et al., 2012). Desta forma, a região do nordeste brasileiro é considerada, um centro de diversidade de famílias, com aproximadamente 50 gêneros e 240 espécies, com muitas espécies endêmicas, que se distribuem, a maior parte, em áreas de Caatinga (LUCENA; ALVES, 2010; OLIVEIRA, 2013).

As Euphorbiaceae são uma das mais importantes famílias vegetais encontradas em áreas de Caatinga (Tabela 1). Espécies desta família pode ser facilmente encontrada em regiões tropicais e subtropicais dos continentes americano e africano (LUCENA; AMORIM; ALVES, 2009). As angiospermas, são uma das mais extensas e complexa família. Espécimes de Euphorbiaceae são bem representadas por cerca de 254 gêneros e 6.300 espécies (HEYWOOD et al., 2007), só no Brasil, já foram registradas ocorrências de 72 gêneros distribuídos em 1.100 espécies, essa informação pode ser confirmada na tabela 1, onde foram registradas algumas das espécies de Euphobiaceae encontrada em uma região do semiárido (WEBSTER, 1994; GOVAERTS; FRODIN; RADCLIFFESMITH, 2000; RADCLIFFE- SMITH, 2001; BARROSO, 2002; SOUZA; LORENZI,2008).

Tabela 1: Lista de espécies de Euphorbiaceae registradas em uma região de Caatinga. A tabela demonstra a riqueza e diversidade de espécies da família Euphorbeaceae

Espécies	**Nome comum**	**Hábito**	**Vocher**
Acalypha multicaulis Müll. Arg	Velaminho	Arbustiva	20577
Acalypha poiretii Spreng.	-	Herbácea	20830
Astraea lobata (L.) Klotzch	-	Herbácea	20573
Cnidoscolus urens (L.) Arthur	Cansanção	Subarbustiva	20378
Croton adenocalix Baill.	Marmeleiro-branco	Arbustiva	20355
Croton blanchetianus Baill.	Marmeleiro	Arbórea	20384
Croton heliotropiifolius Kunth	Velame	Arbustiva	20382
Croton hirtus L' Her	-	Herbácea	25128

Dalechampia scandens L.	Cipó-de-caboclo	Trepadeira	21068
Ditaxis malpighiacea (Ule) Pax & K. Hoffm	Pelo-de-raposa	Arbustiva	20567
Euphorbia heterophylla L.	-	Herbácea	21342
Euphorbia hyssopifolia L	Erva-de-leite	Herbácea	21072
Euphorbia insulana Vell	Maleiteira	Herbácea	20829
Euphorbia prostrata Aiton	-	Herbácea	21357
Euphorbia sp	-	Herbácea	20831
Jatropha molissima (Pohl) Baill	Pinhão	Arbórea	20347
Mabea sp	Pau-de-estralo	Arbórea	20832
Manihot dichotoma Ule	Maniçoba	Arbórea	20346
Sapium glandulosum (L.) Morong	Burra-leiteira	Arbórea	20358
Tragia volubilis L	Urera	Trepadeira	20374

Fonte: OLIVEIRA, 2013, com modificações.

As Euphorbiaceas destacam-se por ser uma família de importância econômica, essa importância torna-se ainda maior quando avaliado a participação em setores industriais, ornamentais, alimentício e farmacológico (ALVES, 1998). Espécies como *Manihot esculenta Crantz,* utilizada para a produção de farinha, no setor da indústria podemos destacar a Hevea Aubl, conhecida por seringueira, foi bastante explorada durante o período do ciclo da borracha (CORRÊA et al., 2002).

Espécies como: *Croton blanchetianus Baill (*marmeleiro*)*, *Phyllanthus niruri L.* (quebra pedra) (CORRÊA et al., 2002), *Ricinus communis L.* (mamona) utilizado para extração de óleo aplicado como lubrificantes e compostos biodegradáveis (SILVA, 1998), *Cnidosculos urens (L.) Arthur* (urtiga), entre outras tantas (CORRÊA et al., 2002). Atualmente muitas dessas espécies são alvo de diferentes estudos, acerca de suas propriedades química e biológicas, importância etnobotânica, contribuindo para melhor compreensão sobre as espécies (SECCO, 2005).

O gênero *Cnidoscolus* Phol é nativo do Brasil e 50-70 espécies com alastramento neotropical (ARAÚJO; LEAL; QUIRINO, 2012). A espécie *Cnidoscolus urens,* pertencente à família Euphorbiaceae, com importância econômica, apresenta distribuição no semiárido (FERREIRA, 2011). Muitas são as causas, as quais espécies do gênero *Cnidoscolus* Phol, são aplicadas pela medicina popular, entre as formas populares de utilização podemos citar a preparação de chás de raízes com efeito cicatrizante, tônico, diurético e até diarreico (BRAGA, 1976).

Conhecida como urtiga, de ocorrência comum na Caatinga, porém também apresenta ocorrência no México e Argentina (ARAÚJO; LEAL; QUIRINO, 2012). Estas plantas apresentam pelos urticantes distribuídos pelo seu caule, nas pesquisas etnobotânicas (PEIXOTO SOBRINHO, 2011), esta espécie é a mais relatada, segundo Albuquerque et al., (2007). Estudos realizados por Alves et al., (2007), identificaram que entra as espécies citadas sendo utilizadas como medicinais, em uma feira livre no estado da Paraíba, a espécie *C. urens*, estava entre as mais relatadas. Na maioria das vezes são administradas por via oral utilizando-se dá raiz para tratar diversas enfermidades: Inflamação da próstata, cisto de ovário e útero.

Entre tanto, ainda se torna escasso a bibliografia científica que informem as propriedades presentes em diferentes extratos desta espécie, corroborando com a necessidade de iniciar estudos e avaliar os potenciais efeitos biológicos que podem ser atribuídos aos produtos sintetizados pelo *C. urens*, a fim de investigar seus metabólitos secundários para que suas atividades sejam comprovadas, até que possam ser utilizados como terápicos seguros.

2 REVISÃO DE LITERATURA

2.1 Plantas Medicinais

A evolução da humanidade apresenta uma relação muito íntima com o mundo dos vegetais. A espécie humana iniciou a domesticação de espécies vegetais e animais quando processos evolutivos da ciência biológica, cultural e técnicas, tiveram início, acerca de 200 mil anos. Os povos mais antigos passaram a reconhecer os produtos naturais, inicialmente, como fonte alimentícia. Com o passar dos anos, a humanidade começou a despertar outros tipos de

interesse a esses produtos e reconhecer que as plantas tinham propriedades curativas E a partir desta prática, cada localidade desenvolveu e criou sua própria cultura, tradição e crenças (MAZOYER; ROUDART, 2010; MINISTÉRIO DA SAÚDE, 2006).

A fitoterapia, que tem como base uma variedade inesgotável de produtos farmacológicos para fins terapêuticos sem isolamento de substâncias ativas. (CARTAXO et al., 2010; TAN et al., 2010; OLIVEIRA et al., 2012). Os fitoterápicos são produzidos de plantas frescas ou secas, processadas por destilação, maceração, percolação, entre outras metodologias. Por tanto a procura por fitoterápicos a nível mundial, tem crescido abundantemente. Esse crescimento dá-se pelo conhecimento acerca das plantas medicinais as quais apresentam um alto valor econômico e são principais fontes para a retirada de recursos voltados para a produção de fármacos (OLIVEIRA et al., 2012).

O conhecimento sobre a ação terapêutica das plantas, tem sido acumulado, aperfeiçoado e reproduzido, passado de geração em geração. Sendo assim, à sabedoria é passada entre pessoas de uma mesma família. Esta prática garante a imortalidade do reconhecimento dessas espécies com ação terapêutica e que é influenciada pela idade, onde os mais antigos de uma determinada comunidade eram o portador de maior conhecimento, capaz de identificar a planta indicada para determinada doença, a parte da planta (raízes, cascas, folhas, frutos e sementes) que podem ser utilizada e a melhor forma de preparo como chás (onde a plantas é fervida junto com a água), decocção ou infusão (o qual a água é fervida e depois colocada sobre a planta, com agitação) (REZENDE; COCCO, 2002).

A utilização de plantas como tratamento de enfermidades ocorreu nas civilizações chinesas, assíricos, egípcios e hebreus desde 2.300 a.C, e que ainda hoje fazem uso desta prática. A fitoterapia cruzou toda a existência das mais antigas civilizações, iniciando na antiguidade seguindo até à idade média, prosseguindo pela idade moderna e contemporânea, sendo utilizada até hoje em todo o mundo. No Brasil não poderia ser diferente. A origem da medicina popular com a utilização de plantas foi influenciada de maneiras diferentes: (i) Os conhecimentos deixados pelos índios que habitaram os territórios brasileiros; (ii) A introdução de tratamentos alternativos foram deixadas pelos negros escravos trazidos para o Brasil e europeus; (iii) Durante o período que o Brasil foi colonizado e seguiu como colônia de Portugal (BIESKI, 2005).

O Brasil encontra-se entre os 10 maiores consumidores de fitoterápicos, onde 91,9% dos brasileiros utilizam ou já utilizaram plantas para algum tipo de tratamento e quase 50% desses possuem um cultivo medicinal caseiro, sendo 30% dos medicamentos, disponíveis e registrados, de origem natural (OLIVEIRA et al., 2012).

A prática da medicina caseira, além do incentivo realizado pelo exercício político, é estimulada entre as comunidades e sem supervisão médica, a procura pela medicina tradicional, nestes casos, só acontece se os sintomas persistirem. Outra causa que leva a escolha do tratamento por métodos alternativos da-se quando a medicação tradicional não se encontra acessível ou é muito cara (RIBEIRO; LEITE; DANTAS- BARROS, 2005).

Diante da grande importância mundial, nota- se a necessidade de incorporar a associação entre a medicina tradicional e a medicina alternativa, visto que nem sempre é possível atingir um potencial terapêutico ideal utilizando somente a medicina tradicional. Por isso, cada vez mais vem sendo estimulado programas de saúde adaptados especificamente para cada localidade de acordo com o perfil socioeconômico (OLIVEIRA et al., 2012).

Deste modo, políticas governamentais estimulam a divulgação de plantas com potencial terapêutico e que poderão ser utilizadas como fitoterápicos e implementadas no cotidiano do Sistema Único de Saúde (SUS), com a finalidade de ampliar o número de fitoterápicos financiados por verbas federal, a fim de torná-los acessíveis à população. (PINHO; PICHONELLI, 2009). Também foram incluídos a criação de leis como o Decreto n 5.813, que regula o programa de Política Nacional de Plantas Medicinais e Fitoterápicos (PNPMF) (BRASIL, 2007).

A fim de concretizar a utilização de fitoterápicos, o governo brasileiro criou em 2006, a Política Nacional de Práticas Integrativas e Complementares do SUS, a qual aprova o uso de medicamentos fitoterápico. Mais tarde, em 2008, o Programa Nacional de Plantas Medicinais e Fitoterápicas foi implantado, garantindo a população brasileira, o acesso seguro aos fitoterápicos (Instituto Ethos, 2016). Em 2009, o Ministério da Saúde do Brasil divulgou uma relação de 71 plantas (Tabela 2), as quais podem ser utilizadas como fitoterápicos (Ministério da Saúde, 2014).

Tabela 2: Relação Nacional de Plantas Medicinais de Interesse ao SUS.

Nome científico	Nome popular	Utilidade
Achillea millefolium	Mil-folhas, Dipirona	Combate úlceras, feridas e é analgésico.
Allium sativum	Alho	anti-séptico, antiiflamatório e anti-hipertensivo.
Aloe spp (A. vera ou A. barbadensis)	Babosa, áloes	Combate a caspa, calvície e é antisséptico. Retira lendia de piolhos e é cicatrizante .
Alpinia spp (A. zerumbet ou A. speciosa)	Colônia	Anti-hipertensivo.
Anacardium occidentale	Caju	Antisséptico e cicatrizante .
Ananas comosus	Abacaxi	Mucolítica e fluidificante das secreções e das vias aéreas superiores.
Apuleia ferrea = Caesalpinia ferrea	Jucá, pau-ferro-verdadeiro, Ibirá-obi	Infecção catarral, garganta, gota, cicatrizante
Arrabidaea chica	Crajirú, carajiru	Afeções da pele em geral (impigens), feridas, Antimicrobiano
Artemisia absinthium	Artemísia	Estômago, fígado, rins, verme (lombriga e oxíuru, giárdia e ameba)
Baccharis trimera	Carqueja, carquejaamargosa	Combate feridas e estomáquico
Bauhinia spp (B. affinis, B. forficata ou variegata)	Pata de vaca	
Bidens pilosa	Picão	Combate úlceras
Calendula officinalis	Bonina, calêndula, flor-de-todos-osmales, malmequer	Feridas, úlceras, micoses
Carapa guianensis	Andiroba, angiroba, nandiroba	Combate úlceras, dermatoses e feridas
Casearia sylvestris	Guaçatonga, apiáacanoçu,bugre branco, café-bravo	Combate úlceras, feridas, aftas, feridas na boca
Chamomilla recutita = Matricaria chamomilla = Matricaria recutita	Camomila	Combate dermatites, feridas banais
Chenopodium ambrosioides	Mastruz, erva-de-santa- maria, ambrosia, erva-debicho, mastruço, menstrus	Corrimento vaginal, antisseptico local
Copaifera spp	Copaíba	Antiinflamação
Cordia spp (C. curassavica ou	Erva baleeira	Antiiflamatorio

C. verbenacea)		
Costus spp (C. scaber ou C. spicatus)	Cana-do-brejo	Combate leucorréia e inflamação renal
Croton spp (C. cajucara ou C. zehntneri)	Alcanforeira, herva-mular, péde-perdiz	Combate feridas, úlceras
Curcuma longa	Açafrão	
Cynara scolymus	Alcachofra	Combate ácido úrico
Dalbergia subcymosa	Verônica	Auxilia no tratamento de inflamações uterinas e da anemia
Eleutherine plicata	Marupa, palmeirinha	Hemorróida, vermífugo
Equisetum arvense	Cavalinha	Diurético
Erythrina mulungu	Mulungu	Sistema nervoso em geral
Eucalyptus globulus	Eucalipto	Combate leucorréia
Eugenia uniflora ou Myrtus brasiliana	Pitanga	Diarréia
Foeniculum vulgare	Funcho	Anti-séptico
Glycine max	Soja	Sintomas da menopausa e osteoporose
Harpagophytum procumbens	Garra-do-diabo	Artrite reumatóide
Jatropha gossypiifolia	Peão-roxo, jalopão, batata-de-téu	Antisseptico e feridas
Justicia pectoralis	Anador	Cortes, afecções nervosas, catarro bronquial
Kalanchoe pinnata = Bryophyllum calycinum	Folha-da-fortuna	Furúnculos
Lamium album	Urtiga-branca	Leucorréia
Lippia sidoides	Estrepa cavalo, alecrim, alecrim-pimenta	
Malva sylvestris	Malva, malva-alta, malva-silvestre	Furúnculos
Maytenus spp (M. aquifolium ou M. ilicifolia)	Concorosa, combra-de-touro, espinheira-santa, concerosa	Antiséptico em feridas e úlceras
Mentha pulegium	Poejo	
Mentha spp (M. crispa, M. piperita ou M. villosa)	Hortelã-pimenta, hortelã, menta	
Mikania spp (M. glomerata ou M. laevigata)	Guaco	Broncodilatador
Momordica charantia	Melão de São Caetano	
Morus sp	Amora	
Ocimum gratissimum	Alfavacão, alfavaca-cravo	

Orbignya speciosa	Babaçu	
Passiflora spp (P. alata, P. edulis ou P. incarnata)	Maracujá	Calmante
Persea spp (P. gratissima ou P. americana)	Abacate	ácido úrico, prevenir queda de cabelo, anti-caspa
Petroselinum sativum	Falsa	
Phyllanthus spp (P. amarus, P.niruri, P. tenellus e P. urinaria)	Erva-pombinha, quebra-pedra	
Plantago major	Eanchagem, tanchás	Feridas
Plectranthus barbatus = Coleus barbatus	Boldo	
Polygonum spp (P. acre ou P. hydropiperoides)	Erva-de-bicho	Corrimentos
Portulaca pilosa	Amor-crescido	Feridas e úlcera
Psidium guajava	Goiaba	Leucorréia, aftas, úlcera, irritação vaginal
Punica granatum	Romeira	Leucorréia
Rhamnus purshiana	Cáscara sagrada	
Ruta graveolens	Arruda	
Salix alba	Salgueiro branco	
Schinus terebinthifolius = Schinus aroeira	Araguaíba, aroeira, aroeira-do-rio-grande-do-sul	Feridas e úlceras
Solanum paniculatum	Jurubeba	
Solidago microglossa	Arnica	Contusões
Stryphnodendron adstringens = Stryphnodendron barbatimam	Barbatimão, abaremotemo, casca-da-virgindade	Leucorréia, feridas, úlceras, corrimento vaginal
Syzygium spp (S. jambolanum ou S. cumini)	Jambolão	
Tabebuia avellanedeae	Ipê-roxo	
Tagetes minuta	Cravo-de-defunto	
Trifolium pratense	Trevo vermelho	
Uncaria tomentosa	Unha-de-gato	Imunoestimulante e antiinflamatório
Vernonia condensata	Boldo da Bahia	
Vernonia spp (V. ruficoma ou V. polyanthes)	Assa-peixe	
Zingiber officinale	Gengibre	Tosse

Fonte: MINISTÉRIO DA SAÚDE, 2014. Modificada.

Em decorrência da implementação desses programas, o MS iniciou convênios com a indústria e pequenos agricultores, além de selecionar 12 municípios distribuídos entre Pernambuco, Paraná, Minas Gerais, Pará, Goiás, Rio de Janeiro e São Paulo, objetivando estimular a produtividade desses produtos, sendo esta a primeira vez em que recursos federais foram investidos nesta área (Instituto Ethos, 2016). Hoje 21 unidades federativas (Tabela 3) já disponibilizam fitoterápicos assegurados por recursos do governo, estado e município (Ministério da Saúde, 2015).

Tabela 3: Vinte e uma unidades federativas pactuaram a disponibilização de fitoterápicos com recurso tripartite, conforme a Portaria GM/MS nº 1.555/2013:

UNIDADES DA FEDERAÇÃO		
ACRE (AC)	ESPIRITO SANTO (ES)	PARANÁ (PR)
ALAGOAS (AL)	GOIÁS (GO)	RIO DE JANEIRO (RJ)
AMAZONAS (AM)	MATO GROSSO DO SUL (MS)	RONDÔNIA (RO)
BAHIA (BA)	MINAS GERAIS (MG)	RIO GRANDE DO NORTE (RN)
CEARÁ (CE)	PARÁ (PA)	RIO GRANDE DO SUL (RS)
DISTRITO FEDERAL (DF)	PERNAMBUCO (PE)	SANTA CATARINA (SC)
SÃO PAULO (SP)	SERGIPE (SE)	TOCANTINS (TO)

Fonte: MINISTÉRIO DA SAÚDE, 2015.

Os ramos de fitoterápicos movimentam cerca de US$ 22 bilhões de dólares, no mundo, com um crescimento anual de 12%. No Brasil, a fitoterapia é detentora de uma parcela de 5% do mercado farmacêutico (MINISTÉRIO DA SAÚDE, 2003). Os fitoterápicos apresentam uma potencialidade inesgotável, baseados nos recursos retornáveis. E através dos conhecimentos obtidos por estudos realizados com matéria prima vegetal e levantamentos etnobotânicos, novas descobertas, e desenvolvimento de novas moléculas com aplicabilidade tecnológica, poderão fazer parte do leque para a busca de terapêuticos com maior eficiência e com menor ou nenhum efeito adverso (MINISTÉRIO DA SAÚDE, 2006b).

No Brasil encontramos uma ampla biodiversidade e vinculada a essa biodiversidade, temos a riqueza do conhecimento popular acumulado por pessoas que têm acesso direto a natureza. Portanto, é um país que somou seus conhecimentos sobre a medicina popular de uma mistura cultural entre os indígenas, europeus e africanos, sendo, hoje uma nação que é dita

como base para o desenvolvimento de novas pesquisas para descoberta de novas fontes de medicamentos utilizando plantas medicinais como ponto de partida (SANTOS et al., 2012). Os altos custos e o potencial adverso com efeitos colaterais que fármacos industriais apresentam e incentiva a tendência para a procura de plantas que apresentam alto potencial biológico (HALBERSTEIN, 2005).

O Brasil abriga a diversidade química vegetal. Sendo o país que aloca a maior floresta úmida. Com seis Biomas bem distribuídos por todo o seu território. No território brasileiro, estão localizados seis Biomas ricos em fauna e flora: Amazônia, Caatinga, Cerrado, Pantanal, Pampa e Mata Atlântica, dentre eles destacamos o Bioma Caatinga, que atinge cerca de 70% do Nordeste (PINTO et al., 2002; SANTOS; JUNIOR; PRATA, 2012). Plantas nativas da Caatinga são vastamente utilizadas como medicinais e algumas espécies são comercializadas como fitoterápico. Este cenário é bem representado pela *Amburana cearenses e Erythrina velutina*, referendadas por Albuquerque e seus colaboradores em 2007.

2.1.1 Características do Bioma Caatinga

Entre 1801 a 1900, século XIX, marcado por muitos conflitos militares, foi também, um período marcado pelo aumento desenfreado de interesses em explorar áreas inexploradas ou pouco conhecidas, como o Cerrado, a Floresta Amazônica e a nossa inestimada Caatinga. Os desbravadores que aqui chegaram, alimentavam uma expectativa de deparar- se com uma vegetação verde e florida, quando na verdade, encontraram uma vegetação seca, cheia de galhos espinhos e sem folhas e nem flores (CARRARA, 1996).

Sendo assim, a Caatinga se destaca dos outros Biomas, por apresentar uma vegetação arbustiva e grandes árvores, repletas de espinhos (Ministério do Meio Ambiente, 2016). De aparência seca, mas com grande capacidade auto renovável, basicamente localizado no Nordeste do Brasil. Toda a extensão territorial da Caatinga, encontra- se bem distribuída em 09 estados brasileiros: Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Bahia e Sergipe, além de atingir uma parte de Minas Gerais (TRENTIN et al., 2011; PINHEIRO et al., 2013).

Para atribuir a real importância da vegetação do semiárido brasileiro, tornou- se necessário que pesquisadores em suas minuciosas observações, notassem o perfil adaptativo que esses vegetais exibiam. Num dia de sol ardente incidindo sobe aquela terra seca, com árvores quase morta, fixadas em baixo da terra por raízes que se estendiam por quilómetros em busca d'água, bastava um rápido contato com a umidade para que esta vegetação desenvolvesse folhas e frutos (MARTIU, 1996).

Provavelmente, hoje, a Caatinga é uma área que tem sofrido com as intervenções do homem, com sua atuação explorativa. Com 844.453 Km² de extensão pelo território nacional, rico em biodiversidade, o Bioma ampara 178 espécies de mamíferos, 591 de aves, 177 de répteis, 79 espécies de anfíbios, 241 de peixes e 221 de abelhas. Milhares de pessoas carentes dependem deste Bioma para sua sobrevivência (MINISTÉRIO DO MEIO AMBIENTE, 2016).

Cartaxo et al. (2010) e Pinheiro et al., em 2013, observaram e relataram a existência de muitos estudos em torno do Bioma, Caatinga, os quais comprovam uma potente capacidade terapêutica, com competência para ampliar o arsenal de moléculas bioativa. Em conjunto a este Bioma está a importância do conhecimento popular que pode auxiliar nesta busca. Vários estudos populacionais relatam que muitas espécies são utilizadas para tratar diversas enfermidades, incluindo doenças de pele, gastrointestinais, infecciosas, entre outras (CARTAXO et al., 2010; PINHEIRO et al., 2013).

Em pesquisa realizada por Agra, Freitas e Barbosa- Filho, em 2007, retratam informações onde a vegetação espinhosa da Caatinga, abriga em média, 483 espécies que tem suas propriedades bioativas reveladas. Em contrapartida, muitas dessas espécies ainda não foram estudadas a nível de composição de seus constituintes e de suas atividades biológicas.

Enxergando a relevância dessa vegetação rica e com baixíssima reserva de água, passamos a compreender o seu importantíssimo papel, pois é altamente vulnerável a ação do homem sobre o mesmo e as modificações dela resultante, esse Bioma que hospeda mais de 20 milhões de habitantes, que sofrem com as mudanças drásticas de estação, principalmente com o período da seca (CARTAXO et al., 2010; PINHEIRO et al., 2013).

A Caatinga não é um Bioma que serve de alojamento para apenas espécies animais e vegetais, servindo também de abrigo para famílias. Aproximadamente 27 milhões de pessoas vivem em regiões de Caatinga, e a maior parte dessas pessoas, são pessoas carente e dependente dos recursos do Bioma para sobrevivência. Essa mata seca tem um imenso potencial para a conservação de serviços ambientais, uso sustentável e bioprospecção. Condições estas que, se bem dirigidas e exploradas de forma conservativa, poderão contribuir para o desenvolvimento da região e do país. Já é sabido que, essas regiões (Ministério do Meio Ambiente, 2016).

A Caatinga dá suporte para vários tipos de atividades econômicas voltadas para fins agrosilvopastoris (Sistema integrativo de lavouras, com espécies florestais e pastagens) e industriais, especialmente nos ramos farmacêutico, de cosméticos, químico e de alimentos. Entretanto, o Bioma de importância inestimada, tem sido devastado de forma acelerada. Uma das principais causas do rápido desmatamento pode ser atribuída ao consumo de lenha nativa de forma ilegal e insustentável, para fins domésticos e indústrias, ao pastoreio e a conversão da vegetação nativa para pastagens e agricultura (ALMEIDA ET AL, 2010; MINISTÉRIO DO MEIO AMBIENTE, 2016).

Na tentativa de cessar esse desmatamento que já atinge 46% da área do Bioma, conforme informações fornecidas pelo Ministério do Meio Ambiente (MMA), o governo luta pela criação de mais unidades de conservação federais e estaduais e promove alternativas para o uso sustentável da sua biodiversidade. Parcerias também são seladas entre o governo e os estados, para garantir a integridade e preservação, além da promoção de ações para a utilização sustentável de espécies nativas, manejo florestal e eficiência energética nas indústrias (Ministério do Meio Ambiente, 2016). Mesmo com todas essas parcerias, a Caatinga continua sendo um dos Biomas menos protegidos do país (Ministério do Meio Ambiente, 20016).

Diante desta vasta biodiversidade, inúmeras espécies têm uma representatividade de valor inestimado, outras dessas espécies são comercializadas em feiras livres como fitoterápicos, mas algumas poucas destas têm propriedades medicinais conhecidas (ALBUQUERQUE et al., 2007; CARTAXO et al., 2010).

Dentre todas as classes e espécies de vegetais já conhecidas podemos destacar a notável contribuição das plantas superiores (CALIXTO, 2003). São cerca de 60 mil espécies de plantas

superiores, onde somente 8% já foram alvo de pesquisa quanto as ações de seus produtos e pouco mais de 1000 espécies tiveram suas propriedades medicinais avaliadas. O Programa das Nações Unidas para o Desenvolvimento (2010) estima que no Bioma Caatinga, possam existir mais de 1500 exemplares de plantas superiores (entre Angiospermas e Gimnospermas) (BRASIL, 2006).

As plantas superiores desempenham um papel fundamental para o desenvolvimento de novas drogas, embora só se tenha 10% da biodiversidade mundial estudada. Aproximadamente 140 mil metabólitos intermediários são derivados em sua maioria, de plantas superiores (CALIXTO, 2003). Esse grupo vegetal, que é composto por 55.000 espécies (SEVERIANO et al., 2010; DUARTE et al., 2004), que apresentam uma heterogeneidade de tipos estruturais como, por exemplo, os flavonóides, os terpenóides e os alcalóides, os quais podem exercer atividades biológicas (GOTTLIEB; BORIN, 2012; DUARTE et al., 2004).

Estima- se que 520 novos fármacos aprovados pela Food and Drug Administration (FDA), aproximadamente 39% tenha partido de recursos naturais. Demonstrando um pouco mais da importância desses derivados, 33% dos medicamentos mais prescritos e vendidos no mundo são de origem vegetal. Este percentual atinge a margem de 70% quando essas drogas são direcionadas para o combate contra o câncer e infecções (CALIXTO, 2003).

2.2 Caatinga e plantas medicinais

A maior responsável pela produção das substâncias orgânicas é a natureza e o reino dos vegetais sendo o produtor da maior parte desta fração. Seja oriundo de qual quer fonte, os produtos de origem natural, podem servir como base para estudos de novos padrões de moléculas benfeitoras para a descoberta de novos fármacos (VIEGAS-JUNIOR; BOLZANI; BARREIRO, 2006).

Apresentando- se como um dos mais importantes Biomas do Brasil, a Caatinga, tem se tornado alvo de grandes pesquisas. É neste lugar onde muitas pesquisas são iniciadas. A fauna e flora que compõem esta reserva instiga muita curiosidade, em especial as plantas. Esta constatação pode ser confirmada pelo grande número de estudos já existente na literatura científica, que correlacionam a utilização dessas plantas como terápicos e a elas tem sido

reportada muitas ações: anticolinesterásica, gastroprotetora, analgésica, anti-inflamatória, anticoagulante, ansiolítica, antiplasmodica, antitumoral, antidiarreica, atividade inseticida, entre outras numerosos potenciais (CARVALHO et al., 1996; DINIZ, 2013; PEREIRA et al., 2012; TREVISAN; MACEDO, 2003).

Trevisan e Macedo (2003) em seus estudos, puderam comprovar que ao utilizar a casca de *Amburana cearenses*, conhecida por umburana, e extraindo da casca os princípios ativos com etanol, aplicando a uma atividade de inibição enzimática por teste de microplaca, poderiam obter excelentes resultados. Sendo assim, o extrato da umburana é um potente inibidor de acetilcolinesterase. Agentes que atuem neste sentido são bastante utilizados para o tratamento de pacientes portadores da doença de Alzheimer (DA).

A indução de úlcera crônica por ácido acético, em roedores, é uma metodologia utilizada a fim de se verificar a ação gastroprotetora dos fármacos. E foi através deste método que Pereira et al (2012) identificaram que *Handroanthus impetiginosus* (ipê roxo), quando utilizou- se extrato hidroalcoólico da casca do caule para tratar animais induzidos por ácido acético, obteve resultados significativos quanto a proteção da mucosa gástrica e ainda pode sugerir que o mecanismo de ação esteja envolvido com a manutenção da integridade da mucosa e estimulo de proliferação celular.

O extrato aquoso de *Libidibia ferrea* (pau-ferro), foi testado quanto sua ação analgésica e anti-inflamatória. Nos modelos testes, o extrato apresentou resultados significantes quando comparados ao controle negativo tanto para os modelos de edema de pata, contorção abdominal e placa quente, entre tanto os melhores resultados foram obtidos para os modelos que avaliaram a ação analgésica do extrato (CARVALHO et al., 1996).

Em 2011, Araújo e colaboradores, conseguiram isolar uma lectina da casca de trapiá (*Crataeva tapia*), a qual foi testada com relação a sua atividade anticoagulante. Existe indicativo de que a lectina isolada tem ação direta nos fatores VIII, IX, X e XI da coagulação. Este mesmo estudo ainda identificou que este composto isolado da trapiá desempenha dupla ação: inibição e anticoagulação.

Testes que avaliam a utilização de plantas da Caatinga, com ação sobre o sistema nervo central (SNC) também foram testadas. O extrato etanólico de folhas de *Annona vepretorum*, mais popularmente conhecida como " pinha da Caatinga", foi empregado para avaliar a atuação sedativa do SNC em camundongos. Segundo resultados obtidos pelo estudo, a pinha da Caatinga apresenta ação sedativa, sem afeta a coordenação motora dos indivíduos testados (DINIZ, 2013).

Quando testado frente a *Plasmodium falciparum*, os compostos extraídos do caule de *Hymenaea coubaril* (jatobá) demonstrou ter atividade. Segundo Köhler et al, 2002, uma concentração de aproximadamente 11 µg/mL, foi a dose encontrada e necessária para induzir a inibição do crescimento desta população em 50%.

Peixoto (2011), conseguiu identificar e comprovar que algumas espécies de *Cnidoscolus*, entre elas: *C. infestus e C. quercifolius,* desempenharam potencial citotóxico e apresentaram a capacidade de inibir o crescimento celular. Extratos produzidos com a utilização de partes aéreas e raiz de *C. insfestus*, apresentaram atividade antiproliferativa contra as linhagens Hep-2 e NCI- H292, numa concentração de 50µg/ mL. Quanto ao extrato da casca de *C. quercifolius*, demonstrou-se ativo frente as linhagens celulares HT-29 e Hep-2, também na mesma concentração. Ainda segundo Peixoto, estas espécies são amplamente utilizadas popularmente para o tratamento de processos inflamatórios e tumorais.

Segundo Beserra, 2014 extratos de *Cissus sicyoides*, já conhecida pela sua ação hipoglicemiante, também chamada de insulina vegetal, foi capaz de inibir a diarreia provocada por óleo de rícino, tanto no que refere- se a frequência de evacuações quanto ao percentual de fezes liquefeitas, quando realizado o pré-tratamento com doses iguais a 250 e 500 mg/Kg. Na visão do autor, o extrato pode apresentar a ação antidiarreica, estando envolvido com a indução de uma alteração na motilidade intestinal, em consequência, inibição do trânsito e aumento da absorção de água, contribuindo para menor secreção do TGI.

Extratos da *Myracrodruon urundeuva* (Anacardiaceae), *Ziziphus joazeiro* (Rhamnaceae) e *Croton blanchetianus* (Euphorbiaceae), foram testados quanto sua atividade de repelência contra *Tetranychus bastosi*. Ao fim do experimento, verificou- se que os extratos

das folhas das três espécies em teste, desempenharam além da atividade repelente, apresentaram também um índice de mortalidade significativa (XAVIER et al., 2015).

Durante muito tempo as plantas medicinais foram negligenciadas e só na década de 80 esse cenário começou a mudar. Na atualidade, como pode ser comprovado pelos estudos realizados por Xavier et al. (2015) são constantes as descobertas e as publicações na literatura científica, sobre plantas como produto terapêutico. A importância da medicina alternativa, baseada na utilização de plantas, tradicionalmente, assume que esses vegetais devem ser empregados para finalidades curativas, ao ser utilizado qualquer parte do vegetal (FONTE, 2004).

2.3 Família Euphorbiaceae

A família Euphorbiaceae é composta por 5 subfamílias, 49 tribos, 300 gêneros e 8000 espécies. Entre as famílias de plantas com flores, é uma das maiores, de alta complexidade e diversificada. (SECCO et al., 2012; WMINE; DAMME, 2011). Essa família está entre as mais importantes por apresentar valor econômico histórico, como por exemplo, o gênero *Hevea brasiliensis* (seringueira), o qual economicamente fez parte do clico da borracha, que aconteceu no Brasil, por volta do século XX e a *Manihot esculenta* (macaxeira) que está entre os mais importantes alimentos (SECCO et al., 2012). E mais recentemente a *Jatropha curcas* (pinhão-manso), matéria prima utilizada para a produção do biodiesel (GARG; KHATRI; GANDHI, 2011).

No Brasil, a família é representada por mais de 1000 espécies, dentro de 80 gêneros (SÁTIRO; ROQUE, 2008). No Nordeste a família é registrada com uma das mais importantes do semiárido e se distribui em 211 espécies e 45 gêneros (LUCENA; ALVES, 2010). A Caatinga possui 17 espécies endêmicas como representante das Euphorbias (SÁTIRO; ROQUE, 2008).

As Euphorbiaceae exibem importância terapêutica e características peculiares que atribuem a esta família uma excelente atividade medicinal. Por apresentarem ampla distribuição em todas as regiões tropicais de todos os continentes, a família apresenta características de sobrevida excepcional, como adaptações climáticas, altas cargas de mutações,

além dos estímulos ambientais. A disposição para sofrer adaptações, confere a esta família a capacidade de produzir diversos metabólitos secundários (WMINE; DAMME, 2011). Compostos tais como fenóis, ésteres, ricina, flavonoides, saponinas, terpenos, alcalóides e taninos foram encontrados em espécies desta família e relatados por Kiem et al. (2009); Duarte; Lage; Ferreira (2008); Gressler et al. (2008); Goel et al. (2007); Liu et al. (2007); Yang et al. (2007) e Rossi et al. (2003) respectivamente.

Os achados de inúmeros pesquisadores em nível de moléculas derivadas do metabolismo das Euphorbiaceaes demonstram a alta eficiência desses derivados, ao desempenhar função medicinal, já que esses metabólitos são produzidos pelas plantas para desempenharem papel protetor contra as adversidades encontradas no ambiente em que vivem. (WMINE; DAMME, 2011). Assinalando assim, um bom ponto de partida para uma pesquisa de novos fitoterápicos os quais possam ser utilizados na medicina humana e veterinária ou ainda na agricultura contra pragas.

Muitas espécies de Euphorbiaceae são alvos de muitos estudos científicos e são designadas para numerosas finalidades como alimentação, ornamentação, predecessor de compostos químicos, lubrificantes, resinas, além das utilidades medicinais. O látex que essas espécies produzem apresentam grandes concentrações de diterpenos de caráter complexo, estes apresentam atividades biológicas diversas como citotóxica, anti-inflamatória, analgésica, antiespasmódica, antitumoral, etc. Outras substâncias bem características desta família são os esteróides, lectinas, as hemaglutininas e compostos cianogênicos (FELIU, 2011).

Trindade e Lameira (2014), puderam comprovar que a maioria das espécies da Euphorbiaceae, que foram citadas por populares em estudos etnobotânicos, das 149 espécies, a grande parte foram indicadas com potencial medicinal. Sabe- se que a utilização dessa família é descrita por civilizações orientais e ocidentais. A tabela 4 abaixo destaca algumas das 149 espécies citadas por Trindade e Lameira e suas respectivas indicações de utilização.

Tabela 4: Lista de espécies da família Euphorbiaceae que foram citadas em estudo etnobotânicos e suas respectivas aplicações.

Nome Científico	**Nome comum**	**Utilização/ propriedade**
*Acalypha accedens*Mull.Arg.	Acalifa	Medicinal, ornamental
Acalypha amblyodonta(Müll. Arg.) Müll. Arg.	Pêlo-vermelho	Medicinal
*Acalypha arvensis*Poepp.	Rabo-de-macaco	Medicinal, ornamental
*Alchornea discolor*Poepp.	Supiarana	Madeira (embalagem), ornamentação
Alchornea sidifolia Müll. Arg.	Urucurana	Reflorestamento, madeira (tabuás)
Alchornea triplinervia(Spreng.) Müll.Arg.	Tapiá	Reflorestamento, madeira (tabuás), medicinal
Aleurites moluccanus(L.) Willd.	Nogueira-da Índia,	Ornamental
Aparisthmium cordatum(A.Juss.) Baill	Marmeleiro	Medicinal, paisagismo
Bernardia pulchella(Baill.) Müll. Arg	Canela-de-virá	Medicinal
Caryodendron amazonicum Ducke	Mamaluco	Medicinal
Caryodendron orinocense H. Karst.	Tacai	Alimento, perfumaria, madeira, medicinal, fertilizante
Cnidoscolus phyllacanthus (Müll. Arg.) Pax & L. Hoffm.	Favela, faveleiro, quimadeira	Alimento gado, medicinal, forrageira
Cnidoscolus quercifolius Pohl	Favela-de-cachorro	Medicinal
Cnidoscolus urens (L.) Arthur	Cansanção	Medicinal
Croton heliotropiifolius KUNTE	Velame	Medicinal e reflorestamento
Croton hirtus L'Hér.	Croton	Medicinal
*Euphorbia heterophylla*L.	Erva-de-leite	Medicinal
Jatropha curcas L.	Pião-branco	Ornamental
Jatropha gossypiifolia L	Pinhao-roxo	Medicinal, cosmético, alimento animal, combustível, ornamental
Manihot esculenta Crantz	Mandioca	Alimento
Ricinus communis L.	Mamona	Codimento, óleo, biocombustível, medicinal
Sebastiania jacobinensis(Müll. Arg.) Müll. Arg.	Leiteiro	Combustível

Fonte: TRINDADE; LAMARIA, 2014.

A família Euphorbiaceae tem um amplo espectro na sua utilização na medicina alternativa, bem como o gênero que também está compreendido nesta família, *Cnidoscolus*, gênero este que é bastante citado em estudos etnofarmacológicos, em contrapartida, pouca são as comprovações e estudos científicos que revelem suas propriedades biológicas.

2.3.1 O Gênero *Cnidoscolus* Phol

Este gênero proposto por Pohl (MELO; SALES, 2008), apresenta uma distribuição por todo o território brasileiro no Norte (Pará, Tocantins), Nordeste (Maranhão, Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Bahia, Alagoas, Sergipe), Centro-Oeste (Mato Grosso, Goiás, Distrito Federal, Mato Grosso do Sul), Sudeste (Minas Gerais, Espírito Santo, São Paulo, Rio de Janeiro) e no Sul (Paraná) (Figura 1) (CORDEIRO; SECCO, 2012).

Cnidoscolus, do grego: knide = urtiga, skolos = ponta, são popularmente conhecidos como "urtiga", uma das características peculiares deste gênero é a presença de tricomas urticantes, os quais provocam irritações severas, na área de contato, severas. Seus representantes são encontrados principalmente na América Tropical, e praticamente predominantes no México e no Nordeste Brasileiro. Para este gênero são relatados cerca de 50 a 75 espécies, em toda América tropical (PEIXOTO SOBRINHO et al., 2012).

No estado de Pernambuco o gênero *Cnidoscolus* é representado por oito espécies, segundo Melo; Sales (2008), *C. bahianus* (Ule) Pax & K. Hoffm., *C. loefgrenii* (Pax & K. Hoffm.) Pax & K. Hoffm., *C. obtusifolius* Pohl, *C. oligandrus* (Müll. Arg.) Pax, *C. quercifolius* Pohl, *C. urens* (L.) Arthur, *C. urnigerus* (Pax) Pax e *C. vitifolius* (Mill.) Pohl. *C. urens* ocorre praticamente em todos os Biomas. *C. leofgrenii* pode ser encontrado na Zona da Mata e Caatinga, *C. quercifolius, C. obtusifolius, C. vitifolius, C. bahianus e C. urnigerus*, com resistro na Caatinga e *C. oligandrus* presente somente na Zona da Mata (MELO; SALES, 2008). No entanto, Peixoto Sobrinho et al. (2011), traz a informação que na Caatinga, o gênero é representado por quatro espéces que se destacam por apresentar uma imensurável diversificação quanto a sua utilização, entre elas, *C. infestus* Pax & K. Hoffm., *C. pubescens* Pohl, *C. quercifolius* Pohl e *C. urens* (L). Arthur.

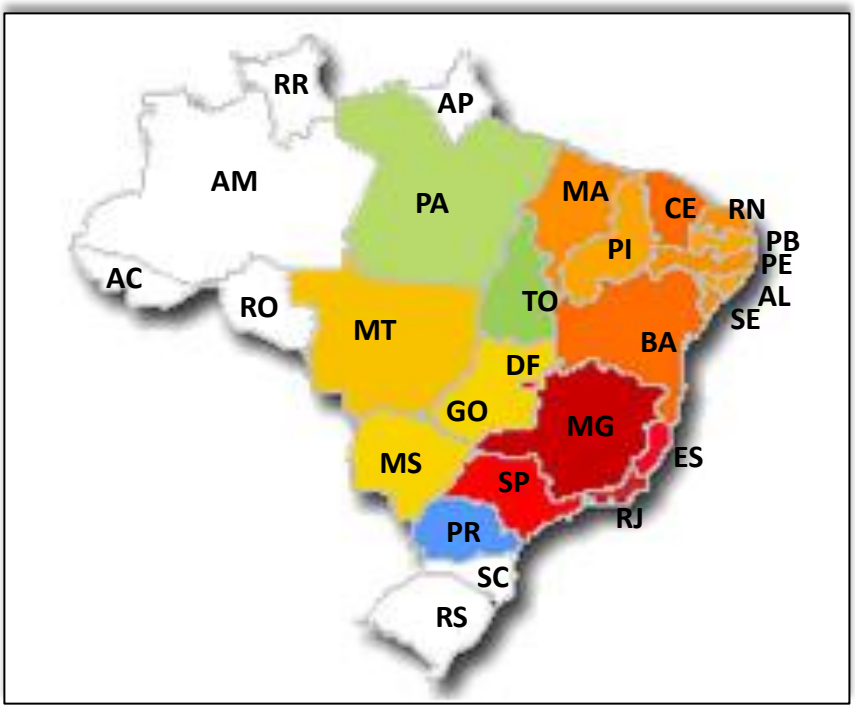

Figura 1. Distribuição geográfica do gênero *Cnidoscolus sp* no Brasil. Áreas coloridas nas cinco regiões (Norte, Nordeste, Centro- Oeste, Sudeste e Sul) do Brasil demonstram a presença do gênero.

Fonte: CORDEIRO; SECCO, 2012. *Cnidoscolus in* Lista de Espécies da flora do Brasil. Disponível em: http://floradobrasil.jbrj.gov.br/2012/index?mode=dp&tid=17491.

Estudos sobre o perfil fitoquímico, a partir de extratos metanólicos, deste gênero revelaram a presença de cumarinas, flavonóides, taninos, fenóis e terpenóides como metabólitos secundários (PEIXOTO SOBRINHO et al., 2012). Várias são as doenças em que os *Cnidoscolus* são indicados através dos conhecimentos populares. Essas indicações incluem atividades anti-inflamatória (ALBUQUERQUE, 2006), antitumoral, para tratamento do sistema genito-urinário, como anti-séptico e no tratamento de infecções renais, lesões dermatológicas e oftálmicas, contusões, fraturas, ferimentos, verrugas, disenteria, hemorragia, apendicite, reumatismo (PEIXOTO SOBRINHO et al., 2012; ALMEIDA et al., 2005) e contra micoses (ALBUQUERQUE et al., 2007). Além da empregabilidade que o gênero apresenta na medicina caseira, este ainda pode ser utilizado como forrageiras, oleífera, laticífero e ornamental (MELO; SALES, 2008).

Por ser utilizada na medicina popular, de uso comum no Nordeste e em outras regiões do mundo e sempre citada em levantamentos etnobotânicos, a espécie *C. urens* se destaca dentro do gênero.

2.3.2 Espécie *Cnidoscolus urens* (L.) Arthur

Largamente distribuída em todo território brasileiro, chamada popularmente por cansanção, arrediabo, cansanção de leite, pinha queimadeira, urtiga de mamão, urtiga cansanção ou simplesmente urtiga, o *C. urens* é uma planta invasora (SÁTIRO; ROQUE, 2008).

Esta espécie pode variar de subarbustos a arbustos, medindo entre 0,5- 2,5 m de altura (SÁTIRO; ROQUE, 2008). É uma espécie nativa do Brasil e apresenta distribuição geográfica nas regiões do Nordeste (Piauí, Rio Grande do Norte, Paraíba, Pernambuco, Bahia, Ceará, Alagoas, Sergipe), Centro-Oeste (Mato Grosso, Goiás, Distrito Federal), Sudeste (Minas Gerais, Espírito Santo, São Paulo, Rio de Janeiro) e Sul (Paraná) (**Figura 2**) (CORDEIRO; SECCO, 2012).

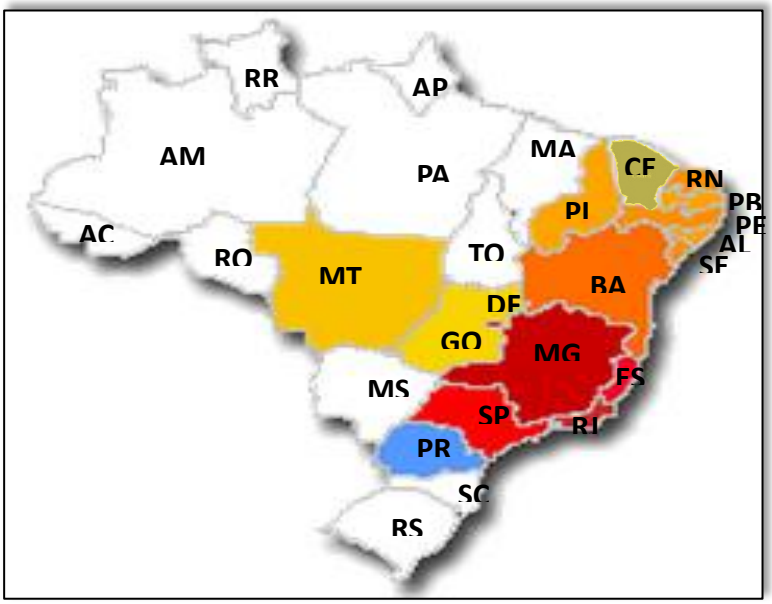

Figura 2: Distribuição geográfica da espécie de *C. urens* no Brasil. Áreas coloridas nas quatro regiões (Nordeste, Centro- Oeste, Sudeste e Sul) do Brasil demonstram a presença da espécie.

Fonte: Cordeiro; Secco, 2012. *Cnidoscolus* in Lista de Espécies da Flora do Brasil. Disponível em: http://floradobrasil.jbrj.gov.br/2012/index?mode=dp&tid=17495.

A espécie é bastante conhecida pela presença de pêlos urticantes chamados de tricomas urticantes, que são quebradiços e quando o tricoma é quebrado ocorre uma liberação instantânea de cristais de oxalato de cálcio os quais provocam irritações quando em contato com a pele (MALHEIROS, 2012).

O *C. urens* tem como característica que auxilia no seu reconhecimento, numerosas glândulas papiliformes na junção do pecíolo com a lâmina, perianto das flores pistiladas com segmentos livres ou raramente unidos (MELO; SALES, 2008). As folhas da espécie são membranáceas, lâmina foliar lobada, ovada, com lobos centrais e laterais, da ápice agudo e margens serradas, estípulas triangulares. A inflorescência com 16 dicásios, brácteas, ápice agudo e bubescente. Suas flores estaminadas e pistiladas, sésseis, cálice tubular-hipocrateriforme, lobos elípticos a oblongos, glabros, não urticantes, com disco nectarífero proeminente, estames em 10 e desprovidas de odor (ARAÚJO; LEAL; QUIRINO, 2012; SÁTIRO; ROQUE, 2008). Quanto às sementes, são ditas oblongas, do tipo cápsula que se abrem para dispersão das sementes odor (ARAÚJO; LEAL; QUIRINO, 2012) **(Figura 3)**.

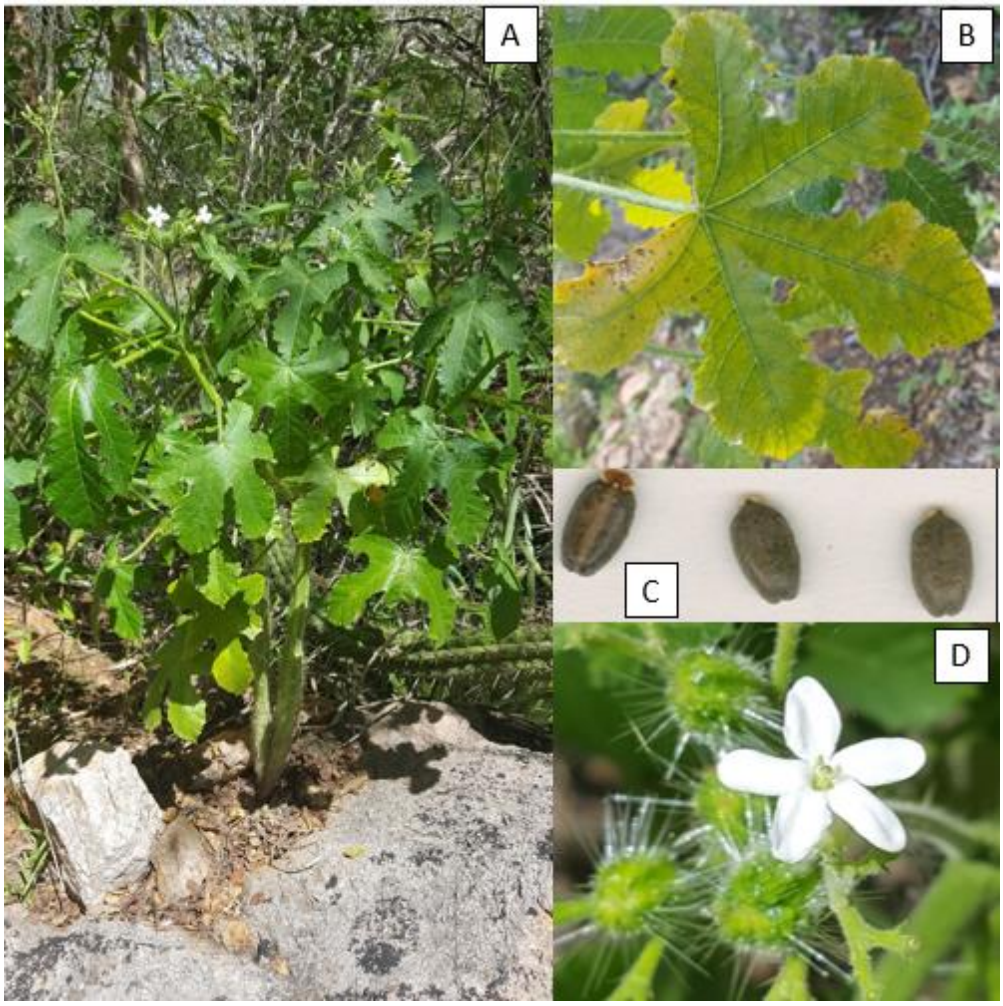

Figura 3: Aspectos gerais de partes de *Cnidoscolus urens.* (A) Planta inteira, (B). Folha, (C) Semente, (D) Flor.

Fonte: SOUZA, P.G.V.D., 2016.

A ocorrência de *C. urens* se dá durante todo o ano e as maiores florações juntamente com a frutificação ocorrem durante os meses de abril e julho, mas durante os outros meses podem-se encontrar arbustos floridos, essa constante floração atribui a espécie importantes recursos alimentares. Assim sendo, espécies com este tipo de característica de floração, atribuem importantes fontes alimentares, já que ofertam estes recursos durante todo o ano (ARAÚJO; LEAL; QUIRINO, 2012).

Estudos prévios de Peixoto Sobrinho et al. (2012) identificaram a presença de antocianinas, derivados de antraquinonas, antraquinonas, cumarinas, flavonóides, taninos, triterpenos, esteróides, mono e diterpenos, xantinas pela extração com metanol de partes aéreas

de *C. urens* e na extração da raiz, pelo mesmo processo foram identificados derivados de antraquinonas, cumarinas, lignanas, mono, di e triterpenos, saponinas e esteróides.

Esta espécie nativa revela-se de grande acuidade, sendo a mais relatada em estudos etnofarmacológicos, onde populares utilizam suas raízes e cascas do caule para tratamento das mais diversas doenças, pois acreditam que esta espécie tem um potencial terapêutico contra canceres, processos inflamatórios variados, dores generalizadas, problemas renais, disenteria, hemorragias, apendicite e reumatismo (AGRA et al., 2007b; ALBUQUERQUE, 2006; ALBUQUERQUE et al., 2007). Porém, até o momento não foram encontrados resistros de sua utilização na agricultura, com ação inseticida.

Os conhecimentos populares são estímulos que incentivam a ampliação dos conhecimentos sobre as ações biológicas e mecanismos, apontando o potencial terapêutico que *C. urens* desempenha. Assim faz- se importante à realização de novos estudos não só sobre a espécie, mas também sobre a família, pois sua presença é notada em estudos etnobotânicos realizado por todo o Nordeste.

2.4 Atividade Biológicas

2.4.1 Atividade Antitumoral

Por haver muitas referências à neoplasia, na literatura médica da Grécia Antiga, a palavra "neoplasia", significa novo crescimento, Hipócrates criou os termos *Karkinos* e *Karkinõma*, derivados de *Karkinos* que denotam úlceras não cicatrizadas e tumores malignos sólidos, respectivamente. (PASCOAL et al., 2010; CERDEIRA FILHO, 2008). Outros relatos existentes, também da Grécia Antiga entre 138 e 201 d.C. aproximadamente, Galeno, médico de origem grega, tenha feito analogia entre o tumor e o caranguejo. (PASCOAL et al., 2010). A palavras neoplasia é a definição científica empregada para referendar o "câncer". (ALMEIDA et al., 2005; CERDEIRA FILHO, 2008). A expressão câncer surgiu um pouco mais tarde, advinda do latim da palavra *cancrum* que quer dizer "caranguejo", essa designação foi atribuída ao câncer por apresentar-se com veias intumescidas e profundas, sendo comparadas as patas de um crustáceo quando firmasse na areia prendendo-se e dificultando sua remoção. (MINISTÉRIO DA SAÚDE, 1971; ALMEIDA et al., 2005).

Entretanto, podemos definir a palavra neoplasia como sendo, o crescimento descontrolado de uma nova massa celular/ tecido onde sua origem pode ser benigna ou maligna, já o câncer está relacionado ao crescimento desordenado de uma massa celular/ tecido de ascendência maligna (BOGLIOLO, 1998; MOURA et al., 2001).

Com a falta de conhecimento, sobre o comportamento desta doença, somente no final do século XVIII, o câncer iria começar a ser entendido, a cerca de como ele poderia progredir e destruir órgãos. Sua ação invasiva, agressiva e progressiva atribuiu ao câncer um protótipo estigmatizado segundo o qual, de fato, pessoa que estivesse acometida com tal doença, na certa teria uma morte precoce e seriam rejeitadas pelo seu grupo de convivência. Essa rejeição dar-se-ia devido à crença de caráter contagioso do câncer incentivada pela ciência, que perdurou até as duas primeiras décadas do século XX (CARVALHO, 2006).

O termo câncer/neoplasia tornou-se generalizado e agora é utilizado para identificar mais de uma centena de doenças. É definido e caracterizado por um histórico clínico-patológico de uma enfermidade multifatorial crônica, com perda de peso (cerca de 30% dos pacientes com câncer, apresentam perda de peso superior a 10%) e desnutrição, além do crescimento desordenado, proliferativo e difuso de células transformadas, as quais podem invadir tecidos e órgãos levando a formação de um novo tecido atípico: o tumor. Células de um tumor primário podem disseminar-se para diversas partes do corpo e diferentes órgãos levando ao surgimento de tumores secundários, assinalando-se como metástase. (MINISTÉRIO DA SAÚDE, 1971; ALMEIDA et al., 2005; GARÓFOLO et al., 2004; GARÓFOLO; PETRILLI, 2006; KOWATA et al., 2009).

O câncer ganha relevância pelo perfil epidemiológico que apresenta, até os dias atuais por ser considerada uma das doenças que mais causa mortes no mundo. Segundo a Organização Mundial de Saúde (OMS) em 2012 houve um total de 8,2 milhões de mortes por câncer e 14,1 milhões de novos casos de câncer. De acordo com essa estimativa o percentual de câncer continuará aumentando em países em desenvolvimento e também crescerá ainda mais em países já desenvolvidos. No Brasil, para o ano de 2014 e 2015 existiram aproximadamente 576 mil novos casos de câncer (INCA, 2014). Os tipos de câncer mais incidentes na população brasileira para o ano de 2014, são demonstrados na figura 4.

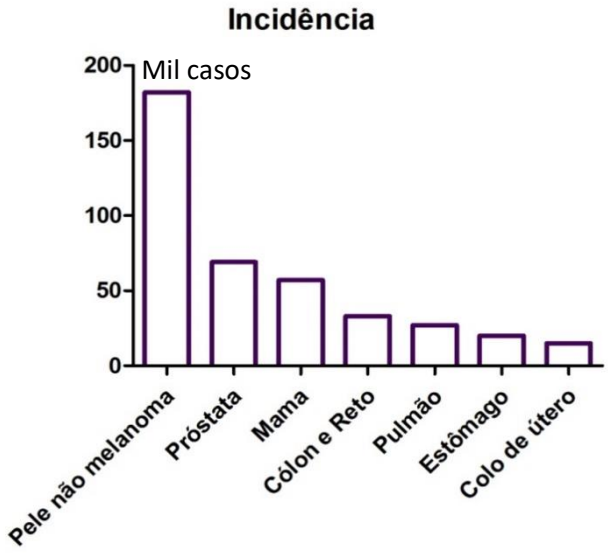

Figura 4: Incidência de diferentes tipos de câncer no Brasil.

Fonte: INCA (2014).

O processo complexo da formação e desenvolvimento das neoplasias, denominado carcinogênese, surge de uma série de alterações genéticas (alterações de genes ou cromossomo) e epigenéticas (expressão ou não de um determinado gene) (Figura 5) e ocorre em três etapas: iniciação, promoção e progressão. A iniciação é provocada por uma desregulação genômica, devido a um agente carcinogênico que incita modificações permanentes em alguns de seus genes, transformando a célula (BOGLIOLO; BRASILEIRO FILHO, 2006).

Esta, agora iniciada, se apresenta menos responsiva a fatores que interfira no crescimento celular ou aos indutores de diferenciação celular ou à apoptose (BOGLIOLO; BRASILEIRO FILHO, 2006). A etapa de promoção consiste na proliferação ou expansão das células devido a uma série de interações entre citocinas, fatores de crescimento e seus receptores. Para que o processo de transformação em célula maligna ocorra é necessário a exposição longa e continuada com o agente cancerígeno promotor.

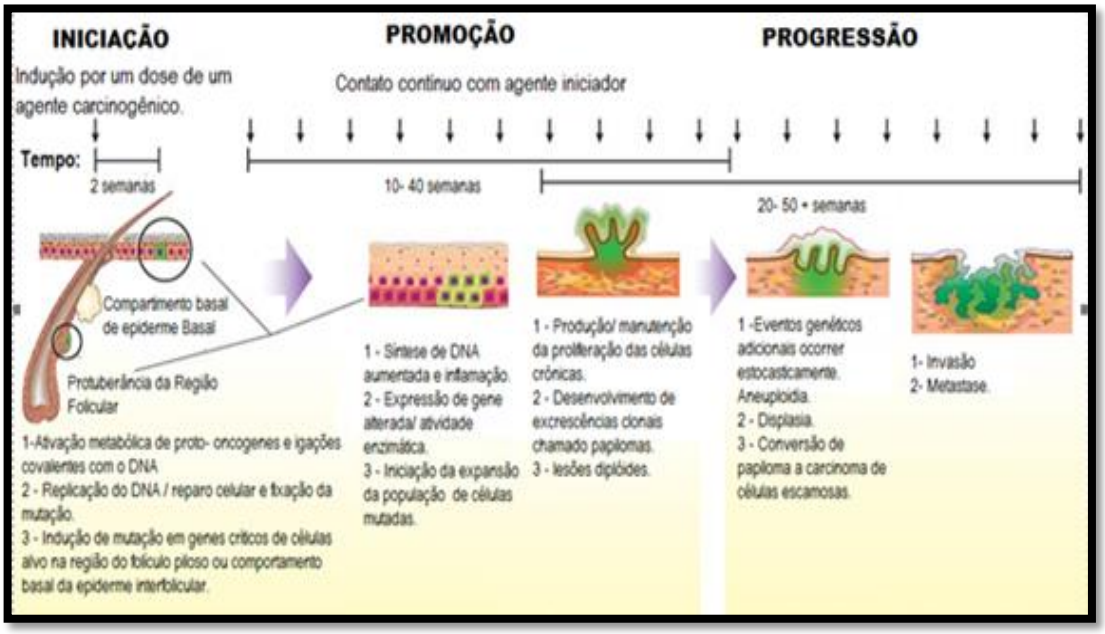

Figura 5: Etapas de instalação do câncer.

Fonte: ABEL et al, 2009.

Os promotores são substâncias que apresentam uma propriedade em comum, de provocar irritação dos tecidos desencadeando reações inflamatórias e proliferativas, como por exemplo o promotor mais conhecido, o 12-O-tetradecanoilforbol-13-acetato (TPA) (BOGLIOLO & BRASILEIRO FILHO, 2006).

Sendo complexo processo, a carcinogênese inicia-se e se estabelece por danos genéticos induzidos por agentes carcinogênicos como: a radiação, vírus ou agente químicos (Figura 6). Os danos ocorrem nos genes conhecidos como, proto- oncogenes, que são promotores do crescimento celular, e genes supressores de crescimento celular e ainda alterações em genes que controlam o processo de morte programada das células (Figura 7). Por fim, o tumor após seu surgimento sofre modificações biológicas que o tornam mais agressivo e maligno, este processo é designado progressão tumoral e é caracterizado pela multiplicação descontrolada irreversível (ONCOGUIA, 2014).

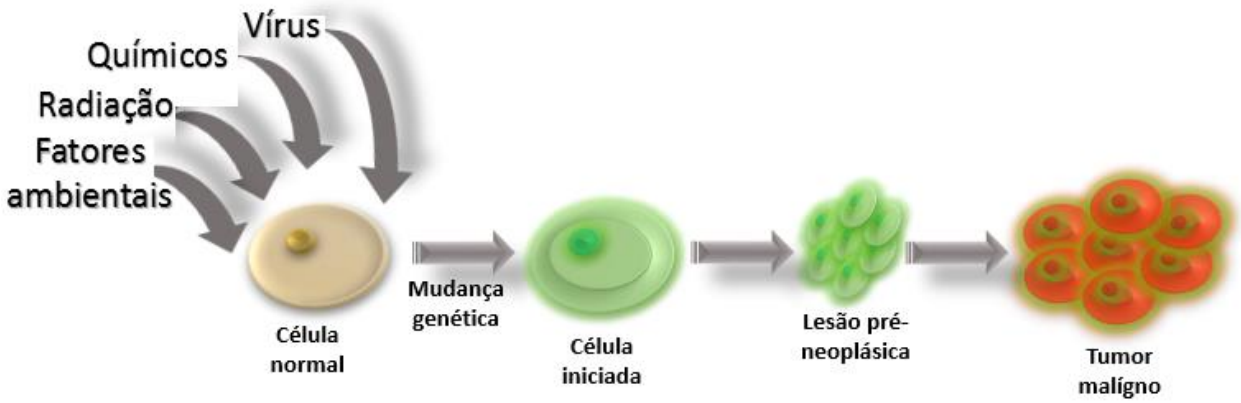

Figura 6: Desenvolvimento e modificações genéticas para o surgimento da carcinogênese.

Fonte: SOUZA, P. G. V. D., 2016.

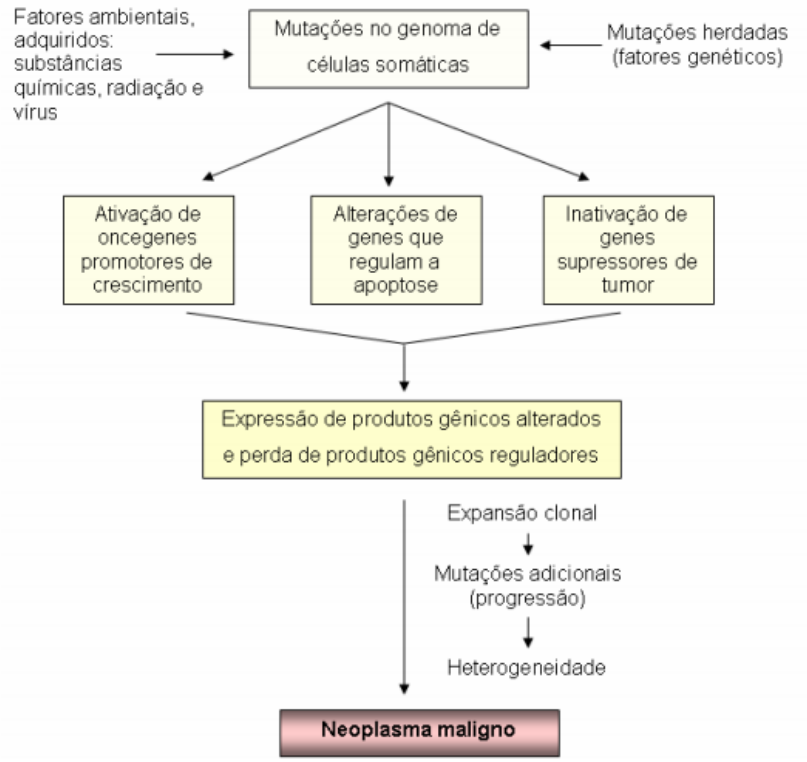

Figura 7: Esquema simplificado da patogênese do câncer. Demonstração dos genes que podem sofrer mutações e que estão envolvidos com o surgimento do câncer.

Fonte: GOMES, 2008.

As neoplasias podem ser divididas de acordo com vários critérios: comportamento clínico (benignas e malignas), aspectos microscópicos (histomorfológico) e pela origem do tumor (histogenético). As neoplasias benignas possuem suas células bem diferenciadas, discretas atipias celulares, baixo índice mitótico, com crescimento lento e expansivo,

delimitação e geralmente não reicidivam após cirurgia de remoção. Já as malignas apresentam alterações na expressão gênica com tendências à síntese de isoformas de enzimas predominantes na fase embrionária, rápida captação de aminoácidos, resistência a hipóxia, são indiferenciadas e provocam metástase (BRASILEIRO-FILHO et al., 2000). Têm como componentes básicos todas as neoplasias, o parênquima constituído de células tumorais e estroma, composto principalmente de vasos neoformados e células inflamatórias e de tecido conjuntivo (DVORAK,1986).

Inúmeras são as razões que levam a perca de função dos genes que regulam o ciclo celular, podendo ser por múltiplos fatores. A predisposição genética, fatores físicos, químicos e biológicos são exemplos. E os riscos envolvidos com a predisposição para o desenvolvimento de um câncer podem ser identificados por alterações moleculares. A carcinogênese se instala lentamente, levando anos, até que o primeiro sinal seja percebido, sendo mais comum em pessoas de idade avançada, devido ao acúmulo de mutações, danos oxidativos e diminuição da resposta imune (GOMES, 2008).

Existem hoje várias formas de tratamento para o câncer com maior eficácia, como esquemas terapêuticos associados a cirurgia, radioterapia e quimioterapia (RAINER et al, 2009). A remoção do tumor com intuito curativo pela cirurgia oncológica, realiza uma drenagem linfática dos linfonodos locais e do tecido normal em quantidades suficientes mantendo uma margem de segurança adequada (TEICH; FRANKS, 1990). A radioterapia é um método no qual aplica-se feixes de radiações ionizantes a um determinado tempo e volume de tecido que engloba o tumor com o intuito de destrui-lo, porém não age de forma específica e resulta também na lesão de células saudáveis. A quimioterapia emprega a utilização de medicamentos com o objetivo de destruir as células cancerosas bloqueando o seu desenvolvimento.

Um dos principais fármacos utilizados no tratamento de diversos tipos de câncer é o 5-Fluorouracil (5-FU) (Figura 8), é um análogo da uracila, contendo um átomo de flúor na posição 5 ao invés de um hidrogênio, amplamente usado a mais de 50 anos para tratar câncer de cólon, de mama, de cabeça e de pescoço. Ele age incorporando Fluoronucleotídeos ao DNA ou RNA, bem como inibindo a ação enzimática da Timidilato sintetase (responsável pela síntese de DNA) (LONGLEY; HARKIN; JOHNSTON, 2003).

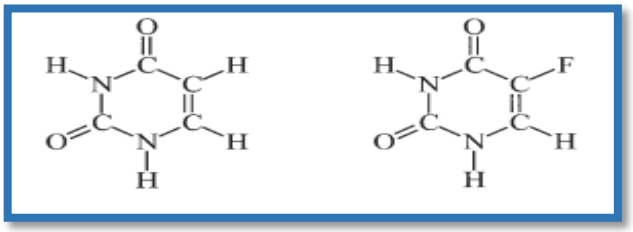

Figura 8: Estrutura química da uracila, à esquerda e do 5- fluorouracil, à direita.

Fonte: GREM, 2000.

Após sua administração, 80% do fármaco é catabolizado no fígado por ações enzimáticas, a massa biodisponivel é transportada para o interior da célula convertida em três metabólitos ativos: Fluorodesoxiuridina-monofasfato (FdUMP), trifosfato de fluorodesoxiuridina (FdUTP) e fluorouridina trifosfato (FUTP). Após a conversão dos metabólitos, sua ação pode ser direta pela incorporação causando danos celulares ou irá ainda ativar a via alternativa pela inibição da timidilato sintetase (Figura 9 e 10). (LONGLEY, HARKIN; JOHNSTON, 2003). Os diferentes medicamentos anticancerígenos agem por diversos mecanismos e podem ser derivados também de organismos marinhos, microrganismo e plantas.

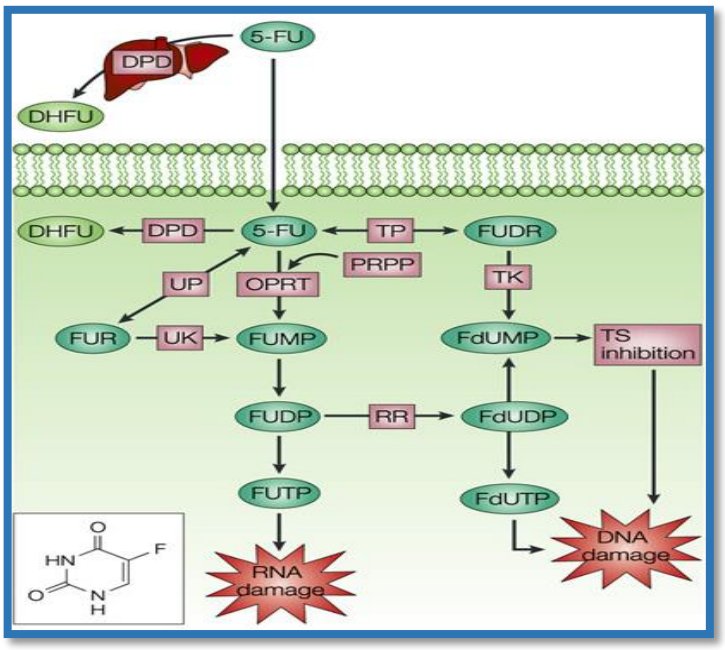

Figura 9: Esquema do mecanismo de ação do 5- Fluorouracil. Reação anabólica e catabólica do 5- FU.

Fonte: LONGLEY; HARKIN; JOHNSTON, 2003.

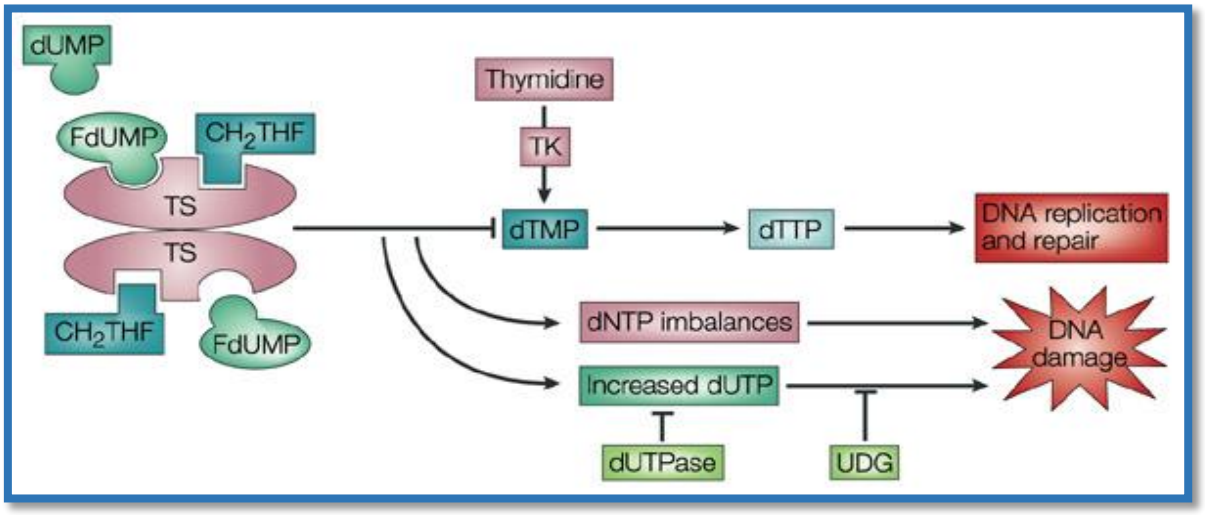

Figura 10: Via alternativa envolvendo a timidina sintetase, resultando em dano ao DNA.
Fonte: LONGLEY; HARKIN; JOHNSTON, 2003. 5-Fluorouracil: Mechanisms of Action and Clinical Strategies.

Os produtos naturais têm se destacado entre outros recursos, como sendo um dos mais utilizados na busca de novos componentes antineoplásicos. E segundo Lima (2006), das 250.000 espécies de plantas existentes em todo mundo, aproximadamente cerca de 1000 espécies diferentes de vegetais apresentam ação antitumoral.

E muitas espécies e compostos isolados dos vegetais já foram testados quanto a sua ação antitumoral: terpenos, alcalóides, cumarinas, lignanas, flavonóides, taninos, stilbenos, curcuminóides, polissacarídeos (PEI-WEN et al, 2005, GOMES, 2008). Segundo Gullo et al. (2006) das novas drogas utilizadas para o tratamento dos tumores, 60% desses novos tratamentos são de origem natural. Um exemplo são os alcaloides (vinblastina e vincristina) extraídos da *Catharanthus roseus* e paclitaxel, extraído de *Taxus brevifolia*.

2.4.2 Anatomia, Fisiologia, Motilidade, Secreção do trato grastrointestinal e a Diarreia

Em seu pleno funcionamento, por sua ação contrátil e secretora, o TGI tem sua função relacionada com a absorção, secreção e motilidade (SILVERTHORN, 2010; CHEN et al., 2010). Em sua fisiologia, o TGI é formado por 4 camadas (Figura 11): mucosa, submucosa, camada muscular circular e longitudinal e por último a camada serosa, sendo coordenadas por uma rede de nervos do sistema nervoso entérico (SNE) (SILVERTHORN, 2010). O estímulo da motilidade do intestino é iniciado em decorrência do esvaziamento do estômago, sendo

modulada por fatores nervosos e humorais, sendo a acetilcolina (ACh) o principal neurotransmissor (GUYTON; HALL, 2006).

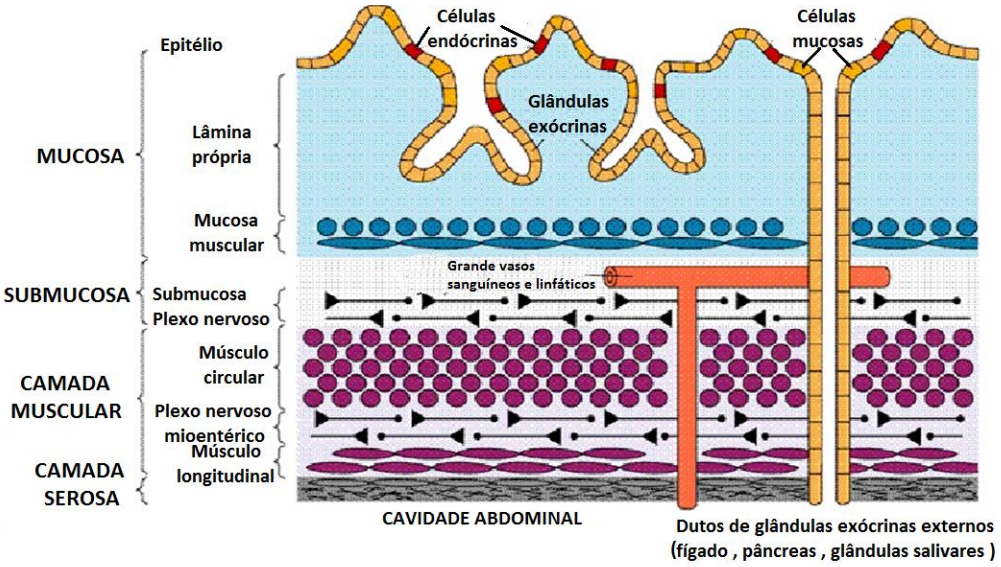

Figura 11: Lúmen do trato gastrointestinal. O trato gastrointestinal possui quatro camadas que auxiliam a desempenhar as funções durante o processo digestivo.
Fonte: <http://magicnumbers-parussolo.blogspot.com.br/2011/05/sistema-digestorio.html>, traduzido por Souza, P. G. V., 2016.

Atuando nos receptores muscarínicos (M3), a ACh liga-se a proteína G, provocando mudanças conformacionais e causando fosforiliação por uma molécula de GTP, a qual induzirá uma dissociação da proteína G. A proteína G que foi subdividida, liberará a subunidade alfa-GTP, e ativará a fosfolipase C- β1 (PLCβ1) e que atuará hidrolisando o fosfatidilinositol bifosfato (PIP2) em inositol trifosfato (IP3) e diacilglicerol (DAG). O IP3 se liga aos seus receptores que estão localizados no retículo sarcoplasmático das células e induz a liberação de Ca^{2+}, o qual irá atuar liberando mais Ca^{2+}. O aumento da concentração de Ca^{2+} intracelular mais o DAG ativa proteinocnase C (PKC) que contribuirá para a inativação dos canais de potássio (SILVERTHORN, 2010; BRUNTON et al., 2011).

A concentração de K^+ aumentada conduz a uma despolarização da membrana e abertura dos canais de cálcio dependentes de voltagem (Ca_v), levando ao influxo de cálcio na célula muscular lisa. O Ca^{2+} se liga a proteína ligadora de cálcio (CaM) ativando MLCK que por sua vez estimula a contração muscular (SILVERTHORN, 2010; BRUNTON et al., 2011).

Por ação da SERCA, que bombeia o Ca^{2+} do citosol para dentro do retículo, diminuindo a concentração de Ca^{2+}, assim como, a ativação da PMCA que empurraram sódio para o interior da célula e retira o cálcio, bem como a atividade da MLCP, ocorre o relaxamento do tecido musculares liso intersticial (SILVERTHORN, 2010; BRUNTON et al., 2011). Processo ritmado e executado por células chamadas de células de Cajal, localizadas na camada muscular (TACK, 2007).

Na luz intersticial, se dá o controle da secreção. Neste processo estão envolvidos o Ca^{2+}, Cl^-, e HCO_3^-, o controle da secreção desses íons é realizado por vários transportadores transmembranares e a secreção do Cl^- ocorre pela abertura dos canais iônicos, que é regulado pelo AMPc ou CMPc, por ativação de proteínas cinases, a qual estimula o bombeamento dos íons através dos canais. Assim, qualquer processo patológico que resulte no aumento de AMPc, promove abertura dos canais com secreção de Cl^-, Na^+ e H_2O para o lúmen intestinal (VANNUCCI; GUEDES, 2009; DONOWITZ et al., 2012).

Processos patológicos como, infecção bacteriana, toxinas, antibióticos, antiácidos contendo magnésio, produtos contendo lactose, análogos de prostaglandinas, colchicina, antineoplásicos, gastrina, serotonina, bem como inibidores, a noradrenalina, colecistocinina, NO, peptídio YY, prostaglandinas, dopamina e peptídeo intestinal vasoativo e agentes colinérgicos, provocam a diarreia (CHASSANY et al., 2000; KOEPPEN; STANTON, 2009; CHEN et al., 2010).

Estes agentes contribuem para o aumento de AMPc ou GMPc que estão envolvidos com a ativação de PKA, PKG e por sequencia fosforilação dos reguladores de bombeamento de íons transmembrana. Havendo abertura dos canais de reguladores transmembrana de fibose cística (CFTR), um aumento o bombeamento de íons (Cl^- e Na^+) e água para o lúmen intestinal, acontecerá, induzindo alterações na motilidade e secreção intestinal, causando a diarreia (VANNUCCI; GUEDES, 2009; DONOWITZ et al., 2012).

A primeira estimativa da grandeza do problema das doenças diarreicas agudas, foi realizado por Snyder e Merson, em 1982 (SNYDER; MERSON, 1982). Infecções gastrointestinais, tem como principal sintoma, a diarreia, que mata cerca de 1,5 milhões de crianças todos os anos. Ainda que existam tratamentos baratos e eficazes, o quantitativo de

casos e internações com doenças diarreicas ainda é muito alto e apenas 39% das crianças com diarreia, recebem tratamento adequado. Embora a maioria dos casos não apresentem evolução mais grave, alguns agentes etiológicos podem contribuir para evolução grave, podendo chegar até o óbito. Mundialmente, os números de óbitos por diarreia, podem ser atribuídos à má qualidade da água, inadequação de saneamento e falta de higiene, contribuindo para um percentual de 88% da totalidade (UNITED NATIONS CHILDREN'S FUND e WORLD HEALTH ORGANIZATION, 2009).

Uma das doenças mais comum em quase todos os países de clima tropical do mundo, apresentando fases de agravamento, a diarreia é uma das principais causas de mortalidade nos países em processo de desenvolvimento socioeconômico (SATISH; RANJANA, 2015). O maior índice de mortalidade pode ser encontrado entre crianças de até cinco anos de idade, esses resultados podem ser comprovados na figura 12**.**

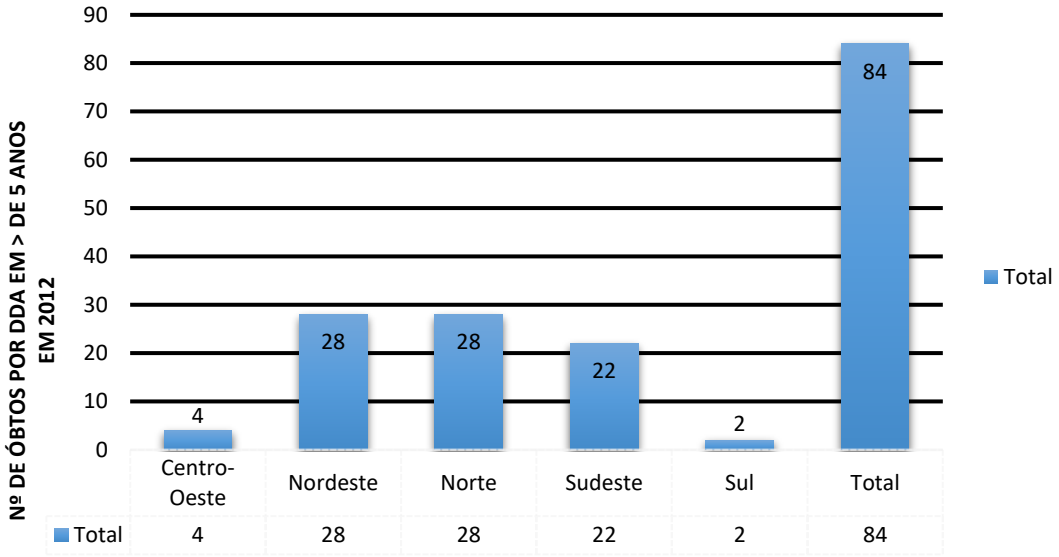

Figura 12: Proporção de óbitos por doenças diarreica aguda em > de 5 anos de idade.

Fonte: DATASUS (2012) modificado por Souza, P.G.V.D., 20016.

As afecções que levam a uma alteração intestinal, evoluindo para um quadro aumentado de números de evacuações, é uma das causas mais comum de morte nos países como a Nigéria e a segunda causa de morte entre crianças no mundo. Só na Europa, em 2001, foram constatados 1.793,000 casos de morte. A diarreia infecciosa é considerada uma doença fatal e grave, com o resultado de 4 a 6 milhões de óbitos/ano no mundo (BAMISAYE et al, 2013).

A diarreia pode ser desencadeada, na maioria das ocasiões por infecções gastrointestinais, podendo ser causadas por várias etiologias Tabela 5 (MINISTÉRIO DE SAÚDE, 2014).

Tabela 5: Tipos de agentes etiológicos desencadeadores de diarreia.

INFECCIOSA	**NÃO INFECCIOSA**
Bactérias e suas toxinas	Intolerância a lactose e glúten
	Ingestão de grandes quantidades de adoçantes
Vírus	Ingestão demasiada de alguns alimentos
	Sais mal absorvidos (Ex: laxantes e antiácidos)
Toxinas naturais	Ácidos biliares (após ressecção ileal)
	Gorduras não absorvidas
Parasitos	Algumas drogas (Ex.: catárticos, óleo de rícino, prostaglandinas)
	Hormônios peptídicos produzidos por tumores pancreáticos.

Fonte: Portal Saúde, MINISTÉRIO DA SAÚDE (2014), modificado por Souza, P. G. V. D., 2016.

A síndrome diarreica é caracterizada clinicamente, por uma alteração no volume, consistência e frequência, associada a uma liquidez das fezes. Podendo ainda estar acompanhada por intensas dores abdominais, febre e ainda vômito. Por tanto, caracterizando-se por uma quantidade anormal de fezes malformadas, aumento do número de evacuações, podendo durar até 14 dias. O comprometimento no equilíbrio do bom funcionamento do sistema digestivo, equilíbrio entre a reabsorção e secreção da mucosa intestinal, leva a um aumento da perca de água e de eletrólitos, pela liquefação, malformação das fezes e uma massa fecal superior a 200g/ dia (WGO, 2012; THIELMAN; GUERRANT, 2004).

Sendo chamados de antidiarreicos, esses fármacos atuam reduzindo os sinais da diarreia, melhorando a frequência, o volume e a consistência das fezes. Desta forma, os antidiarreicos exercem seus efeitos sobre a regulação da motilidade intestinal e da secreção de água e íons (BRUNTON et al., 2011). A terapêutica abordada para as doenças diarreicas deve ser iniciada pela hidratação oral, alimentação adequada e medicamentos que auxiliam na regulação da reabsorção de nutrientes, como a loperamida e o difenoxilato. A exemplo, a loperamida atua

diminuindo a motilidade e a secreção do fluido gastrointestinal (WANG et al., 2005; MARCOS; DUPONT, 2007). Fármacos com ação no retardamento do trânsito intestinal devem ser evitados em casos de infecções bacterianas, a fim de evitar a evolução da doença, com riscos de infecções sistêmicas (BRUNTON et al., 2011).

Os medicamentos utilizados para a terapêutica das DDA, são de classes diversas, desde antibióticos, opiáceos até antagonista de receptores muscarínicos. Entretanto, alguns desses fármacos, como a loperamida, podem desenvolver efeitos não desejados: constipação e distensão abdominal (RANG et al., 2011).

A Organização Mundial de Saúde, estimular a pesquisa com produtos naturais a serem utilizados como possível e potentes terapêuticos para diversas patologias, a fim de proporcionar tratamento eficaz, de baixo custo e com menor ou até mesmo nenhum efeito adverso. Assim, fica exposta a necessidade de novas pesquisas que possam comprovar os conhecimentos populares, tencionando a produção de novos fármacos com ação otimizada e mais acessível.

2.4.3 Controle de Insetos Praga

A guerra que o homem travou para habituar-se a natureza, faz parte da história da botânica e para tal adaptação o homem utiliza- se dos recursos naturais, em diversas áreas, como na medicina, na indústria e na agricultura. E desde da antiguidade a defesa contra pragas agrícolas, que se caracteriza por um surto de determinada espécie nociva ao desenvolvimento agrícola, é realizada com a utilização de plantas e mais recentemente, aplicação de substâncias sintetizadas, como os agrotóxicos (GALLUN, KHUSH, 1984; VIEGAS-JÚNIOR, 2003). A etimologia aplicada, remete desde os tempos mais remotos, estudos que retratam esta realidade além de apresentar a resistência de plantas a insetos (ORTMAN; PETERS, 1984)

A prática de controle de insetos é realizada pela aplicação de uma substância conhecida como inseticida, substâncias assim chamadas por ter ação letal, inibidora ou repelente contra essas pragas. Esses agentes são utilizados para controlar infestações de pragas que atacam tanto na zona rural e quanto na zona urbana (ADDOR, 1994) e começaram a ser utilizados após a Segunda Guerra Mundial e assim muitos desses compostos orgânicos passaram a ser sintetizados (LIU et al., 2011).

Organoclorados, organofosforados e carbamatos são inseticidas sintéticos e são bastante utilizados para o controle de inseto- pragas. A grande questão se dá pelo emprego desta prática em larga escala. O uso indiscriminado contribui para o aparecimento de populações de insetos resistentes, prejuízo ao meio ambiente, efeitos sobre outras populações que não são alvo, além de danos à saúde humana, em específico para aqueles que trabalham diretamente com esses produtos (BARROS et al., 2006; LEITE et al., 2007).

Inicialmente, dosado pela sua eficiência quanto ao controle de pragas em associação aos custos, eram considerados os melhores defensivos, mais posteriormente, a busca acontece de forma diferente, e a eficiência e economia, já não eram mais o padrão. Os interesses estavam em alternativas que não apresentassem prejuízo à saúde humana e ambiental. Recentemente, mais exigências, é preciso avaliar mais propriedades, tais como: mecanismo de ação, especificidades, degradação ao ambiente e segurança à saúde e ao ambiente (SALAZAR, 1997).

Com o interesse crescente pela agricultura, o homem desenvolveu maiores preocupações acerca das pragas com potencial em atacar suas plantações ou ainda causar qualquer outro tipo de dano e por isto passou a compreender melhor o que representa as pragas. As infestações por insetos, ameaça as culturas trazendo consigo ameaças devastadoras, como a fome e ameaçando também a saúde humana e ambiental. E isto tem levantado uma certa inquietação e têm sido acompanhadas pela criação de estratégias mais adequadas. Neste cenário atual, tem- se percebido a busca por novos métodos, que envolve a aplicação de biocidas (CASIDA; QUISTAD, 1998; BESTETE et al., 2011).

Fatalmente o emprego de inseticidas convencionais acarreta em desequilíbrio biológico, como por exemplo, a morte ou repelência de espécies polinizadoras (AZEVEDO, 1998; KNAAK et al., 2012). A alternativa seria a substituição de técnicas convencionais, por técnicas de origem biológica a qual, seria uma alternativa para diminuir tais danos (SALAZAR, 1997). E talvez por isso, o controle biológico de pragas, seja uma área promissora.

A matéria prima vegetal se constitui uma nova fonte e praticamente inesgotável para a pesquisa de novas moléculas biologicamente ativas e com potencial toxicológico a serem aplicadas ao manejo e controle de pragas (SIMÕES et al., 2004; GRZESIUK, CATELAN,

GEBARA, 2013). E o Brasil detêm uma das maiores biodiversidades em espécies (COSTA, 2001). Algumas dessas espécies já foram investigadas quanto ao seu mecanismo de ação, com relação a reguladores do crescimento ou inseticida.

Em 2003, Junior, verificou que isolados de *Croton cajucara*, apresentou potencial inibidor de crescimento em *Heliothis virescens* e neurotóxico em *Cylas formicarius*. O potencial inseticida do extrato etanólico do caule de *Croton linearifoliu* contra *Cochliomya macellaria* foi evidenciado por Cunha et al, em 2010. Segundo Grzesiu, Catelan e Gebara (2013), relataram que os extratos de folhas e galhos de *Croton florinbundus*, podem ser utilizados como isca para *Periplaneta americanas.* Vale destacar que todas essas espécies supracitadas fazem parte da mesma família, Euphorbiaceae, revelando sua importância como novas alternativas promissoras não apenas como inseticidas, mas também para outros fins.

Mesmo havendo potentes inseticidas, nos últimos anos o que se têm visto é a capacidade espantosa de resistência e adaptação dos insetos aos inseticidas sintéticos (MAIRESSE, 2005). Na atualidade, aparentemente existe um sucesso no controle biológico, pela utilização de técnicas de melhoramento genético para a resistência a pragas, através da engenharia genética, mas ainda assim, são técnicas caras e pouco exploradas (OLSON et al., 1996).

Não é por acaso que a utilização de inseticidas vem crescendo nas últimas décadas (TSUNECHIRO, FERREIRA, 2000), porém já se conhece os danos causados pela utilização extensiva dos agrotóxicos, como intoxicação e contaminação do meio ambiente, do aplicador e dos alimentos. Este contexto tem contribuído pela busca de novas alternativas, através de bioativos (BESTETE et al., 2011). Torna- se importante destacar que os produtos alternativos, apesar de apresentar menor toxicidade, podem afetar as plantações, por já existir alguns estudos que comprovem a ação na alteração no ciclo germinativo de algumas espécies. Partindo desta prerrogativa, informações sobre este tipo de interação ainda é bastante escasso, fazendo- se necessário estudo mais específicos que contribuam para a seleção de produtos mais adequados para serem aplicados como fitoinseticida (SMANIOTTO et al., 2013). E ainda apresenta vantagens como, alta especificidade em relação à praga alvo, sem causar danos a outros insetos, plantas e animais, sendo mais facilmente degradados que os sintéticos (KOUL; DHAWIWAL, 2001)

Apenas 1% do mercado mundial de inseticidas, é composto por fitoinseticidas. Essa realidade é atribuída a algumas barreiras encontradas. A escassez de recursos vegetais com atividade comprovada, o controle e padronização das formulações, são fatores que dificultam o registro e a comercialização destes produtos. Mesmo assim, é evidente que inseticidas vegetais é uma ferramenta vantajosa, inclusive no que se trata da obtenção de novos compostos bioativos contra novas pestes que possam surgir. Além disso, o interesse crescente por produtos naturais, conscientização de produtores e consumidores inclinando na direção de atitudes ecologicamente correta (ISMAN, 1997; VIEGAS- JUNIOR, 2003; KRINSKI, MASSAROLI, MACHADO, 2014). E ainda apresenta vantagens como, alta especificidade em relação à praga alvo, sem causar danos a outros insetos, plantas e animais, sendo mais facilmente degradados que os sintéticos (KOUL; DHAWIWAL, 2001).

2.4.3.1 O Gorgulho do Milho (*Sitophilus zeamais),* **Insetos Praga de Grãos Armazenados**

O Brasil é um dos maiores produtores mundiais de frutas (BOTTON et al., 2005a) e de grãos, principalmente de milho o qual é direcionado para alimentação humana e produção de ração animal. Esses produtos podem ser atacados por pragas levando a prejuízos (FONTES, FILHO ALMEIDA, ARTHUR, 2003). Aproximadamente 10% da produção de grãos são descartadas, devido aos danos causados pelo ataque de pragas, em especial pelo gorgulho do milho, o *Sitophilus zeamais* (PAIXÃO et al., 2009). Cerca de 20% da safra de milho, arroz, trigo e feijão, do Brasil entre o ano de 1991- 1992 foi desperdiçada pelo ataque de insetos praga (FONTES, FILHO ALMEIDA, ARTHUR, 2003).

Diante deste cenário, torna- se notória a perda qualitativa e quantitativa desses produtos, que geralmente acontecem no período de armazenamento dos grãos (PAIXÃO et al., 2009) ou ainda durante o período de amadurecimento (pré-colheita) das frutas susceptíveis aos ataques (BONETI; RIBEIRO; KATSURAYAMA, 2002). Das causas e fatores que contribuem para estas perdas, estão os insetos praga, entre eles: mosca- da- fruta *(Anastrepha fraterculus)* (Figura 13 A), mariposa oriental *(Grapholita molesta)* (Figura 13 B), e o gorgulho *(S.s zeamais)* (Figura 13 C), que são um dos principais agentes causadores de danos em grãos armazenados e frutas, no Brasil, segundo Bonetti et al., (1999) e Botton; LORINI; AFONSO, (2005).

Figura 13: Espécies de insetos pragas que estão associados com os prejuízos e perdas da produção de grãos e frutas no Brasil. (A) *Anastrepha fraterculus* (Mosca das frutas Sul Americana), (B) *Grapholita molesta* (Mariposa oriental), (C) *Sitophilus zeamais* (Gorgulho de milho)

Fonte: [A] http://quintaisimortais.blogspot.com.br/2013/07/anastrepha-fraterculus-mosca-das-frutas.html

[B] http://agrolink.com.br/agricultura/problemas/busca/mariposa-oriental_1505.html

[C] http://www.beefpoint.com.br/cadeia-produtiva/dicas-de-sucesso/pragas-de-armazenamento-de-milho-4575/

Uma das maiores dificuldades encontradas no combate desses insetos, está envolvido com a forma com que os vegetais são parasitados. Como o gorgulho apresenta um período de ataque justamente próximo ao período de colheita, além, os insetos adultos ficam protegidos dentro dos frutos ou grãos, dificultando o contato com os inseticidas e podendo estes permanecerem depositados nos alimentos (BOTTON et al., 2005b).

O *S. zeamais* (Figura 14), sendo no Brasil, a principal praga para o milho, pode causar danos na reprodução deste grão, redução da biomassa, da qualidade e germinação das sementes (MARSARO JÚNIOR, et al., 2005; PAIXÃO et al., 2009). Foi descrito pela primeira vez em 1885, por Mostschuescky (TAVARES, 2002), hoje bem distribuído em regiões tropicais e temperadas (VILARINHO, 2012). Os insetos adultos (Figura 14 A) são pequenos, medindo em torno de 3 mm, de coloração castanha, possuem manchas avermelhadas nas asas (Figura 14 A-2), cabeça com projeção frontal e rostro curvados (Figura 14 A-1) apresentando peças bucais, as quais são mais curtas e grossas nos indivíduos machos (Figura 14), (VILARINHO, 2012) e ainda alimentam- se dos grãos. As fêmeas ainda utilizam os grãos para oviposição e proteção de seus ovos (Figura 14 B) (ALVES et al., 2008; SANTOS et al., 2010).

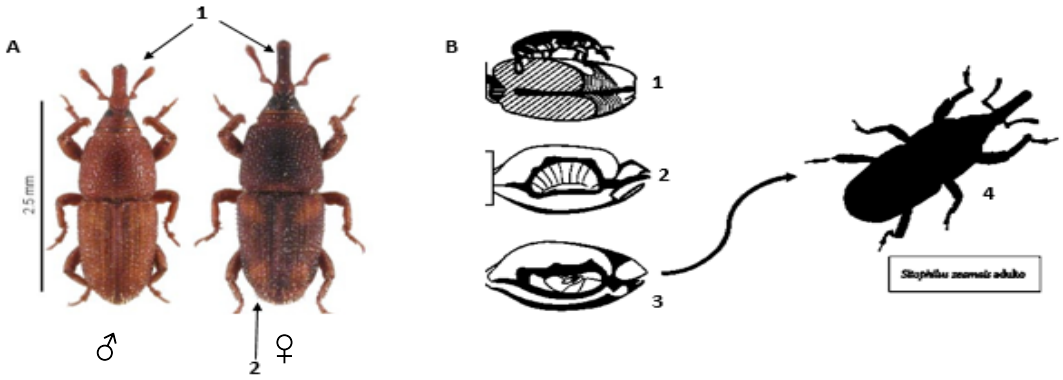

Figura 14: Características das fases do *Sitophilus zeamais.* [A]- Inseto adulto com destaque para o rostro (1) e manchas avermelhadas nos élitros (2), [B]- Estágios de desenvolvimento do inseto, (1) Fêmea ovopositando no interior do grão, (2) Larva em desenvolvimento no interior do grão, (3) Pupa, (4) Fase final, adulta, do inseto.

Fonte:

[A]http://www.azoresbioportal.angra.uac.pt/listagens.php?lang=pt&sstr=8&id=A00470,

[B] http://www.agronegocios.eu/noticias/sitophilus-zeamais-e-sitotroga-cerealella-pragas-do-milho/

As fêmeas da espécie do *S. zeamais,* com o período de pré-oviposição que dura cerca de 6 dias, logo após iniciando o ciclo de oviposição com duração de 104 dias, sendo 282, o número médio de ovos depositados por cada fêmea. Os ovos são depositados no interior dos grãos, através de um orifício aberto pelo aparelho bucal da fêmea. O tempo de incubação varia entre 3 a 6 dias, passando por 4 fases larvais, levando 34 dias até atingirem a fase adulta. Ao deixarem o grão, os novos insetos cruzam e dão início ao novo ciclo (GALLO et al., 2002; ANTUNES, DIONELLO, 2010).

A aplicação de produtos químicos para o combate e controle das pragas, normalmente é alcançado com o uso dos inseticidas do tipo fumigante ou com resíduos protetores, este segundo é o principal método preventivo (VASQUEZ- CASTRO, 2006). Segundo Coitinho et al. (2011), outras práticas também têm sido aplicadas de forma paralela, como a limpeza e secagem dos grãos, aeração e manutenção da temperatura. Porém, todas as alternativas, por mais viáveis que sejam, apresentam alto poder de causar intoxicações, deixar resíduos impregnados nos grãos e indução do aparecimento de espécies resistentes (VASQUEZ-CASTRO, 2006). Porém uma outra alternativa, aparentemente bastante promissora, se faz pela

utilização de espécies vegetais e por isto, o uso de extratos de espécies vegetais, como práticas alternativas podem ser mais sustentáveis (TAVARES et al., 2009; TAVARES et al., 2010).

Sendo assim, as aplicações indiscriminadamente desses inseticidas sintéticos têm desencadeado problemas com a produção de insetos resistentes e contaminação de alimentos com resíduos de agrotóxicos (MARSARO JÚNIOR et al., 2005) e envenenamento dos operadores (VILARINHO, 2016). Desvantagens como essas têm contribuído para impulsionar a busca por alternativas de menor impacto prejudicial para o homem, os grãos e o ambiente. Dentre as alternativas mais atuais são encontrados os inseticidas de origem vegetal (Figura 15). A utilização destas práticas tem como principais vantagens a redução de resíduos impregnados nos alimentos, dos efeitos nocivos a outros organismos não alvo, da intoxicação humana e problemas ambientais. Para tanto, essas pesquisas apresentam expressividade quando objetivam a descoberta de novas moléculas, as quais poderão ser aplicadas para o controle e combate de insetos praga (VILARINHO, 2016).

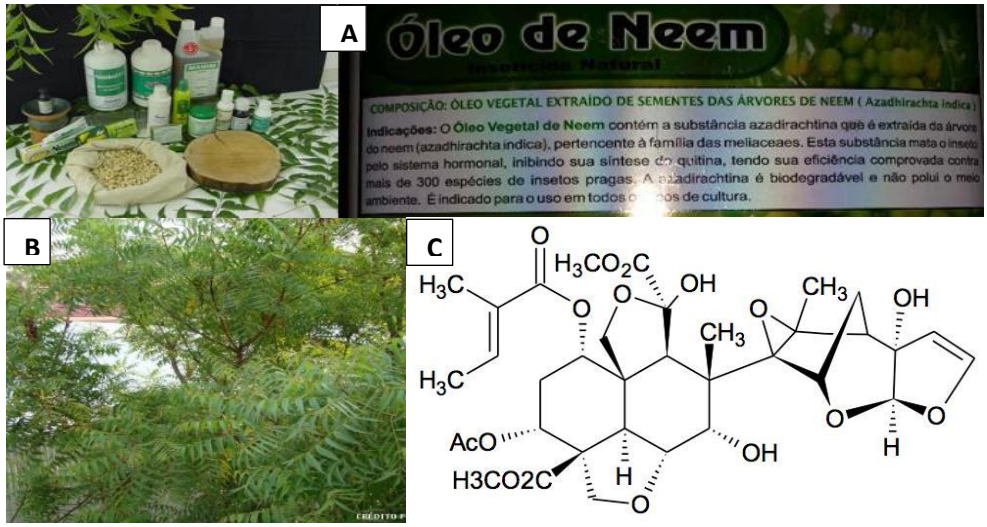

Figura 15: (A) Exemplar de inseticida de origem vegetal, (B) *Azadirachta indica,* (C)Estrutura química da azadiractina.

Fonte: [A]https://www.google.com.br/search?q=inseticida+de+origem+vegetal&source=lnms&tbm=isch&sa=X&ved=0ahUKEwin_tL2o6jNAhWHjJAKHcnXAFAQ_AUICSgC&biw=1360&bih=643#imgrc=2Ln6KPqR4DY5NM%3A

[B] https://www.cpt.com.br/cursos-agricultura/artigos/nim-um-agente-biologico-eficaz-no-controle-natural-de-pragas

[C] http://orquideascomocuidar.com/inseticida-natural-para-combater-pragas/

Vários autores têm demostrado e constataram muitas espécies vegetais com atividade inseticida. Liu, Goh e Ho (2007), testaram quanto a atividade inseticida de contato, deterrente e de fumigante de 40 espécies de erva medicina de 32 famílias botânicas, frente duas espécies de insetos: *S. zeamais e Tribolium castaneum*, dentre as famílias, destaca-se a espécie da família da Euphorbiaceae. Liu e colaboradores relataram que 30 das espécies exibiram atividades inseticidas.

Devappa et al. (2010) sugeriram que inseticidas contendo ésteres de forbol, desempenham atividade inseticida importante e esses produtos são produzidos como metabólitos secundários, pelos vegetais, principalmente por espécies da família Euphorbeaceae, principalmente pelo gênero *Jatropha*. Silva et al., (2012), constataram efeito inseticida sobre *S. zeamais e Rhyzopertha dominica.* Em estudos realizados por Silva et al. (2012), apresentaram mortalidade dos insetos para os grupos tratados com extrato aquoso das sementes, comprovando que as ações pesticidas também podem varias de acordo com as partes das ervas.

A utilização de plantas medicinais como *C. urens* e a sua impactante representação encontrada em citações nos estudos etinobotânicos, desperta curiosidade e são alavancas que impulsionas a busca pelo conhecimento sobre esta espécie, a cerca de sua potencial ação biológica e seus mecanismos, apontando o potencial terapêutico e agrículo que não apenas *C. urens,* mas outras espécies possam desempenhar. Assim faz- se importante à realização de novos estudos e pesquisas, afim de ampliar os conhecimentos e tornar está espécia uma possível alternativa para a prevenção e o tratamento de enfermidade com menor efeito adverso e também seu emprego na agricultura diminuindo as chances de resistência e permanência de ressisduos em grãos.

3 OBJETIVOS

3.1 GERAL

Investigar o perfil fitoquímico e avaliar o potencial biológico dos extratos aquoso e orgânico de *Cnidoscolus urens* coletadas em Caruaru/ área de Caatinga- PE.

3.2 ESPECÍFICOS

Realizar triagem Fitoquímica das frações acetato de etila, N- butanol e aquosa de extratos de *Cnidoscolus urens;*

Identificar as principais classes de constituintes químicos presente em extratos orgânicos e aquoso, derivados do metabolismo de *C. urens*, por análise em cromatografia em camada delgada (CCD);

Investigar a capacidade antioxidante dos extratos aquoso e orgânico de *C. urens*;

Determinar o perfil de segurança, através do estudo da toxicidade aguda (*in vivo*) de extratos aquoso e orgânico de *C. urens;*

Avaliar o efeito de citotoxicidade *in vitro* dos extratos aquoso e orgânico de *C. urens* frente à linhagem celular HELA;

Averiguar a atividade inibitória dos extratos aquoso e etanólico de *C. urens* frente a tumor sólido de carcinoma de Ehrlich;

Interpretar o perfil hematológico e bioquímico nos grupos experimentais com tumores sólidos induzidos e tratados com os extratos aquoso e orgânico de *C. urens*;

Estabelecer a capacidade de interferência na normalidade do sistema digestivo de extratos orgânicos de *C. urens*, em modelos animais;

Comprovar a ação antidiarreica de extratos orgânicos de *C. urens* frente a modelo de diarreia induzida por óleo de castor;

Identificar a influência dos extratos orgânicos de *C. urens* sobre a capacidade de interferir no acúmulo de fluido intestinal, além de sua capacidade de inferir sob o peristaltism em modelos experimentais;

Verificar o efeito dos extratos orgânicos de *C. urens* sobre insetos praga.

Determinar a atividade inseticida de extratos orgânicos de *C. urens* e os índices nutricionais de *S. zeamais*.

Avaliar a toxicidade por ingestão de dieta artificial e da toxicidade ppor contato de extratos orgânico *C. urens* sobre *S. zeamais*

Avaliar a toxicidade por contato de extratos orgânicos de *C. urens* sobre *S. zeamais.*

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CAPÍTULO II

Manuscrito 1: *In vitro* **and** *in vivo* **antineoplasic activity of** *Cnidoscolus urens* (L). Arthur **extracts.**

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***In vitro* and *in vivo* antineoplasic activity of** *Cnidoscolus urens* (L). Arthur **extracts.**

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ABSTRACT

Ethnopharmacological relevance: Brazil comprises 20% of biodiversity in the world and one the six major biomes, the Caatinga, is a highly threatened biome, covering an area of 60% in Northeast region, totally restricted this country. *Cnidoscolus urens* (Euphorbiaceae) is a plant commonly known as "urtiga" by rural community of Northeast of Brazil. This species has been used for rubber production, are widely used, empirically, alternatively paw treating diseases by many popular.

Aim of the study: Evaluate the antitumor activity *in vivo* and *in vitro* of aqueous and ethanol extracts of *C. urens*.

Materials and Methods: Aqueous and ethanolic extracts were obtained from total parts of *C. urens*. Ehrlich carcinoma (EC) solid tumor which induced in mice and then treated with aqueous or ethanolic extracts at 200 mg / kg. Tumor weight, biochemical and hematological parameters of the animals were analyzed. Toxicity of the extracts was tested antineoplastic *in vitro* test HELA cells and antioxidant activity was accessed by 1,1- diphenylpicrylhydrazyl (DPPH) method at different concentrations 50, 100, 200 e 500 µg/mL of extracts. Phytochemical screening of *C. urens* was realized by Thin Layer Chromatography (TLC).

Results: Flavonoids, terpenoids, coumarins, tannins and reducing sugars were found in aqueous, ethyl acetate and n-butanolic extracts of *C. urens*. Aqueous and ethanolic extracts at 200 mg / kg were effective in inhibiting the tumor growth 84.4% and 79.2%, respectively and no adverse side effects due to the treatment were observed in biochemical urea, creatinine, total cholesterol, HDL-cholesterol, glucose and triglycerides and hematological red and white blood cells and hemoglobin parameters. *C. urens* extracts did not show any *in vitro* toxicity effect against HELA, however showed great antioxidant potential > 50% at 50, 100, 200 and 500 µg/ mL.

Conclusions: Aqueous and ethanolic extracts of *C. urens* have a notorious antitumoral effect and could explain the basis for traditional use to treatment of cancer by rural community of Northeast of Brazil.

Keywords: *Cnidoscolus urens* (Euphorbiaceae), antitumoral activity, EC solid tumor, antioxidant, HELA cells, toxicity.

1 INTRODUCTION

Cancer is a particular group of diseases, characterized by uncontrolled cellular growth, tissue invasion and metastases **(Dashora et al., 2011; Instituto Oncoguia, 2015; SBCO, 2015).** This is one leading causes of death in world **(Ferlay et al., 2015)** and accounted for 7.9 million deaths in 2007, with 38% in developed countries and 62% in developing countries. Projections to 2030 show that almost 21.4 million of new cancer cases and more 13.2 million deaths will occur worldwide **(GLOBOCAN, 2008).** According to INCA, it is esteemed 600.000 new cancer cases only in Brazil, a country in Development **(INCA, 2016).**

Chemotherapy is an effective treatment against various types of cancer singly or in combination with surgery and radiotherapy. However, chemotherapeutic effects of most the drugs showed a limited efficacies due to the development of many side effects, nausea causing, pain, neurotoxicity, nephrotoxicity and Other So many effects (**Asirvatham et al., 2012; Ostadhadi et al., 2015**). This have been stimulated to evaluate new products against cancer **(Syamala et al., 2015)** capable to cause less serious side effects **(Aranjani et al., 2013)**. The plant kingdom is a potential source of bioactive compounds of varying composition (tannins, coumarins, phenols, flavonoids, catechins, anthocyanins and proanthocyanins) **(Jeevananthan et al., 2011; Ashraf et al., 2015)** with antitumor activities and various plants which traditionally have been used in the treatment of infectious diseases and in particular cancer **(Noolu et al., 2013; Servin Wesley et al., 2013; Ramasamy et al., 2013 and Ashraf et al., 2015**).

Brazil comprises 20% of biodiversity in the world and the six major biomes (Amazon, Atlantic Forest, Pantanal, Cerrado, Caatinga and Pampa) comprise the highest levels of

diversity in the on world (**Pilatti et al., 2011; Rada, 2015**). The Caatinga vegetation is a highly threatened biome, covering an area of 60% in Northeastern region of Brazil. This ecosystem is dominated by one of the few types of vegetation whose distribution is totally restricted to the country (**Silva et al., 2012**).

Euphorbiaceae is a family of plants which has high medicinal use, as well as the genus *Cnidoscolus,* popularly known as "urtiga" or "favela", and highly cited due its properties **(Crepaldi et al., 2016)**. This genus has 50 to 75 representatives, whose characteristic is the presence of stinging trichomes that when stimulated by contact with skin, can cause severe and localized pain (**Sátiro and Roque, 2008; Melo and Sales, 2008**). In the Caatinga biome the genus is represented by various species, among them *Cnidoscolus urens* (L). Arthur (**Sobrinho et al., 2012**). Popularly this species have been used orally for the treatment of hemorrhage, inflammation, antiseptic, for treat kidney infections and antitumoral in the Brazilian region Northeastern (**Sobrinho et al., 2012; Albuquerque and Andrade, 2002; Sobrinho et al., 2011, Menezes et al., 2014**).

In this way we can suggest that this species can present effective therapeutic properties, based on its popular use and the presence of metabolites such as flavonoids and terpenoids, which are cited with properties in studies demonstrating its antiinflammatory, analgesic and antitumor action.

This study was carried out to evaluate the antitumoral activity of the ethanolic and aqueous extracts of total plant of *Cnidoscolus urens* against *in vivo* Ehrlich tumor solid model and *in vitro* toxicity of HELA cells and determine the antioxidant activity of these extracts.

2 MATHERIALS AND METHODS

2.1 Chemicals

1,1-diphenylpicrylhydrazyl (DPPH), Neutral Red, 3-[4,5-Dimethylthiazol-2-yl]-2,5-Diphenyltetrazolium Bromide (MTT), 5-Fluorouracil (5-FU) and standard for Thin Layer chromatography (TLC) was purchased from Sigma-Aldrich Chemical Company, St. Louis, MO. Solvents for this work were purchased from Merck, Darmstadt, Germany. All the chemicals were of analytical grade.

2.2 Collect of Botanical Material

C. urens were collected in the county Agrestina, Pernambuco, Northeasth Brazil in Caatinga area (Lat 8 ° 23'04.8 "S; Long 35 ° 58'49.7". W), from June 2011. A sample of collected material is archived as voucher specimen number 84.190, at the herbarium of *Instituto Agronômico de Pernambuco (IPA).*

The total plant (root, stems, leaf, seeds and flowers) materials were dried at room temperature, followed by milling of the dry plant material, was carried out in a Willey type mill, guaranteeing the standardization of the vegetal fragments. The crushed amperial was used for the preparation of the extracts.

2.3 Preparation of Extracts

2.3.1 Preparation of Extract for Phytochemistry

Screenig Extract: 50 g of dried plant material were homogenized for 20 minutes in a mechanical stirrer with 100 mL of methanol in ebulition and filtered with Whatman filter paper (No. 1). The extract was concentrated to dryness, by rotary evaporator pressure Buchler Instruments, Fort Lee, NJ, USA, and resuspended in water (100 mL). The aqueous extracts (Aq) obtained was subjected to partition into separatory funnel with an equal volume of ethyl acetate (EA). Obtaining 2 phases, where the EA phase was separated. This process was repeated twice in order to obtain 2 fractions: Aq and AE. The EA fraction was reserved and Aq was used to obtain N-butanolic fraction that was performed in the same manner of EA fraction. At the end was obtained three fractions: Aq, EA and N-bu **(Wagner; Bladt, 1996)**.

2.3.2 Preparation of Extracts for Biological Activities

Aqueous Extract: Dried plant material 100 g were homogenized for 1 hour in a mechanical stirrer with 200 mL of water, kept under refrigeration (4 – 10ºC) during 24 hours and filtered with Whatman filter paper (No. 1). This process was repeated twice more. The extract was lyophilized (Liofilizador L101, LIOTOP®, São Paulo, Brasil).

Ethanolic Extract: 100 g of dried plant material were homogenized for 1 hour in a mechanical stirrer with 200 mL of absolut ethanol, kept under refrigeration (4 – 10ºC) and filtered with Whatman filter paper (No. 1). The extract was concentrated to dryness, by rotary evaporator pressure Buchler Instruments, Fort Lee, NJ, USA.

The extracts EA, N-bu and Aq were selected according to **Wagner; Bladt (1996)**, for a better elucidation of the chemical constituents of the extracts, while the extracts aq and EtOH were chosen for the toxicity, antioxidant and antitumor experiments in vivo and in vitro, based on ethnomedical (ethnopopulational) information available in ethnobotanical studies.

2.4 Phytochemical Screening of Cnidoscolus. urens

Thin layer chromatography (TLC) assays were performed for qualitative detection of the substances contained in *C. urens*. Samples of Aq., EA and N-bu extracts were applied to specific Merck silica gel $F_{254+366}$ chromatoplates developed in ascendenting unidimensional, according **Wagner; Bladt (1996)**. Spots were revealed by spraying and revealing specific followed by visualization under UV light of wavelength of shorter (256 nm) and long (366 nm). In order to identify the main chemical groups of secondary metabolites. The results were compared to the standards specific for each metabolite analysis.

2.5 *Animals*

The study was carried out with adult male albino Swiss mice (30 ± 5g), obtained from Biotherium of Laboratory of Immunopathology Keizo-Asami (LIKA), UFPE, Brazil. There were housed at a temperature of 22 ± 2°C with a schedule of 12 h light and 12 h dark cycle. Mice were allowed to feed on chow (Labina®, Purina, Brazil) and water *ad libitum.* All experiments reported herein are in accordance with the Animal Care and Use Committee at the Federal University of Pernambuco n° 23076.041082/2011-25 and Guidelines for Care and Use of Laboratory Animals.

2.6 Acute Toxicity in Mice

Acute Toxicity study performed as per Organization for Economic Co- operation and Development (OECD Guidelines) **(OECD, 2004)**. Swiss Albino mice of either sex were used. The animals were fasted for 4 h, allowed free access water. Dose maximum of the extracts of *C.s urens* were from 2000 mg/kg, solubilized in saline solution, through the intraperitoneal route of administration. The mices were observed continuously for behavioral changes for the first 4 h and then observed for mortality if any 24 h after the extract administration.

The dose used for other tests was determined from the maximum dose (2000 mg / kg) for the acute toxicity test and the dose applied, corresponding to 10% of the maximum dose.

2.7 Antitumoral Activity of Extracts of Cnidoscolus urens in Mice

Ehrlich carcinoma (EC) solid tumor cells were supplied by the Department of Antibiotics, Federal University of Pernambuco (UFPE), Brazil. The cells were maintained *in vivo* in mice by intraperitoneal transplantation. EC cells aspirated from the peritoneal cavity of mice were washed with saline and injected subcutaneously to develop EC solid tumor. For the experimental tumor induction mice were injected with EC (5.0×10^7), subcutaneously in the left footpad to obtain the EC solid tumor (**Stock; Clack; Philip, 1955).**

The mice (n=36) were divided into 6 groups (n=6, for group) as follows:

Group (I) – Control mice without induction of tumor, receiving saline solution 0.9%, named normal control (NC);

Group (II) – Control mice with induction of tumor, treated with saline solution 0.9%, named saline treated (Saline);

Group (III) – Control mice with induction of tumor, treated with 5-FU (20mg/kg- according to the manufacturer), named 5- FU treated;

Group (IV) – Group of mice with induction of tumor, treated with aqueous extract 200mg/kg, named aqueous fresh treated (Aq200);

Group (V) – Group of mice with induction of tumor, treated with ethanolic extract 200mg/kg, named aqueous dried treated (EtOH200).

All the groups were treated for seven days, beginning after the tumor inoculation. On the 8th day, after fasting overnight, all mice were anaesthetized with urethane 1.25g/kg and blood samples were withdrawn by retro-orbital venipuncture technique. After, all mice were euthanized by cervical dislocation and the tumor, liver, kidney and spleen were collected and weighed.

The blood was stored in two separate tubes: (**1**) containing the anticoagulant ethylenediamine tetraacetic acid (EDTA) and other (**2**) without anticoagulant, called dry tube (VACUETTE^{TM}, Greiner, Kremsmunster, Austria). The dry tube was immediately centrifuged 2500 rpm / 15 min to obtain serum. Serum was utilized for determination of levels of glucose, total cholesterol, HDL-cholesterol, triglycerides, urea, creatinine, total protein, albumin and globulin by chemical automatic analyzer (COBAS$^{®}$ 6000, Roche Diagnostics, England). The tube containing EDTA was used to determine the hemathological parameters: total white blood cell (WBC), red blood cell (RBD) and hemoglobin (Hb) were determined by standard method using haemocytometer (SYSMEX XT-4000*i*^{TM}, Sysmex Corporation, Curitiba, Brazil).

2.8 Antiproliferative Activity of Extracts of Cidoscolus urens in HELA Cells

Antiproliferative activity of extracts of *C. urens* was assessed by cell culture method used to epithelial cell carcinoma of human cervix (HELA). HELA is a line of human cell oldest and most used in scientific research since 1951 **(Masters, 2002)**. The cells were obtained from the Cell Bank of the Rio de Janeiro, Brazil. The HELA cells were maintained in 90% Dulbeccos's Modified Eagle (DMEM) supplemented with 2mM L-glutamine, peniciline 100 IU/mL, streptomycin 25mg/mL and 10% heat-inactivated fetal bovine serum.

The cell viability was assessed by Trypan Blue staining with assistance of an inverted microscope. The cell suspension 105 cells/mL was plated in 96-well flat bottom plates 220 µL/well. Cells were allowed to adhere to the wells overnight, and then the extracts of *C. urens* were added to triplicate wells 22µL/well. These plates were incubated at 37 °C, 5% CO_2 for 72 hours, then assayed from growth inhibition using a colorimetric method of MTT, according to protocol of National Cancer Institute, such method, allows to measure the ability to metabolize tetrazolium, by viable cells. A second colorimetric method used to measure cell viability was neutral red, capable of measuring the retention of the dye by the lysosomes of viable cells. and also by the neutral red method. The percentage of cell viability was calculated as:

% Cell viability = ABS mean extract wells X 100/ ABS mean control well and

% Cell inhibition = 100 - ABS mean extract wells/ ABS mean control well x 100.

2.9 In vitro Antioxidant Activity of Extracts of Cnidoscolus urens

The method was carried out as described by **Brand- Williams et al. (1995).** Various concentrations 50, 100, 200 and 500 µg/mL of the ethanolic and aqueous extracts of total plant

of *C. urens* were used. The DPPH solution 150 µM was prepared with methanol solvent. The assay mixture had a total volume of 1 mL (500µL of the extract + 500µL of DPPH solution). α-tocopherol (50, 100, 200, 500 µg/mL). was used as the positive control. After 30 min it's incubation at 25 °C, the absorbance was measured at 490 nm (EL_X 800uv Universal Microplate Reader, BIO-TEK INTRUMENTS, INC., Vinooski, VT). The radical scavenging activity was calculated from the equation:

$$\% \text{ Scavenging} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

2.10 Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical significance was determined by one-way ANOVA followed by Turkey's tests. $P < 0.05$ implied significance. All analyses were carried out using software PRISMA (GraphPad Software, Inc., San Diego, CA, version 5.01).

3 RESULTS

3.2 *Phytochemical Screening*

Phytochemicals assays were used to determine the presence of the main secondary metabolites present in the Aq, EA and N-bu extracts *C. urens*. Through the tests can reveal the significant presence of chemical groups such as flavonoids and terpenoids in EA extract and reduction of sugar in extract Aq. At lower concentrations, we can also detect a presence of tannins and coumarins without N-bu extract, in addition to traces of proanthocyanidins in EA. In the meantime, alkaloids and saponins can not be detected in the samples used for the

phytochemical experiment. In previous studies carried out in 2007 by Yuan et al., Also indicated the presence of flavonoids, terpenoids and coumarins in methanolic extract of *C. texanus.* More recently Peixoto Sobrinho, et al. (2012), identified the presence of anthocyanin, coumarins, flavonoids, tannins and terpenoids in methanolic extracts of aerial parts and root of *C. urens*, corroborating our results. In disagreement, the author referended above detected the presence of saponins in methanolic extract of the *C. urens* root, such a metabolite can not be detected in our studies. As early as 2016, Gomez et al., identified a presence of eight different flavonoids, nine phenolic acids, as well as saponins and alcalde in aqueous extract of *C. chayamansa* leaves. As well as Pérez-González et al., 2017, determine a presence of phenolic compounds and flavonoids in methanolic extract of *C. chayamansa* leaf.

3.2 Acute Toxicity

Intraperitoneal administration of a maximum dose 2000 mg/kg of the *C. urens* did not result in any mortality or observable behavioral changes such as writhing, gasping, palpitation, pilo-erection, hyperventilation and decreased respiratory rate, in the treated mice. Additional studies about chronic toxicity are necessary, which are needed for the best solution on the effects that may or may not be induced by treatment with extracts of *C. urens.*

3.3 Antiproliferative Effect of Cnidoscolus urens Extracts

As shown in **(Figure 1)** the Aq and EtOH extracts of *C. urens* exhibited no satisfactory growth and inhibitory activity against a HELA cells. However, ethanolic (100µg/ mL) extract showed greater inhibition of cell growth by the MTT method, with a percentage of approximately 40% **(Figure 1- A)**. The aqueous (100µg/ mL) extract was more effective during

antiproliferative activity with neutral red method, demonstrated a percentage of inhibition of approximately 25% **(Figure 1- B)**. In the meantime, if we compare the MTT and NR methodology, the ethanolic extract shows to have developed a simple improvement of the antiprologative activity. **(Figure 1- A, B)**.

3.4 Antitumor Activity of Cnidoscolus urens Extracts

After induction of EC solid tumor, the footpad thickness curve presented a significantly behavior from the fourth day post inoculation **(Figure 2- A)**. The saline treated group (negative control) showed significant differences in tumor growth between 6^{th} and 8^{th} days. There was a significant reduction of tumor growth in mice treated with 5-FU, Aq200 and EtOH200 at 6^{th} and 8^{th} day, different from control group that increased the tumor weight.

The EC solid tumor weight in the saline treated group was 2.31 ± 0.35g (**Figure 2- B**). The treatment with Aq200 and EtOH200 showed a significant antitumor activity which was further evidence by percentage reduction (84.4% and 79.2%, respectively). Administration of 5-FU, a commercial drug for treatment of cancer, also lowered tumor weight in 65.4%. However, treatment with Aq200 was significantly ($P<0.05$) greater than the treatment with 5-FU.

The antitumor nature of Aq200 and EtOH200 was accompanied by the significant reduction in organ weights of animals treated with the extracts ($P<0.05$ compared to EC solid tumor bearing mice). It was also supported by the significant reduction in liver EtOH200 and kidneys Aq200 and EtOH200 in the extracts treatment when compared to the EC solid tumor treated with saline, named Saline Group (**Table 1**).

Urea, creatinine, total cholesterol, HDL-cholesterol and triglycerides were significantly decreased in animals treated with Aq200 as compared to the saline treated group (**Table 1**). The glucose levels alteration were significantly in all groups with EC solid tumor, and treatment with Aq200 was able to reverse this condition. The 5-FU is a potent agent against tumor (**Figure 2- B)**, however, biochemical parameters such as urea, creatinine, total cholesterol, HDL-cholesterol and triglycerides were higher in this group as adverse effects. These parameters were significantly (P<0.05) lower in the groups treated with Aq200 when compared with the group treated with 5-FU. The treatment with EtOH200 showed a significant reduction in the creatinine, total cholesterol and triglycerides levels when compared with the 5-FU group.

The time of tumor development 7 days, which already allows us to see some changes in white blood cells in the treated groups 5-FU and Aq200 (**Table 2**). Also, the treatment with 5-FU promoted significant (P<0.05) decreasing in white blood cells number when compared to the saline treated group. The Aq200 and EtOH200 showed significantly increased in the WBCparameter when compared to the 5-FU treated group.

3.5 Antioxidant Activity of Cnidoscolus urens Extracts

The antioxidant method performed by DPPH can be achieved by the elimination of its radicals, being determined by the absurd. The antioxidant capacity of extracts of *C. urens* and of α-tocopherol can be evidenced in **Figure 3**, showing a decrease (p <0.05) in the concentration of DPPH radicals, this is due to the ability of extracts and α- tocopherol to eliminate reactive species. The radical scavenger activity of the standard and extracts followed the order α – tocopherol > aq > EtOH, with a percentage of 85, 65 and 52% in the concentration of 500 µg / mL. These results indicated that the extract Aq of *C. urens* has a better effect by eliminating the free radicals than the extracted EtOH of *C. urens*, independent of concentration

4 DISCUSSION

Bioactive products of plants have served as a good source of antitumor treatment. Recently, several studies have been conducted and a large number of plants possessing anticancer properties have been documented (**Aranjani et al., 2013; Noolu et al., 2013; Servin Wesley et al., 2013; Ramasamy et al., 2013; Thummar et al., 2015 and Aliya, et al., 2016**). The *Cnidoscolus* genus was traditionally used for the treatment of many diseases **(Bijekar, Gayatri, 2014)**, especially against cancer in Northeaste Brazilian population **(Jiménez-Arellanes et al, 2014)**. Administration of the Aq200 and EtOH200 extracts of *C. urens* for 7 days showed an expressive and significant antitumoral activity. Similar results were found by **Ribeiro et al., (2012)**, when inducing solid tumor of Ehrlich in mice and performing the treatment with aqueous and ethanolic extract of leaf of *Arrabidaea chica*. Thus, it can show the smaller development of the tumor mass when compared to the control. The antitumor activity was demonstrated for the methanolic extract of *Euphorbia dendroides* (Euphorbiaceae) when used for the treatment of mice also with Ehrlich solid tumor. Fayad et al. (2017) highlighted reduction of tumor volume and weight in addition to Increase in the mean survival time of the animals treated with such extracts.

The treatment with Aq200 extract of *C. urens* was a significantly better in decreasing the tumor growth in comparison with treatment of 5-FU, and significantly improve hemoglobin, RBC and WBC. Indicating that extract from *C. urens* has antitumor activity and protective action to hematopoietic system. Similar results were found by Pillai et al. (2012), when used ethanolic extract of *C. chayamansa* sheet for the treatment of Dalton's ascitic Lynphoma. In most cases chemotherapy is accompanied by adverse effects as myelosuppression and anemia.

This framework is already well known tumor patients and can be associated with iron deficiency or hemolytic conditions.

Recent studies with other species of the Euphorbiaceae family also collaborate with our findings, indicating a potent anti-tumor action of these species. Acetone extract of aerial parts of *Phyllanthus niruri* (Euphorbiaceae) showed the presence of flavonoids which was used to demonstrate and prove its antitumor activity **(Padmapriya; Poonguzhali, 2015).** The results found by our phytochemical tests also revealed the significant presence of flavonoids in *C. urens* extract. The presence of this compound in the extracts can be directly related to its potent anti-tumor action, as suggested by scientific studies **(Babu et al., 2013; Yu-Ke et al., 2014; Zhong et al., 2015).**

Under normal conditions, the synthesis of new fatty acid molecules isinhibited by the presence of fatty acids derived from food. This condition altered appears in patients with tumors installed. There is a probability that the altered cells lose their sensitivity, fail to notice an increase in the concentration of fatty acids, and continue to synthesize. Researchers propose that enzymes involved in the synthesis of fatty acids (ATP citrate lyase (ACLY), acetyl-CoA carboxylase α (corn meal), fatty acid synthase (FASN), and long chain acyl-CoA fatty synthetases 1, 3 and 5 (ACSL1, ACSL3 , ACSL5)) are increased **(Wu et al., 2014)**, being able to influence the lipid profile.

Elevated triglycerides levels were observed in EC solid tumor group in comparison to the normal control group (**Laza-Jacoby et al., 1984**). This finding may be related to changes in lipid metabolism that occurs during cancer development, where a defect in clearance, due to the decrease in the activity of adipose tissue LPL, may be responsible for the early development

of hypertriglyceridemia during tumor growth (**Laza-Jacoby et al., 1984; Wu et al., 2014**). The treatment with Aq200 and EtOH200 extracts of *C. urens* showed reduction of serum triglycerides and total cholesterol. The previous biochemical studies supported properties of bioactive plant compounds that can contribute to the lipid profile ameliorating (**Kumar et al., 2011; Ramachandran et al., 2012**).

Elevated urea and creatinine levels are well reported as one of the most sensitive markers of kidney damage (**Omara et al., 2012**). In the serum of EC solid tumor animals urea showed elevated, indicating kidney damage in these groups. After 8 days of treatment of aqueous extract of *C. urens*, the levels of urea significantly decreased and this result clearly indicates that Aq200 possess effectiveness to improve kidney damage in EC solid tumor animals. Species such as *Jatropha gossypiifolia* (Euphorbiaceae) were tested in order to assess the toxicological ability of ethanol extract of the leaves. The animals treated with doses of 1-5 g / kg were analyzed for their biochemical profile after 14 days **(Silva et al., 2014)**. The same study showed the ability to reduce the concentration of urea. By contrast observed an increase in creatinine concentration. Data partially corroborate our.

Recent surveys depict glucose metabolism related to the development of tumor cells. Tumor cells are involved in changes in glycolytic and mitochondrial metabolism, providing enough energy for the multiplication of altered cells, to the synthesis of ATP and NADPH, as well as amino acids, lipids and nucleotides. One of the consequences of the change of glucose metabolism, is the decreased level of glucose significantly. This change may be triggered by a disruption in signaling pathways and activation of oncongenes, allowing the production of glucose via the intermediates **(Weinberg; Chandel, 2015).** Decrease in glucose level is a characteristic feature of all types of tumor **(Reinhold et al., 1991)**. This may be attributed to

the multiplication of cells requiring more glucose to produce energy in cancer cells **(Bartrons; Caro, 2007; Weinberg; Chandel, 2015**).

Treatment with Aq200 *C. urens* extract increased glucose levels with treatment after 8 days, corroborating with decreasing in a multiplication of EC solid tumor cells in vivo model. **Shetti; Kaliwal** (2015) was able to prove that different species of Euphorbiaceae can also show an improvement in glycemic profile of animals with diabetes induced by alloxan. After treatment with a single dose of 400mg / kg ethanol extract of *Phyllanthus amarus*, the animals demonstrated a low level of blood glucose and hypoglycemic activity of *P. amarus* extract was maintained until the ending of the experiment. The results of Shetti and Kaliwal help prove the hyperglycemic capacity Aq200 and EtOH200.

Gomez et al (2016), used the *C. chayamansa* extract 2% and were able to prove that at the end of the experiments the animals treated with the extract it sufficient to cause an improvement in glucose levels, suggesting the presence of phenolic compounds from the extract could improve insulin secretion, cellular regeneration and inhibition of digestion and absorption of glucose.

Tumor development time for 8 days was able to induce changes in the hematological profile of the groups, improving the immune response of animals that were treated with extract of *C. urens*, with an expressive increase in the number of white blood cells (WBC). Also, the treatment with 5-FU, promoted significant ($P<0.05$) decreased in white blood cells number. The myelosupression and anemia are the great problems caused by tumors and the anemia encountered in EC solid tumor animals is mainly due to reduction in red blood cells (RBC) or hemoglobin (Hb) percentage and this may occur either due to iron deficiency or due to

hemolytic or myelopathic conditions **(Sreelatha et al., 2011).** Similar results were showed in animals with EC solid tumor in the present study. The treatment with Aq200 extract promoted a reverse condition for these hematological parameters, suggesting that the aqueous extract of *C. urens* may possess protective action on the hematopoietic system.

Preliminary studies with leaf extract *C. aconitifolius* administered (400, 600 and 800 mg/kg) in groups of diabetic rats showed a great improvement in hemoglobin concentration. This improvement is related to the presence of phenolic compounds identified by prior phytochemical analysis. These phytochemicals findings of the study **Obichi, et al.** (2015), as our results are similar and suggest that these species possess potential anti-inflammatory and antioxidant and strong indication for the treatment of cancer. **Pillai et al** (2012) Also found similar results by treating mice with induced tumors with ethanol extract of *C. chayamansa*.

Aqueous and ethanolic extracts of *C. urens* exercised the antioxidant potential and previous studies showed that the antitumor activity of plant extracts may be attributed to the antioxidant principles present in the extract (**Sreelatha et al., 2011**). These findings in the present study are consistent with the earlier reports, which support the antioxidant activity of of methanolic extract of root and leaves *C. urens* (**Sobrinho et al., 2011**). The antioxidant activity is not just a feature of the species *C. urens*, other species of *Cnidoscolus* also showed antioxidant activity, with the antioxidant activity of *C. aconitifolius* demonstrated by **Obichi, et al.,** 2015.

Cytotoxic activity of the extracts was determined according to NCI scale. Extracts are considered without cytotoxic activity when inhibition percentage range was 1-20%, with little activity when such percentage was between 20 and 50%, moderate activity when the inhibition

was 50 to 70% and high activity when the inhibition range was 70 to 100%. Thus, aqueous and ethanolic extracts had their cytotoxicity evaluated by two different methodologies, MTT (access cell with active mitochondrial metabolism) which evaluates the succinate dehydrogenase enzyme ability to reduce MTT to formazan in the mitochondria cell and Neutral Red (soluble in water that accumulates in the lysosomes of viable cells), which assesses the damage caused in the cell membrane. Both extracts showed little cytotoxic activity at the highest concentration (100 ug) by MTT and neutral red dye, similar to that observed by **Sobrinho et al.** (2011). .

The preliminary phytochemical screening of *C.urens* indicated the presence of various compounds. A number of studies reports indicate terpenoids, and phenolic compounds such as tannins, coumarins and flavonoids have a chemo preventive role in cancer through their effects on signal transduction in tumor cell proliferation and many of such compounds are known to possess potent antitumor activity (**Weber et al., 1996; Blois, 2002**). The biochemical studies supported its antioxidant properties and preliminary phytochemical screening showed compounds that can contribute to the *in vivo* antitumor activity of *C. urens* extracts **(Obichi, et al., 2015).**

5 CONCLUSION

The aqueous and ethanolic extracts of *C.urens* were effective in inhibiting the growth of Ehrlich solid tumor models and no adverse side effects due to treatment were observed. However, the cytotoxicity of *C. urens* extracts in HELA cells showed no *in vitro* antiproliferative activity. The biochemical studies supported its antioxidant properties and preliminary phytochemical screening showed the presence of flavonoids, terpenoids, coumarins

and reducing sugar that can contribute to the *in vivo* antitumor activity of *C. urens* extracts. *C. urens* merites further investigation in a tumor model to elucidate its mechanism of action and isolation of its active constituents may prove rewarding in cancer treatment.

The data our pre-clinical suggest that *C. urens* extract is a systematically and nontoxic available angiogenesis inhibitor and should be further evaluated as a potent chemotherapeutic agent. Although *C. urens* be defended as an effective treatment against cancer and widely used for this purpose by the people claiming that in some cases cure the disease, and our preliminary studies have shown promising results, more detailed scientific studies to evaluate its effectiveness and mechanism of action are missing.

CONFLICT OF INTERESTS

All authors declare that they have no competing interests in the present work.

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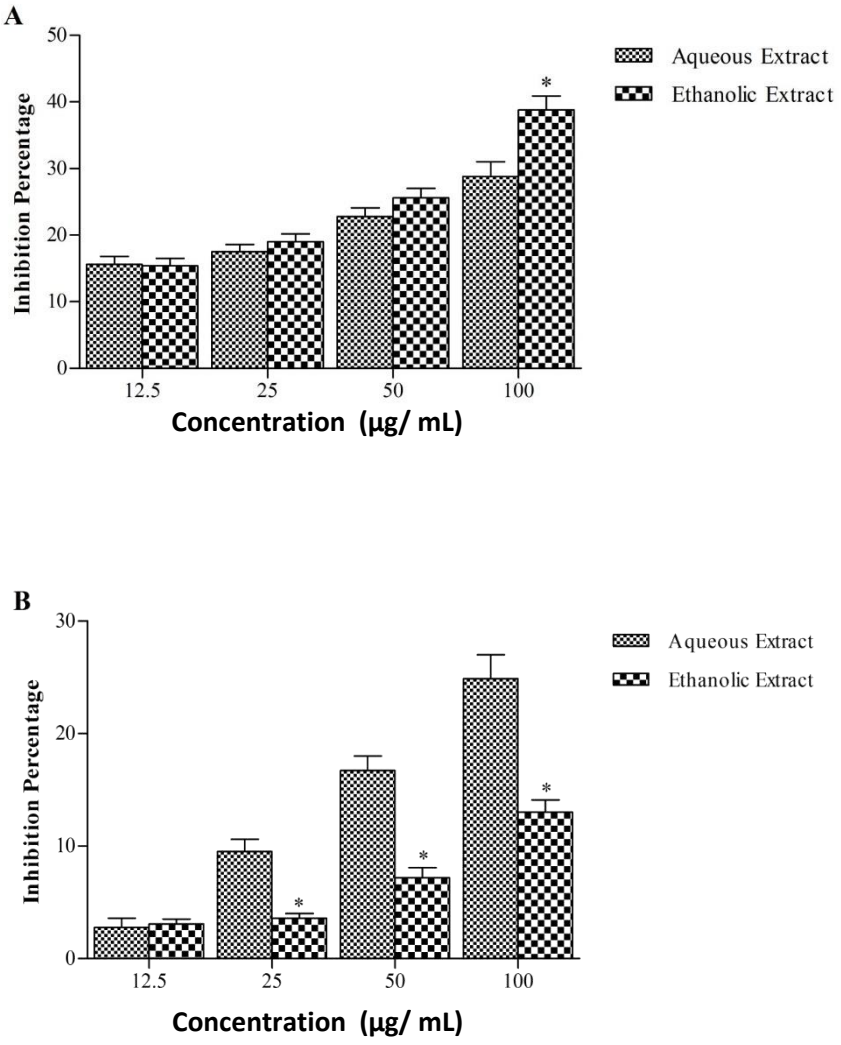

Figure1. Antiproliferative activity of extracts of *Cnidoscolus urens* in HELA cells by MTT method (A) and Neutral Red method (B). **P*<0.05 for all analysis as compared with Aq group.

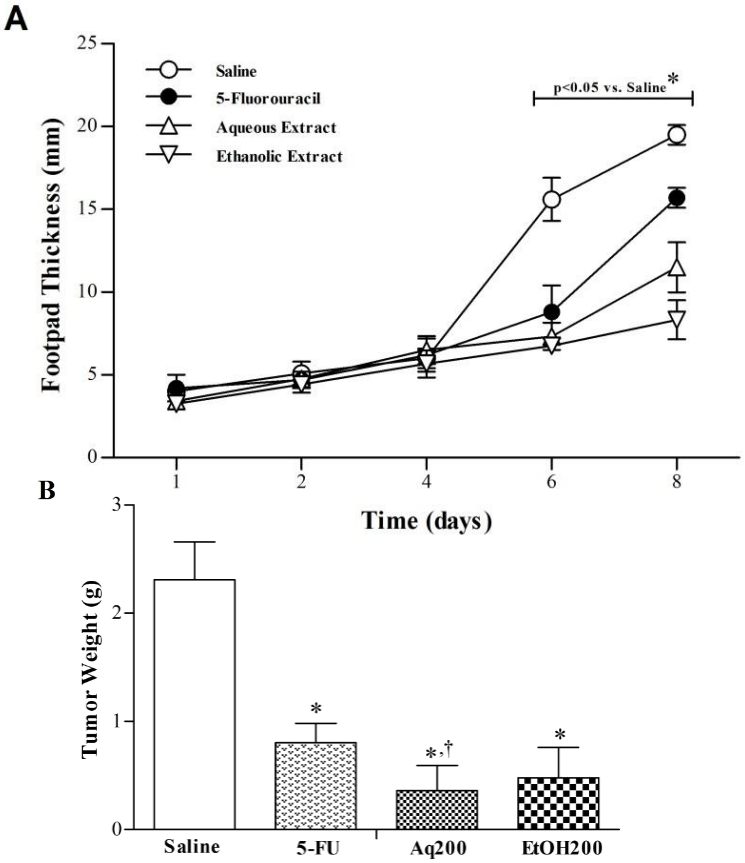

Figure 2. Effect of *C. urens* extracts on footpad thickness (A) and EC solid tumor weight (B). Data are reported as the mean ± S.D. Statistical differences from the controls were determined by ANOVA followed Tukey's Test. **P*<0.05 for all groups as compared saline treated group (negative control); †*P*<0.05 for all groups as compared with 5-FU treated group.

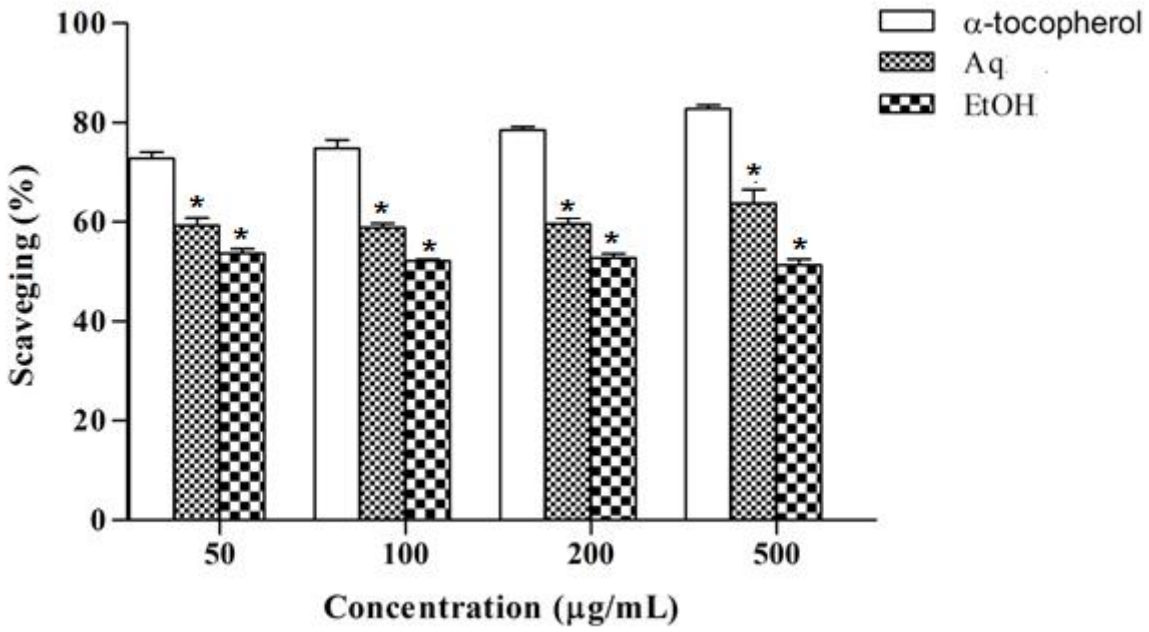

Figure 3. Radical scavenging activity of *Cnidoscolus urens* extracts by DPPH method. **P*<0.05 for all analysis as compared with α-tocopherol; $^{*}P$<0.05 for all analysis as compared with Aq group.

Table 1. Biological parameters in mice bearing EC solid tumor treated with *Cnidoscolus urens* extracts.

Biological Parameters	**Groups**				
	Normal Control	**Saline**	**5-FU**	**Aq200**	**EtOH200**
Liver (g)	1.84 ± 0.15	2.16 ± 0.10	2.27 ± 0.10†	1.97 ± 0.43	1.71 ± 0.29*,†
Spleen (g)	0.17 ± 0.02	0.18 ± 0.02	0.23 ± 0.03	0.23 ± 0.05	0.20 ± 0.07
Kidneys (g)	0.56 ± 0.04	0.51 ± 0.02	0.42 ± 0.03‡,*	0.42 ± 0.07‡,*	0.38 ± 0.06‡,*
Total Proteins (g/ dL)	5.24 ± 0.39	4.25 ± 0.17	4.42 ± 0.15	4.45 ± 0.46	3.53 ± 1.26‡
Albumin (g/ dL)	3.52 ± 0.19	2.37 ± 0.23‡	2.45 ± 0.16‡	1.97 ± 0.21‡	2.13 ± 0.80‡
Globulin (g/ dL)	1.72 ± 0.50	1.88 ± 0.11	1.97 ± 0.05‡	2.48 ± 0.42	1.40 ± 0.46
Urea (mg/ dL)	27.67 ± 3.78	46.55 ± 5.68‡	48.67 ± 5.07‡	27.1 ± 7.80*,†	38.1 ± 11.90
Creatinine (mg/ dL)	0.21 ± 0.01	0.5 ± 0.06 ‡	0.65 ± 0.09‡,*	0.22 ± 0.04*,†	0.25 ± 0.14*,†
Glucose (mg/ dL)	116.1 ± 10.40	81.3 ± 2.04‡	79.5 ± 2.80‡	127.9 ± 47.40‡,†	115.9 ± 17.20
TC (mg/ dL)	120.6 ± 9.10	116.1 ± 10.40	138.1 ± 11.30*	77.6 ± 8.10‡,*,†	88.3 ± 17.00‡,*,†
HDL-c (mg/ dL)	69.1 ± 5.20	63.3 ± 3.10	72.1 ± 8.20	41.1 ± 4.10‡,*,†	58.3 ± 18.90
Triglycerides (mg/ dL)	118.7 ± 5.90	292.5 ± 29.20	209.4 ± 25.30‡,*	167.8 ± 35.70‡,*,†	173.6 ± 31.20‡,*

Data represent the mean ± S.D. (n=6). Statistical differences from the controls were determined by ANOVA followed Tukey's Test. $^{\ddagger}P<0.05$ for groups as compared with normal control group; $^{*}P<0.05$ for groups as compared with saline treated group (saline group); $^{\dagger}P<0.05$ for groups as compared with 5-FU (positive control). TC – total cholesterol; HDL-c – high density lipoprotein cholesterol.

Table 2. Hematologic parameters in mice bearing EC solid tumor treated with *Cnidoscolus urens* extracts.

Hematologic Parameters	**Groups**				
	Normal Control	**Saline**	**5-FU**	**Aq200**	**EtOH200**
Total RBC	98.0 ± 26.90	71.2 ± 28.60‡	88.4 ± 25.60	93.5 ± 0.40*	84.1 ± 0.70
Total WBC	1.31 ± 0.05	1.25 ± 0.09	0.35 ± 0.06‡,*	2.74 ± 0.50‡,*,†	1.51 ± 0.26†
Hb (%)	15.0 ± 0.30	12.8 ± 0.40‡	13.9 ± 0.40	14.8 ± 0.40*‡	13.4 ± 1.30‡,*,†
PLT	1.31 ± 0.08	1.67 ± 0.12‡,*	1.13 ± 0.11	1.48 ± 0.35†	1.00 ± 0.02‡

Data represent the mean ± S.D. (n=6). Statistical differences from the controls were determined by ANOVA followed Tukey's Test. $^{‡}P<0.05$ for groups as compared with normal control group; $^{*}P<0.05$ for groups as compared with saline treated group (saline group); $^{†}P<0.05$ for groups as compared with 5-FU (positive control). RBC – red blood cells (x10^5 cells/mL); WBC – white blood cells (x10^4 cells/mL); Hb – hemoglobin; PLT – platelets (x10^6).

CAPÍTULO III

Manuscrito 2: Effects of methanolic extract of aerial parts of *Cnidoscolus urens* **(Euphorbeaceae) on the spontaneous contractile activity and induced castor oil in an animal model.**

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Effects of methanolic extract of aerial parts of *Cnidoscolus urens* **(Euphorbeaceae) on the spontaneous contractile activity and induced castor oil in an animal model.**

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ABSTRACT

Ethnopharmacological relevance: *Cnidoscolus urens* (Euphorbiaceae) popularly called "nettle" and well known for causing irritating reactions when in contact with the skin. Area native species of Caatinga, always present in sitations in ethnopharmacological surveys. Parts of this plant, root and stem are widely used as herbal medicines. *C. urens* popular believe that can be used for the treatment of cancer, inflammation, pain, kidney problems and diarrhea. But there is limit scientific study on its activity and mechanism against almost any of these diseases which are used as therapeutic, nor for the treatment of gastrointestinal disorders.

Aim of the study: To evaluate the anti-diarrheal activity and acute toxicity *in vivo* of the methanol extract of aerial parts of *C. urens.*

Materials and Methods: The methanolic extract of aerial parts of *C. urens* (MeOH) was tested at two different doses (200 and 400 mg / kg, p.o. in mice), against experimental models which evaluated the effect in normal stools and diarrhea models using the test Castor- oil induced diarrhea, enteropooling and Charcoal meal. The evaluated criteria were whether the administration MeOH (200 and 400 mg / kg) induce a change as the consistency and total number of stools in diarrhea model induced by castor oil; production and fluid accumulation was achieved by enteropooling model and intestinal transit as to the methodology of choice was the Charcoal meal test. The acute toxicity was evaluated following the guidelines of the OECD.

Results: The MeOHC extract *C. urens* was unable to cause toxic effects at the highest dose tested, which was 2000 mg / kg. For the antidiarrheal activity, the doses of 200 and 400 mg / kg MeOHC. urens showed a significant decrease in diarrhea, inhibiting in 68 and 87% respectively, the number of bowel movements. The MeOHC. urens (200 and 400 mg / kg) was also able to decrease to 3.4 and 3.0 respectively, wet number stools on castor oil induced diarrhea, compared to the control, which was 11.2, improving the consistency of the faeces over

of time. In an enteropooling assay of the two doses were capable of inhibiting fluid accumulation induced by oil, given value of 23.4, at a dose of 200mg / kg and 20.8, at a dose of 400mg / kg. Finally the Charcoal meal test demonstrated efficacy as the action of the intestinal transit. In this test it was obtained a reduction in motility percentage equal to 36.3 for the dose of 200 mg / kg and 25.5 to 400mg / kg, a significant reduction when compared to the negative control that reduced to a percentage of 79.3.

Conclusions: These results indicated MeOH extract an anti-diarrheal action, working on improving the consistency of stool, decreased intestinal motility and the accumulation of intestinal fluid.

Keywords: *Cnidoscolus urens* (Euphorbiaceae), Diarrhea, Castor oil, Motility and Charcoal.

1 Introduction

Gastrointestinal infections present as fear symptoms, diarrhea, which may be, caused by a wide array of pathogens. Among the main pathogens, we mention can bacteria, viruses and protozoa. In the meantime a single pathogen, it becomes primarily responsible for about 40% of hospital admissions for diarrhea due to the rotavirus. However, we can, not help but draw attention to other important actors, such as bacteria, including: *E. coli, V. cholerae*, *Shigella* and *Salmonella*. (WHO, 2009; Palla; Gilani, 2015)

Gastrointestinal disorders are usually, accompanied by muscular contractions that cause abdominal pain, constipation, intestinal colic, diarrhea or occlusions leading to an inflammation of the mucosa, this symptomatology is caused by the alteration in the movement of the smooth muscle of the small intestine, which can increase or decrease the speed of the Muscle contractility (Salaga et al., 2015). Intestinal transit, gastrointestinal tract length, muscle tone and myoelectric and contractile activity are a set of actions that contribute to gastrointestinal system (SGI). (Joseph et al., 2015).

According to the World Health Organization (2013), diarrheal disease is a leading cause of child mortality and morbidity worldwide. Developing countries are the most affected by these types of diseases, in these children under three years of age, have on average three episodes of diarrhea / year. The Acute Diarreal Diseases (DDA) is the second leading cause of death in children under five years. It is estimated, about 760 000 children under five will death/ year due to diarrheal disease origin, which is the main cause of malnutrition in children under five years of age, and 1.7 billion cases of diarrhea each year. Diarrhea is a major cause of malnutrition in children under five years of age.

Important cause of mortality and morbidity in Brazil and in developing countries, the DDA has a high prevalence (Ministério da Saúde Brasília/DF, 2004). According Eduardo et al., em 2005, the Brazil, in spite of limitations to how many records DDA cases reached more than 600,000 hospitalizations due to intestinal infections and about almost 8000 deaths. In 2011, there were 49,175 deaths from infectious and parasitic diseases (Ministério da Saúde, 2012a), and these numbers became more significant when analyzing the number of deaths due to ADD in children under 5 years of age, reaching a total of 756 per year In Brazil (Ministério da Saúde, 2012b).

Many countries live a socioeconomic condition of transition, like Brazil. And for this reason their populations coexist with important social problems that end up in severely affect public health. These problems such as lack of sanitation and environmental pollution, contribute to intestinal infections. This type of infection is, considered relevant because it can cause damage to the physical and intellectual development, in particular, children under 5 years (Santos et al., 2014). Other important factors that may be associated diarrheal diseases are reduced productivity at work, morbidity, increased use of medical and hospital resources, and unnecessary expenses (Palla; Gilani, 2015).

To support our research, the Caatinga, exclusively Brazilian biome, presents about 900 plant species (Zanirato, 2010; Pinheiro et al., 2013). They have aroused great interest among researchers from around the world, for its high therapeutic power and their ability to expand the arsenal of bioactive molecules (Cartaxo et al., 2010). The scientific community has shown that many species of plants are, enjoyed by the population as a therapeutic product and the Euphorbiaceae family it stands out for the wide use. Among those belonging to his family, give

emphasis on the gender Cnidoscolus (Gomes et al., 2014; Peixoto Sobrinho et al., 2012). In the Caatinga biome, the *Cnidoscolus* genre is represented by four, *C. infestus, C. pubescens, C. quescifolius and C. urens,* species that are used as therapeutic componentes, such as anti-inflammatory, appendicitis, rheumatism, kidney infections among other utilities (Peixoto Sobrinho et al, 2012; Agra et al., 2008; Coelho et al., 2012; Gomes et al., 2014).

The species *C. urens* gained great prominence in the popular medicinal, for always appear in quotes with therapeutic activities in ethnobotanical studies. To find out its biological activity, methanolic extract of parts of *C. urens* sand was, used to demonstrate its antidiarrheal effect in experimental models in mice.

2 MATERIALS AND METHODS

2.1 Chemicals

Castor oil (SD Fine Chem. Ltd.), loperamide [2 mg] were from square pharmaceuticals limited and Vincristine Sulphate (VINCRIRST ®) from Techno Drugs Ltd, atropine sulphate were purchased from Sigma Aldrich Chemie Gmbh (Taufkirchen, Germany), activated charcoal (Vetec), methyl alcohol (99.8% - Vetec).

2.2 Collect of Botanical Material

C. urens, commonly known as nettle, were collected in Agrestina, located 136 km from the capital Recife, state of Pernambuco, Brazil Northeasth, in Caatinga area (Lat 8 ° 23 '04.08", Long 35 ° 58' 49.7" w) from January 2014. The collected sample was, filed with the voucher specimen number 84.190, in the herbarium of the Agronomic Institute of Pernambuco (IPA).

Aerial parts (leaves, flowers and fruits) were, dried at room temperature (27 ± 28° C) and used to extract preparations.

2.3 Preparation of Extract

Methanol extract: 100 g aerial parts of *C. urens* collected and adequately dried, it was placed in a percolator and added to 1 liter of methanol, left overnight and then filtered with Whatman filter paper (no. 1), this procedure, was repeated until exhaustion.The extract was concentrated to dryness by rotary evaporator pressure Buchler Instruments, Fort Lee, NJ, USA.

2.4 Animals

Swiss male albino mice, weighing 30 to 35 g were, used for all experiments. All animals were, originally obtained from the vivarium Keizo Asami Immunology Laboratory (LIKA), UFPE, Brazil. The animals were, housed in polypropylene cages (47 X 34 X 18 cm^3), with sawdust at a temperature of 22 ± 2 ° C, with a schedule of 12 h light and 12 h dark cycle. Mice were, allowed to feed on Labina® chow, Purina, Brazil and water *ad libitum*. All tests described herein are in accordance with the Animal Care and Use Committee at the Federal University of Pernambuco (No. 23076.041082 / 2011-25) and Guidelines for Care and Use of Laboratory Animals of Laboratory

2.4.1 Study Desing

The study was, designed to evaluate the methanol extract of *C. urens* (MeOH) in different concentrations and different models of diarrhea induction. The activities I and II (Normal defaecation and Castor- oil induced diarrhea, respectively) and III and IV (Enteropooling and Charcoal meal, respectively) followed the same design distribution groups.

The mice (n = 24) Were divided in to 4 groups (n = 6, for group) as follows:

2.4.1.1 Drawing for the activities I and II:

Group (I) - Negative control, inducing diarrhea from castor oil and treated with saline solution only.

Group (II) - Control loperamide (10mg / kg), inducing diarrhea from castor oil and treatment with standard drugs.

Group (III) - Group test, inducing diarrhea from castor oil and treated with MeOH [200mg / kg].

Group (IV) - Group test, inducing diarrhea from castor oil and treated with MeOH [400 mg / kg].

2.4.1.2 Drawing for the activities III and IV:

Group (I) - Negative control, inducing diarrhea from castor oil and treated only with saline.

Group (II) - Control atropine sulfate (0, 25mg/kg), inducing diarrhea from castor oil and treated with standard drugs.

Group (III) - Group test, inducing diarrhea from castor oil and treated MeOH [200mg / kg].

Group (IV) - Group test, inducing diarrhea from castor oil and treated MeOH [400mg / kg].

2.5 Toxicity Study

Before starting any type of test, the extract was, tested for its acute oral toxicity. For the determination of acute toxicity, we follow the guidelines of the Organization for Economic Cooperation and Development (OECD, 2004). Swiss Albino mice, females and males, were, used to evaluate the tocicity of the methanolic extract of *C. urens*. The animals were fasted for 6 h, received free water. After the fasting period, the animals were, treated with a maximum dose of 2.000 mg / kg MeOH and diluted in saline solution. Administration of the extract was, done orally. The treated animals remained under observation for 4 h to detect any changes in their behavior such as: Piloerection, train survey, agitation, respiratory and cardiac frequency and then were evaluated for mortality, if there was mortality up to 24 h after administration of the extract of *C. urens.*

From the acute toxicity test, we can determine the dose of choice used for the treatment of the experimental tests, which corresponded to 10% of the maximum dose of the doxicity test, which is equal to 200 mg / kg. An additional dose, which corresponds to twice the dose of choice, being 400 mg / kg was, also used in order to evaluate if there would be improvement as the dose was increased.

2.6 Evaluation of the effect of the extract MeOH on normal defaecation (I)

The effect of the extract on the production of the normal number of stools was, assessed as described by Melo et al., 1988. Adult rats were fasted for 18 h with free access to water and

divided into four groups (n = 6) and treated according to the design described in item 2.4.1.1. The animals were, individually placed in plastic cages coated with filter paper.

The 4 groups were, treated orally, with saline, loperamide and MeOH (200 and 400 mg / kg). The total number of feces of each group was, counted every hour for 4 hours. The percentage reduction in the number of feces of the treated groups was, compared with the control group.

2.7 Evaluation of the Castor- oil induced diarrhea in mice (II)

Adult mice were, divided into 4 groups randomly fasted for 18 h with free access to water. The groups were, divided and treated in accordance with item 2.4.1.1. After 30 minutes of treatment was, administered to all animals 0.1 mL of castor oil orally. The animals were, placed in polyethylene cages lined with filter paper and evaluated for 4h for the production of diarrheal stools. The total count of diarrheal stools of treated groups was, compared to the control group, which was, considered 100% of diarrheal stools production. Thus, the results were expressed as % reduction of the production of diarrhea stools (Awouters et al., 1978). Percent reduction was, calculated by using equation as follows:

% reduction: [(Mean castor oil diarrhea score (control) – mean score of treatment group)/ Mean castor oil diarrhea score (control)] X 100

2.8 Castor oil induced enteropooling assay in mice (III)

As part of the studies that evaluated the antidiarrheal activity of MeOH extract, the accumulation of intraluminal fluid was determined by the method of Robert et al., 1976; Capasso et al., 2002; Gilani et al., 2005. The animals fasted for 24 h were divided into 4 groups (n = 6) and treated (saline, loperamide and MeOH 200 and 400 mg / kg), as described in activity III of the study desing (topic 2.4.1.2). After 30 minutes of treatment, all animals received 10 ml / kg of castor oil orally. A further 30 minutes after administration of the oil, the animals were, sacrificed by servic displacement and the small intestine was, removed. The small intestine was filled heavy (W1), then emptied and weighed again (W2) and the length, determined (L). The weight difference divided by the length demonstrates the enteropooling in mg / cm when applied the following formula:

Enteropooling: (W1-W2)/L

2.9 Charcoal meal in mice (IV)

To determine the transit of the small intestine, a solution of charcoal suspended in carboxymethylcellulose (CMC), orally administered in mice was, used as previously described by IZZO et al. (1992). The animals were fasted for 18 h with free access to water and divided into 4 groups. The four groups were, treated according to item 2.4.1. 2, being administered as saline treatment, atropine sulfate and MeOH (200 and 400 mg / kg). After 30 minutes of pretreatment, all animals received castor oil (0.2 mL). After a further 30 minutes, after administration of the oil, a suspension of CMC coal (0.5%) was, given in all animals orally. After 30 minutes of administration of the charcoal suspension, the animals were, sacrificed. The

abdomen of each animal was, opened, removing the small intestine from the pylorus to the cecum. The small intestine was, carefully stretched under a tape measure to obtain, in centimeters, the total length of the small intestine and the distance traveled by the charcoal. Intestinal transit was expressed as a percentage of the total intestinal length and the values applied in the following formula: Intestinal transit (%) = (DC / LSI) x 100, where DC is the distance reached by charcoal and LSI refers to the length Total of the small intestine.

2.10 Statistical evaluation

The results were submitted to analysis of variance (ANOVA) followed by Tukey's multiple comparison test, with $p < 0.05$ considered to denote a statistically significance .all data were expressed as mean values ± standard deviation (SD).

3 RESULTS

3.1 Toxicity activity

The MeOH extract was, used to test its possible toxicity at a dose of 2000 mg / Kg in albino camundogos, by oral route, which has shown to be devoid of any toxic effect. Thus, for the antidiarrheal effect studies, doses of 200 and 400 mg / kg of MeOH extract were used.

3.2 Evaluation of the effect of MeOH extract on normal defaecation

Oral administration of MeOH 200 and 400 mg / kg showed that there was an inhibition percentage of the number of evacuations of 68 and 87% respectively, during the 4 h evaluation. In the same way the group that was treated with loperamide showed a percentage of 74% inhibition compared to the negative control.

3.3 Effect of the MeOH extract on the diarrhea induced by castor oil in mice

C. urens extract was, used to test its ability to inhibit the diarrhea caused by castor oil after 4 hours. The negative control group, the castor oil administration promoted early on, an increase in the total number of bowel movements and loose stools. The treatment prior to administration oil with MeOH (200 and 400 mg / kg) and loperamide, caused a reduction in the total number of stools, and the final number of diarrheal stools, plus an increase in the first time defecation. It can be observed an improvement in the consistency of fecal material causing a change from solid to aqueous state. These results can be found in Table 1.

3.4 Castor oil Induced enteropooling Assay in Mice

The castor oil adiministration a dose of 0.2 mL induces an increase in the production and intestinal fluid accumulation in mice, as can be seen by the control group, Figure 1. Treatment with MeOH at doses of 200 and 400mg/kg, was shown to be significantly effective ($p < 0.0001$) inhibition of fluid accumulation induced by the oil, given a value of (23.4 ± 1.9

and 20.8 ± 3.2), respectively, when comparison is made with the control negative that was equal to (30.2 ± 3.9).

3.5 Evaluation of the small intestinal transit model charcoal meal in mice

The effect of MeOH on intestinal motility induced a change in the rhythm of intestinal transit in mice. In the table 2, we can see that, the negative control group showed a distance traveled by coal in % 79.3 ± 4.7. The groups treated with loperamide and MeOH (200 and 400 mg / kg) showed an average 19.7 ± 2.3, 36.3 ± 4.5 and 25.5 ± 4.7 respectively. The groups treated with MeOH and atropine sulfate, produced a significant reduction of motility precentual when measuring the distance traveled by the charcoal in the intestine compared to the negative control. When comparing the average dose was, taken between 200 and 400 mg / kg treated groups MeOH can, not be observed significant effects.

4 DISCUSSION

It affects children more frequently, so diarrhea is a national health and common problem among people with low hygiene standards. The number of causes of morbidity and mortality and diarrhea in all age groups, is higher in developing countries. It is, estimated that 4 million cases of diarrhea occur each year (Sachdeva et al., 2012). Therapies currently used as rehydration has, been indicated with good prognosis in cases of diarrhea Acute. Between both chronic cases are more, serious problems, and can progress to death of the patient.

The disturbing and shocking situation of diarrhea causes, contributes to the search for new drugs, and the plants are targeted at the moment. Despite the wide availability of simple and inexpensive for diarrhea treatments, poor communities and popular still use and rely on herbal remedies, herbal, being extremely contribution the herbal treatments and research the background of its pharmacological action (Heinrich et al., 2005; Smith et al., 2016).

The use of castor oil diarrhea inducer is common and has been reported (Bamisaye et al., 2013). The obtained, oil is accomplished by extraction *Ricinus communis* seeds (Guo et al., 2014). An active metabolite, castor oil constituent is ricinoleic acid. In the intestinal mucosa, ricinoleic acid has an irritant causing inflammation and changes in the permeability of membranes of water and electrolyte (Guo et al., 2014; Bamisaye et al., 2013). Quickly becomes fluid and the aqueous contents of the intestinal lumen, resulting in a hypersecretory response (Guo et al., 2014). These events contribute to the release of substances such as prostaglandin, nitric oxide, platelet-activating factor and cAMP. Luderer et al (1980) found experimentally that mice had a high serum concentration of prostaglandins, after oral administration of castor oil. These substances induce an increase in the motility and secretion of the intestinal fluid, in addition to reducing the reabsorption of sodium and potassium ions. (Guo et al., 2014; Bamisaye et al., 2013). Thus, the induction of diarrhea model castor oil is the motility and secretory diarrhea (Guo et al., 2014).

Some synthetic drugs which act by inhibiting prostaglandin synthesis, are recommended to retard diarrhea induced by castor oil (Bamisaye et al., 2013). A drug sample holding action antagonist to the castor oil is loperamide, used against diarrheal disturbances (Magaji et al.,

2007). It reduces fecal volume, intestinal fluid and the unnecessary excretion of electrolytes (Sarin et al., 2013).

The drug causes almost immediate inhibition peristalsis, by decreasing the circular and longitudinal muscle action in the intestine, besides having antisecretory activity by acting on opipaceos receptors. Atropine is an alkaloid extracted, mainly from the plant Atropa belladona, consists of a powerful muscarinic blocker with action in the central and peripheral nervous system. Being commercially available as atropine sulfate, it is a drug that works by blocking the action of acetylcholine on receptors present on smooth muscles and is therefore an antispasmodic agent (Sarin et al., 2013).

In this way, we can prove that the methanol extract of *C. urens* in the two tested doses, may be, indicated as having antidiarrheal activity since caused decreased frequency of stooling and fecal parameters such as, number of stools, physical condition (dry, or wet pasty), intestinal transit and production of fluid.

Shalaby et al. (2015) demonstrated antidiarrheal activity for the aqueous and ethanolic extracts of *Euphorbia helioscopia* (250 mg / kg and 500 mg / kg, respectively) belonging to the Euphorbiaceae family. Its finding was, based on tests of diarrhea induced by castor oil and charcoal meal, observing a significant decrease in the number of feces and reducing intestinal transit. Antidiarrheal activity induced by oil, was also, achieved when ethanol extract of *Cronton grewioides* (Euphorbiaceae) induced a significant reduction in frequency of stool and the number of liquid stools. The same extract was, tested for its action on the intestinal transit and intestinal fluid accumulation (Silva et al., 2016).

Other authors also reported several other species of Euphorbiaceae with antidiarrheal activity. As is the case of methanol extract of the bark of the stem of *Jatropha curcas* (100 and 300 mg / kg) that has been, shown effective in the treatment of diarrhea (Sacheva et al., 2012). To test for efficacy of another species activity, aqueous extract of the stem bark of *Sapium ellipticum* was used and during the tests, it was observed that animals treated had reduced start time of the first stool, decreased frequency of defecation and intestinal transit, suggesting strong antidiarrheal action (Wansi et al., 2014).

Preparation of aqueous and methanolic extract of *Sebastiania chamaelea* leaves, administered at a dose of 100 and 200 mg / kg were, used to establish the antidiarrheal activity of this species. A significant antidiarrheal activity has, been demonstrated up to 90% for this kind of treatment. Yasodamma et al (2013) suggest that activity may be related to the present tannins and cinnamic acid in the extracts causing astringently and anti-inflammatory action resulting in an effective antidiarrheal action.

Authors suggest that the antidiarrheal activity of Euphorbiaceae family species can this related to the anti-inflammatory capacity and the anti-inflammatory activity, which has also, been proven for other species of this family, such as *Cnidoscolus quercifolius* (Gomes et al., 2014). Ethanolic extracts of bark and leaves of *C. quercifolius* (100, 200 and 400 mg / kg, ip) was used to test the antinociceptive activity, and the end of the experiments was supported popular use of this plant for the treatment of inflammatory pain. Magaji et al. (2007) also portrays the anti-inflammatory activity of extracts from leaves, bark and root *Securinega virosa*

(Euphorbiaceae) exhibited anti-inflammatory action in paw edema induced by carrageenan and writhing induced by acetic acid.

The biosynthesis of prostaglandins has been involved in inflammatory processes. Many plants are, related to inhibitory effect of these substances production, especially those that produce flavonoids as its secondary metabolite. It has been, well described in the literature that flavonoids may alter biosynthesis of cyclooxygenase and lipoxygenase inhibiting inflammation (Magaji et al., 2007).

The activity demonstrated by our studies with methanolic extract of aerial parts of *C. urens*, can probably be, attributed by the presence of flavonoids and tannins with or without combination with other constituents. The presence of such secondary metabolites in extracts of *C. urens* has, already been proven in studies by Peixoto Sobrinho et al. (2012). In the diarrhea induced model castor oil, we had a decrease in the number of wet stool next to loperamide group. We have also achieved a significant decrease as the decrease the accumulation of fluids and intestinal transit. This scenery can corroborate wich the results of other surveys conducted with other species of the Euphobiaceae family, as demonstrated above. We therefore suggest that the results demonstrated that methanol extract of *C. urens* can inhibit the motility and hypersecretion induced by castor oil.

5 Conclusion

This study thus demonstrates that methanol extract of aerial parts of *C. urens* has significant antidiarrheal activitys. This activity is, correlated with its inhibitory effect of

propulsion and intestinal secretion. This study also claims that the dynamic mechanism involved in these findings may be the main inhibitory activity of inducing substances inflammation and hypersecretory activity. Thus, the indication of this herbal medicine for the treatment of diarrhea is justified by the pharmacological properties described herein. Studies about extract mechanism of action should be performed, especially by hardly exist descriptions with antidiarrheal activities for this species.

Conflict of Interests

The authors have not declared an conflict of interest.

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Table 1. Effect of the MeOH (200 and 400 mg/kg) extract of *C. urens* on castor oil induced diarrhea.

Treatment	**Dose (mg/Kg)**	**Total nª of stools**	**Nº of dry stools**	**Nº of pasty stools**	**Nº of wet stools**
Control	-	11.2 ± 2.7	0.5 ± 0.8	0 ± 0	10.7 ± 2.1
Loperamide	2	5.8 ± 3.3	1.5 ± 1.6	0.8 ± 1.6	3.5 ± 0.8
MeOH	200	7 ± 0.9*	2.3 ± 0.5	1.3 ± 1.0	3.4 ± 1.4*
MeOH	400	7.2 ± 1*	2.6 ± 0.5	1.6 ± 1.4	3 ± 1.5*

*Data are presented as mean ± SD.P values are significantly diferente from control using Tukey post hoc test (n=6). P< 0.0001.

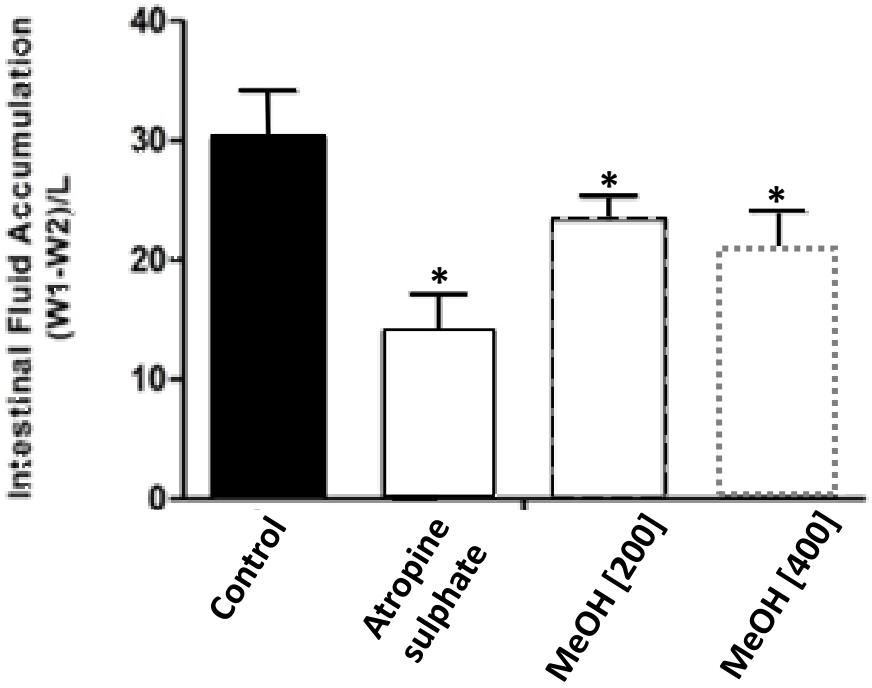

Fig 1: Effect of the MeOH (200 and 400 mg/kg) extract of *C. urens* on enteropooling induced by castor oil in mice. P<0.001 vs. Control.

Table 2. Effect of the MeOH (200 and 400 mg/kg) extract of *C. urens* on small intestinal transit in mice.

Parameters/ dose	**Control**	**Atropine sulphate**	**MeOH**	
			200 mg/Kg	**400 mg/Kg**
LSI (cm)	50.3 ± 2.3	50.8 ± 1.9	57.7 ± 2.7	57.5 ± 4.3
DC (cm)	39.8 ± 2.3	10 ± 1.1	21.2 ± 6.8	14.5 ± 7.5
Intestinal transit (%)	79.3 ± 4.7	19.7 ± 2.3	36.3 ± 10.6*	25.5 ± 15.8*

*Data are presented as mean ± SD. P values are significantly diferente from control using Tukey pos hoc tes (n= 6). P< 0.001.

CAPÍTULO IV

Manuscrito 3: Potential effect of extracts of *Cnidoscolus urens* **on** *Sitophilus zeamais***, an insect Prague Biology.**

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Manuscrito a ser submetido ao periódico Pesticide Biochemistry & Physiology **no formato Original Research Article (FI:** 2. 388**; QUALIS CBII).**

Potential effect of extracts of *Cnidoscolus urens* **on Sitophilus zeamais, an insect Prague.**

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ABSTRACT

Products obtained from botanical material (extracts, oils and purified compounds) were suggested as an excellent alternative for the control of stored grain pests. The deterioration of these grains by pest insects leads to unpredictable economic losses. Species such as *Sitophilus zeamais* are responsible for crop damage of many foods, grains, rice and wheat. Conventional insecticides present high environmental and human toxicity, as well as causing many cases of resistance. Plants are currently target in the alternative search for new products with insecticidal action. In this study, we demonstrated the effect of crude methanolic extract (MeOHC.urens) and fractionated (ether, chloroform, acetone and methanol (MeOHFrac.). The choice of the use of the types of extracts was based on the extracts that presented the best results. From the aerial parts (leaves, flowers and seeds) of *C. urens,* extracts MeOHC. urens and MeOHFrac available from ingestion, which adult insects of *S. zeamais,* divided into 5 groups, were exposed to a diet containing MeOHC. urens (0.18, 0.25, 0.5 mg / g) and MeOHFrac (0.25, 0.5 mg / g) showed an induction of 27 to 90% and 33 to 91% in ingestion mortality, respectively. For the same extracts at concentrations of 50 and 100 μg / mL and presented 45 to 75% and 77 to 87% of mortality respectively.The LC50 was also determined, both by ingestion for MeOHC.urens and MeOHFrac (LC50 = 0.24 and LC50 = 0.11, respectively), and by contact for the same extracts (LC50 = 0.012 and LC50 = 0.026), respectively. These methodologies also allowed us to evaluate that the extracts induced significant changes in the nutricionail indices, leading to a decrease of the biomass, being perceived by the negative values of the relative biomass gain and conversion of ingested foods, in addition to inducing food rejection, inducing a strong deterrence to the food source available during the test. In conclusion, the extract of aerial parts of *C. urens* potential for the control of *S. zeamais*. The deleterious effects of extracts may be associated with the presence of flavonoid and terpenes.

Keywords: *Cnidoscolus urens;* Insecticidal activity; *Sitophilus zeamais*

1 Introduction

The first reported insecticide resistance occurred about a century ago. Soon after, other sporadic cases of insecticide inactivity were reported. In the mid 1940, new management methods were implemented, such as synthetic organic pesticides, for example organophosphates and phosphine, which were then used in biological control. Synthetic compounds were efficient in their insecticidal activity, resulting in large-scale application [6].

Consequently the great use of pesticides, resistance cases have rapidly increased again, along with the number of resistant species and new compounds. Over the decades, herbicides and fungicides have also shown reduced efficiency, but resistance to insecticides has increased [6]. Attempts to control insect pest attacks, by synthetic compounds, are volatile, toxic to the environment and to the human, contribute to insect resistance and target non-target insects. Cleaning, drying, aeration and temperature control of stored grains are also alternatives to combat, however they still do not seem to be enough [4,7].

Populations of agricultural and urban insect pests have demonstrated an evolution in their physiological and behavioral mechanism, becoming increasingly resistant. In view of this condition, the importance of more accurate information before the choice to replace the new control method to be used in plantations, storage places and urban centers [8,9,10,11] is important. Many insect species are being reported and causing post-harvest losses [12, 13].

The three major grain pests belong to the same genus, *Sitophilus* [14]. *S. zeamais*, from brown to reddish color, is described among the three [2,15]. It presents preference for cereals such as wheat, rice and corn, which are the most cultivated, consumed and well distributed in the world [16, 17]. Adult individuals and larvae feed on intact grains and cause damage such as weight loss and economic value [16,18]. Tefera et al. [19], found that the damage begins at harvesting, with about 10% of perches and continues to spread during storage, reaching up to 50% [4]. *S. zeamais* presents a moderate level of resistance to some synthetic insecticides [2]. Meanwhile the increase in the levels of inactivity are expected due to excessive application, in addition to environmental damages [8].

Proved by several studies [1,8,11], the resistance to existing pesticides is very important and in view of the great losses caused, the development of new control strategies, with greater specificity for the target species, low toxicity to the environment Environment and the farmer, in addition to minimizing waste, are increasingly sought after [12,20]. A new way out can be found in our biodiversity.

Pest control is a worldwide practice [1]. Public Health and Agroindustry organs have been faced with major concerns regarding care and protection with food grown and stored [2,3]. Among the foods most attacked by pests are wheat, rice, barley [4], corn and even industrialized foods such the pasta [2]. According to Lira et al. [4], the damages caused by insect pests are devastating, leading to economic and environmental damages. These damages account for about 30% - 60% of total production [5], contributing to the loss of billions of dollars [2]. However, the pest perch occurs from agricultural products and non-renewable source products [2].

Biodiversity and its chemical compounds have been explored for their biological performance. Extracts and purified plant compounds are the main focus of these studies. These products are being reported with high insecticidal potential for many different species of these organisms including species that have created resistance to existing insecticides [21,22].

Some plant species have already been indicated with insecticidal properties, *Jatropha curcas, Ricinus communis* [1], *Tithymaloides pedilanthus, Phyllanthus amarus, Euphobia hirta and Euphorbia tirucalli* [23]. These species, which are part of the Euphorbiaceae family, have been tested against insects such as *Anopheles stephensi, Aedes aegypti* L. and *Culex quinquefasciatus* [1,23], however, the number of studies proving their insecticidal activity on Euphorbiaceae species is still few when tested against *S. zeamais*. This evidence is even smaller, when we speak of some species of the family Euphobiaceae, as is the case *Cnidoscolus urens.*

C. urens is a weed species, which has a wide distribution throughout the Brazilian territory, mainly in the Caatinga area [24,25]. Popularly, this species is used for the treatment of hemorrhage, cancer and inflammation [26,27,28]. Among the rare studies, antibacterial actions [29] and fibrinolytic could be attributed to the use of derivatives of this species [26].

In this study, we report for the first time, the biocidal properties of different extracts of aerial parts of *C. urens*, evaluating its insecticidal action with *S. zeamais*. In addition to suggesting a new low cost and sustainable botanical insecticide [30].

2 Matherials and Methods

2.1 Plant material

The collection of *C. urens* samples was carried out in the city of Agrestina, located approximately 136 km from the capital of the state of Pernambuco, in northeastern Brazil (Lat 8 ° 23'04 .08 ", Long 35 ° 58'49 .7" W). The sample period was collected in January 2014. A small part of the collected botanical material, containing root, leaves, flowers and fruit, was prepared for identification and sent to the Herbarium of the Instituto Agronômico de Pernambuco (IPA). The species collected and used for biological testing was deposited and identified by the number 84.190. The aerial parts (leaves, flowers and seeds) of C. urens were, dried at room temperature (27 to 28 ° C) and used to prepare extracts.

2.2 Preparation of Methanolic Extract

Methanolic extract: Initially, the collected botanical material was sanitized and separated 100 g of aerial parts of *C. urens*. Then, the material already selected was adjusted for drying in the oven at room temperature (27 to 28 ° C) and controlled in a drying oven for botanical material. After drying, the plant material was triturated and placed in a erlenmeyer, 100mL of methanol was added and left overnight, then filtered with Whatman paper (No. 1). This process was repeated several times until complete exhaustion. The methanolic extract of *C. urens* (MeOHC.urens) was concentrated by rotary evaporator pressure Buchler Instruments, Fort Lee, NJ, USA, resulting in the corresponding crude organic extract (Figure 1) [31].

2.2.1 Fractionation of the methanolic extract

Aerial parts (100 g) of *C. urens* dried and crushed were used to obtain fractionated extracts. Initially 100 mL of ether was added to the plant material and kept under stirring for 1h, then left under refrigeration for 24h. After 24 h the solvent was filtered and the same volume of ether added again. This procedure was repeated 3 times with each solvent (ether, chloroform, acetone and methanol) using the same plant material (Figure 1). This method of extraction allowed the probable separation of the major secondary metabolites resulting in a total of 4 different types of extracts.

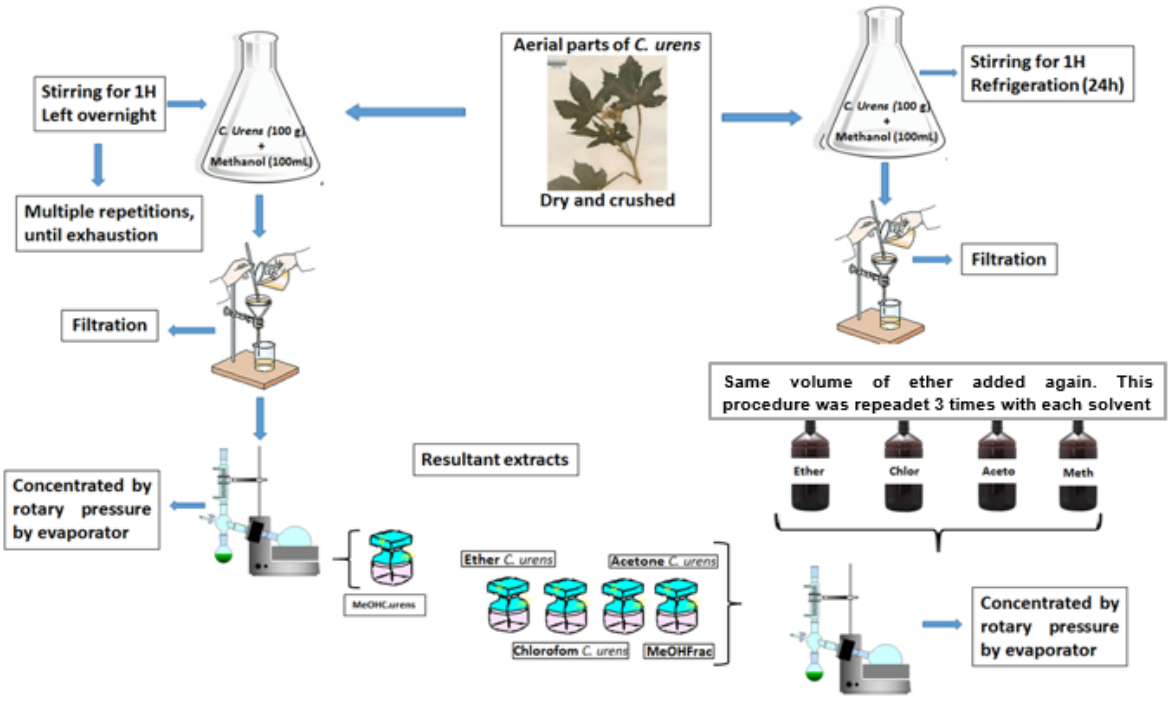

Figure 1: Diagram of production of raw extracts and partitioning of *C. urens.*

2.3 Ethical considerations

All experimental procedure used for the construction of this study were submitted for evaluation and approval by the Animal Experiment Ethics Committee of the Universidade Federal de Pernambuco, from the Center for Biological Sciences (CEEA-UFPE). Under protocol approval 23076.012693/2016-71.

2.4 Insects

Adult insects of *S. zeamais*, taxonomically identified by specialists from the Department of Biology of the Federal University of Pernambuco (UFPE), were obtained from colonies kept in the Department of Mycology of UFPE, located in the city of Recife, Brazil. The insects were kept in breeding grounds at the Natural Products Laboratory, Department of Biochemistry, also from the same university. The creation of the insects was granted permission, Nº. 36301-2, from the Chico Mendes Institute for Biodiversity Conservation (ICMBio) of the Ministry of Environment.

The insects purchased were placed in a glass container (1L), sealed with nonwoven (TNT) fabric, allowing the aeration at a relative humidity of 70%, in addition to photoperiod of 12: 12 ligth: alonk and temperature 28 ± 2 ° C. The individuals of *S. zeamais* were submitted to a diet of corn grains, selected under conditions of health, sanitary and absence of contamination. The insects used for the tests were approximately 30-50 days old.

2.5 Bioanalysis of toxicity by ingestion of extracts of *C. urens*

The toxicity level of the different types of extracts of *C. urens* was determined following the methodology of Xie [32], with adaptations described by Napoleão [2], that was used to determine the insecticidal activity of *C. urens* extracts. For each test, a suspension of 2 mg of properly-prepared wheat flour (Dona Benta, Bunge Alimentos S.A., Benevides, PA, Brazil) was used, homogenized with a solution of 5 mL of the 5 extracts (MeOHC. urens, ether, chloroform, acetone and MeOHFrac), in the concentrations 0.18, 0.25, 0.5 mg / g with sterile distilled water. Five aliquots (200 µL) of the obtained solutions were distributed in Petri dishes (90 mm x 100 mm, of known weight) with the help of micropipette coupled to a disposable and cut tip (2mm internal diameter). Subsequently, placed in a greenhouse overnight, at 37 ° C to dry. Thereafter, each plate was reweighed and the weight of the dough was determined by the difference of the empty plate between the plate and the flour disc. Twenty individuals of *S. zeamais* (n= 20), of known weight, were placed on each plate. Finally, each test was performed in quadruplicate and the tested concentrations of samples on disc at the following final concentrations [0.18, 0.25, 0.5 mg/g] (mg of each extract/g of wheat flour). For comparison, another group of the same *S. zeamais* number was maintained in petri dish containing only the flour solution diluted in sterile water.

All groups were maintained at 28± 2 ° C in the dark for 15 days. At the end of this period, can determine the mortality rate and weights of flour discs.

2.6 Determination of feeding-deterrence index and nutrition

The determination of feeding-deterrence (FDI) was achieved by applying the following formula: FDI (%) = 100 x (A-B) / (A). Where A is the feed mass ingested by the control test insects and B is equal to the feed mass ingested by the groups of insects exposed to the different extracts [33]. The difference between the weight of the plate containing the flour discs (0 days) and the empty plate (15 days), was used to determine the FDI. In agreement with the IDE, the samples were classified: Strong feeding deterrence (FDI ≥ 70%), moderate feeding deterrence (70% > FDI ≥ 50%), weak feeding deterrence (50% > FDI ≥ 20%) or feeding deterrence (FDI < 20%) [34]. The data found by the assay described in the above table can be used to determine the relative consumption rate (RCR) by the formula: RCR = C / (D x Days), where C, the ingested feed mass (mg) and D corresponding to the initial biomass of the insects (mg). The ratio of the biomass gain rate (RBGR) = E / (D x Days), where E is equal to the biomass obtained (mg). Regarding the efficacy of food intake concersion (ECIF) = E / (C x 100).

The concentrations applied in this study and varied from 0.18 to 0.5 mg /g were determined according to the LC_{50} values were cauculated using StatPlus. These concentrations were, applied to the tests for the determination of ingestion toxicity against *S. zeamais*, being able to vary between the types of extracts.

2.7 Evaluation of contact toxicity of *C. urens* extracts

To determine the toxicity potential of *C. urens* extracts on populations of *S. zeamis*, the assay was performed according to Liu and Ho [35]. The extracts MeOHC. urens and MeOHFrac

(50 and 100 μg / mL) were solubilized in distilled water. From the aliquot removal solutions (0.5mL), was applied with a micropipette, on the dorsal surface of the insects thorax. For this test, 5 groups (n= 20) each were created and each experiment was repeated in quadruplicate. The group classified as control only distilled water. The other groups make up the tested groups. Mortality of insects was observed daily until end-point, when the number of dead insects did not increase over time.

The ability to induce toxicity of extracts from aerial parts of *C. urens* was evaluated by 5 different extracts: crude methanol (MeOHC.urens), and the fractions: ether, chloroform, acetone and methanol (MeOHFrac) as described in item 2.2. 1. Initially pilot tests were performed, which helped to select the extract with better efficiency with the tested insects. From the pilot test, two extracts, MeOHC.urens and MeOHFrac, which showed a significant insecticidal effect when compared to the control were selected, the other extracts practically did not exert effective mortality. Regarding to the choice of concentrations to be tested, they were determined according to the lethal dose (LC_{50}).

2.8 Statistical analysis

GraphPad Prism version 5.1 for Windows (GraphPad Software, San Diego, California, USA) was used to express a mean of replicates ± SD. Significant differences between the test treatment groups were developed by the Tukey's test for multiple comparisons (significance $p < 0.05$) using PRISMA software (GraphPad Software, Inc., San Diego, CA, version 5.01). For determination of lethal concentration to kill 50% of insects (LC_{50}) Probit was used with a 95% confidence interval with StatPlus ® 2008 computer software (AnalystSoft, Canada) [36].

3 Results

3.1 Determination of mortality by ingestion of *S. zeamais* from extracts of *C. urens*

Thus, it was possible to identify that MeOHC.urens extracts at all concentrations (0.18, 0.25; 0.5 mg/ g) and MeOHFrac (0.25; 0.5 mg/ g) had a significant effect ($p < 0.05$) on the percentage of ingestion mortality when compared to the control (Figure 2), during the ingestion toxicity bioassay. As for the percentage of mortality, MeOHC.urens induced a percentage of 27, 73 and 90% death at concentrations (0.18; 0.25; 0.5 mg / g), whereas MeOHFrac induced 33 and 92% mortality at concentrations (0.25; 0.5mg / g).

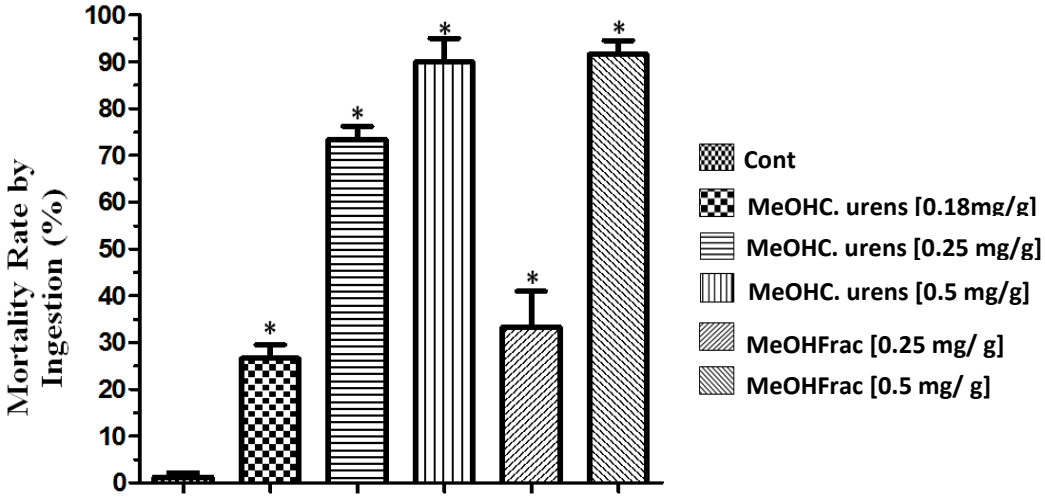

Figure 2: Percentage of mortality by ingestion of *S. zeamais* exposed to different concentrations of MeOHC.ures and MeOHFrac of *C. urens*. * $P < 0.05$ for all groups compared to control.

3.2 Determination of the effects of extracts of *Cnidoscolus urens* on nutritional indexes

The nutritional results obtained through the ingestion methodology, including the application of both extracts of *C. urens* in the diet available for *S. zeamais*, interfered in the nutritional development of the insects distributed in the test groups, significantly affecting nutritional indices (Figure 3). The relative mean biomass gain (Figure 3A) and the average feed conversion efficiency (Figure 3B) were remarkably affected, approaching zero or becoming negative, according to the increase in the concentration of the extracts tested. These findings reveal that insects were unable to ingest and / or digest the available food and still used their nutritional reserves to generate energy. The biomass gain is relevant when we compare the lowest and highest concentration of MeOHC.urens.

Meanwhile, with respect to feed intake conversion efficiency (ECI), only MeOHFrac (0.25 mg / g) was not significant when compared to the control. In addition, the relative consumption rate (RCR) was also affected, observing a significant and significant reduction in insect food consumption (Figure 3C) in all groups that received both extracts and in all concentrations when compared to the group control ($p < 0.05$).

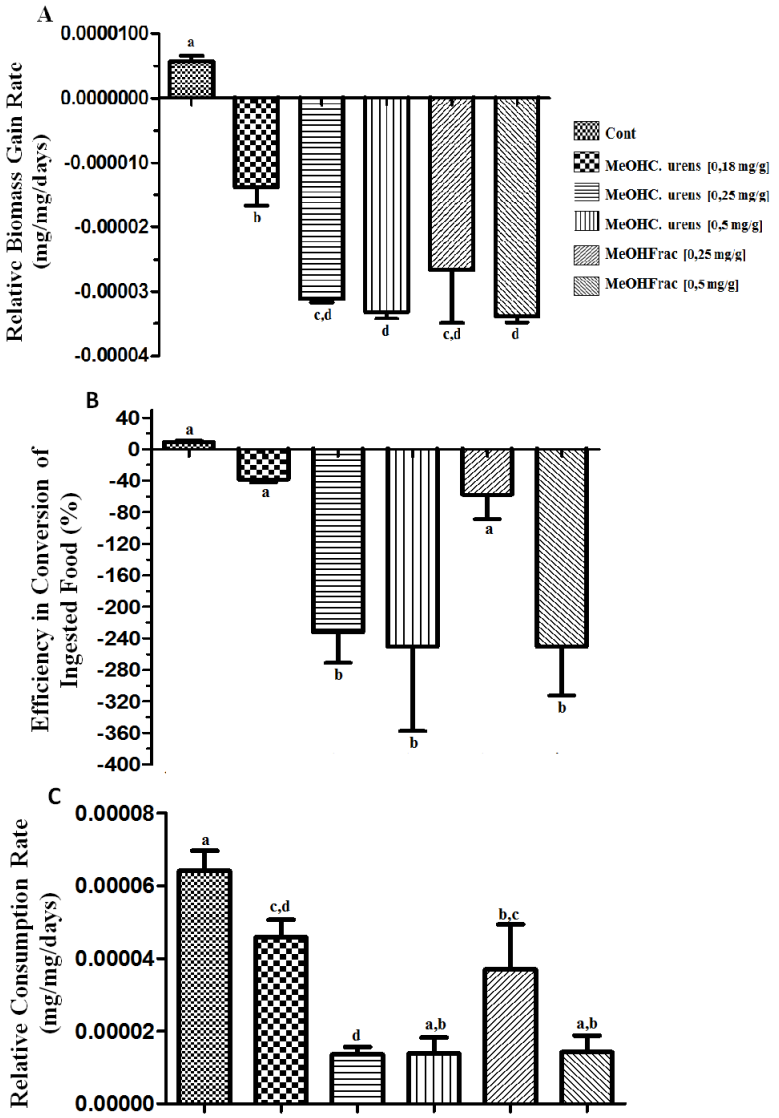

Figure 3: Evaluation of the anti-feeding action of *S. zeamais* grown in artificial diets (0.18 - 0.5 mg / g of wheat flour). (A) Rate relative to biomass gain (RBGR) indicates a biomass (mg) gained every day per mg of initial body weight. (B) The percentage of conversion of ingested food (ECI%) indicates the amount of food consumed and converted to insect mass. (C) A relative consumption rate (RCR) indicates an amount of food consumed by *S. zeamais* (mg) per mg of body weight per day. Each column corresponds to the mean ± SD of five replicates. The different letters are indicative of the significant differences ($p < 0.05$) between the treatments by the Tukey's test.

3.3 Feeding-deterrent index (FDI)

As regards the feeding-deterrent effect, representing the capacity of repulsion to the food, it was observed that the MeOHC.urens and MeOHFrac (0.5mg / g) had an FDI of 72 and 78% respectively, having a strong action effect, according to the Classification of Liu [34]. As the concentration is decreased observed a weak action, corresponding to 21 and 38% (Table 1).

As the concentration increases MeOHC.urens (0.18- 0.5 mg / g) showed a strong effect on the percentage of FDI increasing from 21 to 72% (Table 1). Similar behavior can be identified in the groups receiving food impregnated with MeOHFrac. A 38% feeding-deterrent percentage was found for the concentration of 0.25mg / g, becoming strong, 78%, when there was an increase in concentration (0.5mg / g). For this, the MeOHFrac shows a slightly better effect, but with no significant difference between the two extracts.

Table 1: Effect the feed-deterrence index of extracts of *C. urens* against *S. zeamais*.

Group	Concentration (mg/g)	% FDI (Mean±SD)	Classificação (Segundo Liu et al.[43])
MeOHC.urens	0.18	21 ± 0.003	FDI- Fraca
	0.25	78 ± 0.002	FDI- Forte
	0.5	72 ± 0.004	FDI- Forte
MeOHFrac	0.25	38 ± 0.008	FDI- Fraca
	0.5	78 ± 0.002	FDI- Forte

The data here are expressed, represented by mean ± SD.

3.4 Ingestion toxicity assessment of extracts of *C. urens*

MeOHC.urens has promoted mortality of 50% of the insect population with an LC_{50} of 0.236 ± 0.01mg / g, while MeOHFrac presented an LC_{50} of 0.114 ± 0.03mg / g (Table 2).

Table 2: Determination of the lethal dose (LC50) of extracts of *C. urens*, capable of inducing mortality in 50% of the population of *S. zeamais* by ingestion.

Group	**LC50 (mg)**	**95% Confidence limits**	
		LCL	ULC
MeOHC. urens	0.236 ± 0.01	0.209	0.263
MeOHFrac	0.112 ± 0.03	0.061	0.167

LC50 values were cauculated using StatPlus. The 95% confidence limits between the paragraphs were based on five replicates (P<0.05). LCL: Lower confidence limit. UCL: Upper confidence limit.

3.5 Determination of mortality of *S. zeamais* by contact of *C. urens* extracts

The toxic potential of MeOHC.urens and MeOHFrac was significantly ($p < 0.05$). Meanwhile of all concentrations and types of extracts tested, only MeOHC.urens (50µg / insect) did not present a significant percentage (45%) when compared to the control (38%). MeOHC.urens (100µg / insect) presented an average of 75% (Figure 4), being significantly different from the control.

Better results were observed when the insects were submitted to MeOHFrac contact (50; 100µg / insect) with averages of 77 and 78% (Figure 4) of mortality, respectively, and were therefore significant when compared to the control.

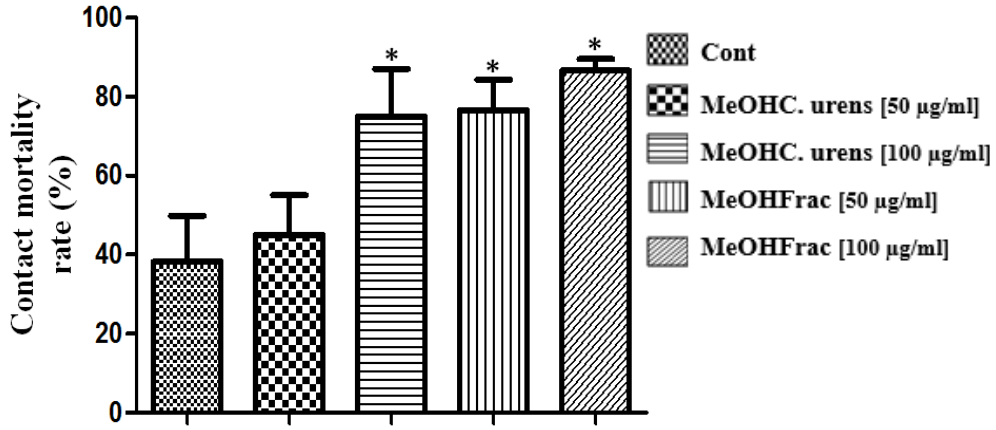

Figure 4: Percentage of mortality by contact of *S. zeamais* exposed to different concentrations of extracts of MeOHC.ures and MeOHFrac of *C. urens*. * P <0.05 for all groups compared to control.

3.6 Assessment of contact toxicity of extracts *Cnidoscolus urens*

The contact toxicity of MeOHC.urens induced a mortality of 50% of the *S. zeamais* population with an LC_{50} of 0.012 ± 0.05 µg / insect. MeOHFrac, however, showed an LC_{50} of 0.026 ± 0.001 µg / insect (Table 3).

Table 3: Determination of the lethal dose (LC50) of extracts of *C. urens*, capable of inducing mortality in 50% of the population of *S. zeamais* by contact.

Group	LC_{50} (µg/ inseto)	95% Confidence limits	
		LCL	UCL
MeOHC. urens	0.012 ± 0.05	0.109	-0.086
MeOHFrac	0.026 ± 0.01	0.009	0.042

LC50 values were cauculated using StatPlus. The 95% confidence limits between the paragraphs were based on five replicates (P<0.05). LCL: Lower confidence limit. UCL: Upper confidence limit.

4 Discussion

With a territorial extension and extensive biodiversity, the Caatinga is a biome still little explored, but it has a huge potential to be an inexhaustible source of bioactive. Currently, many studies, based on the use of extracts, oils and even purified compounds, suggest that plant products from this biome present bioinsecticidal action [30,37]. However, there are few studies that portray the potential of the Caatinga plants as protectors of stored grains, their potential to influence nutritional indices and deterrent capacity, and that few existing studies only determine the potential of mortality [38, 39].

The use of plant extract to control insect-pest infestation is an old practice [40]. Published studies show a growing interest in the alternative search for the use of synthetic products [41]. In the present study, the insecticidal activity at different concentrations and different types of extracts of *C. urens* were tested in order to investigate their ability to act on contact and ingestion toxicity, as well as to analyze their potential to infer the nutrient indices of *S. zeamias.*

As described in the methodology of ether, chloroform and acetone extracts from aerial parts of *C. urens that* showed no insecticidal action at any concentration tested, nor did they interfere with nutritional indices. Different results were obtained on the action of MeOHC. urens extracts and MeOHFrac, which showed influence on biomass gain, consumption rate, feed conversion efficiency and even on mortality. This difference can be related to the different compounds extracted by using solvents with polarity difference, indicating that bioactive with insecticide action, were extracted by using methanol or if they remained in the last fraction,

these different extract may still contain the same compounds, but may differ in relation to their concentration [42].

Meanwhile, the extracts MeOHC. urens and MeOHFrac demonstrated a great insecticidal effect when compared to the control. Numa et al.; Candido et al.; LEE et al. [43,44,45] in their research have indicated the insecticidal potential of some species of the Euphobiaceae family, including *Sebastiana corniculata* and *Cnidoscolus acontifolius* and *Cnidoscolus phyllacanthus*, which are of the same genus of *C. urens*, for different species of insects, *Tetranychus urticae, Aedes aegypti* and *Nilaparvata lugens, Sogatella furcifera, Laodelphax striatellus*. However, this study seems to be a pioneer in presenting the bioside effect of *C. urens*. Both extracts exerted effects on the nutritional parameters in *S. zeamais* population. In addition to proving to be a potent deterrent agent. According to the data found in this study we can suggest that the insecticidal action may be related to an effect linked to the pre-ingestion of the flour treated with the extracts. Sievers, in 1949 [46], had already reported some plants in tropical areas with action to combat insects.

Combating *S. zeamais*, one of the insects with destructive capacity, by consuming the stored grains at high speed [4,14,47,48], may have as an alternative the use of MeOHC. urens and MeOHFrac. For demonstrating anti-nutritional effects, its anti-nutritional effects affected the assimilation of food as a result of rejection of available food. Similar results to those found in these studies were reported by Taghizadeh, the use of aqueous and hydroalcoholic extracts from aerial parts of *Alhagi maurorum*, to demonstrate their anti-nutritional potential against *Tribolium castaneum.* Applying the same methodology (the flour disk bioassay) that we used

the author found a significant decrease in RGR, RCR and ECI, in addition to the increase in FDI, results were improved as concentrations were increased, as were our [49].

In previous studies, a Phytochemical analysis of extract *Cnidoscolus sp* was performed. Among the analyzed species was *C. urens*. In extracts of aerial parts identified a low presence of anthraquinones and xanthine derivatives, in moderate concentration whach were triterpenes and steroids, but among the groups of major compounds: anthocyanins, flavonoids, tannins, mono and diterpenes [29]. Upasani et al. reported the isolation of flavonoids from aqueous leaf extract of *Ricinus communis* L. (Euphorbeaceae) and it exerted insecticidal action against *Callosbruchus chinensis* [50]. These results help us to suggest that extracts of *C. urens*, due to their expressive flavonoid content, their insecticidal activity may be related to the great presence of these compounds. Another recent study shows the repelling action of hydroethanolic extracts of leaves of *R. communis*, which has been tested and proven its repellent action against *Scyphophorus acupunctatus*, also known as weevil [51].

Some species of the Euphorbiaceae family, are already used in the fields with the objective of inhibiting the infestations by pests. On these aspects, it was demonstrated that a mixture of the *R. communis* plant with the beans repelled the weevil [52]. Another study evaluated the potential of inducing mortality of larvae and adults of *Tribolium castaneum* from *R. communis* seed extracts, concluding that mortality increases as a consequence of concentration and time of exposure [53]. Mortality of eggs and larvae of *S. zeamais* was attributed to potent action of *C. quercifolius* extracts [38].

An important aspect for the effectiveness of the insecticidal action of any plant material is its lower lethal dose [54]. In this same concept, we can prove that through the numbers obtained by this study, extracts of *C. urens*, are excellent when the subject is mortality, because the MeOHC. urens and MeOHFrac. They showed high mortality with low concentrations, 75% and 87% of contact mortality, and 90% and 91% mortality from ingestion. Promising results were shown for *Jatropha curcas*, where the petroleum ether extract acted as an antifeedent agent, with an LC_{50} of 6.82 µl / g for a species of *Sitophilus* sp [55]. A 100% mortality was reached by root methanol extract and 70% for the ethereal extract of the *J. curcas* leaf, according to Verma et al. [56]. Silva et al. also showed the bioactivity of powders and aqueous extract of seeds and pericarp of *J. curcas*. The mortality of *S. zeamis* in this study was attributed the presence of toxic compounds such as esters [57], which are commonly found in species of the family Euphorbiaceae [58].

Many studies have shown that many extracts and plant oils may have a representative percentage of mortality against insects [59,60,61], especially against *S. zeamais* [2,4,38,57,62]. Some of the tested plants were part of more than ten different families, among them Anonaceae and Euphorbiaceae, which the species *Anona muricata* and *J. curcas* had a percentage of mortality of 100%, while the other species evaluated had a mortality less than 50%. The results could still be different if we compare with different extracts obtained from different parts of the same plant and if the concentrations are increased [63]. These data corroborate with those found in the present study, where we observed an increase in mortality when the concentration was increased. However, we found no significant difference between MeOHC.urens and MeOHFrac when applied by contact, the significant difference was evidenced when the same extracts were ingested at a concentration of 0.25 mg / g. The presence of diterpenes has been described for

the oil of *J. curcas* [64], and the insecticidal property of the products extracted from botanical material can also be bound by the presence of diterpenes [65].

5 Conclusion

The use as insecticidal agents and application of botanical materials in rural areas, aiming an the protection of stored grains which is a traditional method in these areas. Many farmers use bioavailable resources, especially in areas where commercial resources are difficult to access, such as in the Caatinga area. These farmers have low incomes and need to surrender their production soon after harvest to avoid possible infestations by pest insects.

In conclusion we can suggest that the extracts of aerial parts of *C. urens* have the potential to be used in the control of *S. zeamais* because it has been toxic to adult insects when exposed through contact or through ingestion, besides having exerted anti-nutritional effect. MeOHC.urens and MeOHFrac may be an alternative, without the need to isolate secondary metabolites, but the insecticidal effect may possibly be associated with the presence of flavonoid and terpene fractions, which have been described for this action. Further research should be conducted to increase the efficiency of these extracts.

The results reported here are initial findings and for use of *C. urens* extracts in agriculture as an insecticide, further studies including testing with other insect species are needed.

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4 Conclusão

- Os extratos de *C. urens* revelaram a presença de compostos majoritários tais como: Flavonóides, açúcares redutores e terpenos, além de outros metabólitos como: proantocianidinas, taninos e cumarinas, em menor quantidade.
- Os extratos de *C. urens* apresentaram uma ótima atividade antioxidante, mostrando ação significativa em todas as concentrações 50, 100, 200 e 500 µg/mL quando comparados ao controle.
- No entanto, os extratos aquoso e etanólico não apresentaram resultados satisfatórios quando foram testados contra células de linhagem HELA *in vitro*.
- Os extratos aquoso e etanólico de *C. urens* apresentaram ações antitumoral para o modelo experimental *in vivo* quando testado contra tumor sólido de carcinoma de Ehrlich, numa concentração de 200 mg/Kg, entretanto o extrato aquoso mostrou- se mais eficiente quando comparado com o extrato etanólico, sugerindo que os compostos presentes em extratos de *C. urens* desempenham um potencial inibitório contra células tumorais.
- Além da atuação da inibição do crescimento tumoral pode ser observado que os extratos aquoso e etanólico de *C. urens* também atuaram melhorando a resposta imunológica, bem como o perfil bioquímico dos grupos tratados.
- MeOHC.urens não interferiu na produção do número de fezes normais em camundongos por tratamento oral.
- O MeOHC.urens (200mg/kg; 400mg/kg) demonstrou ter uma eficiência em reduzir o percentual de produção de fezes diarréicas em camundongos com diarreia induzida por oléo de rícino.
- Além disso MeOHC.urens, também se mostrou eficiente em reduzir de forma significativa, o acumulo e fluido intestinal induzido por óleo de rícino *in vivo.*
- Extratos de éter, clorofomio e acetona de partes aéreas de *C. urens* foram testados quanto a sua ação inseticida, porém não demonstraram atividade siginificativo.
- Entretanto, extratos metanólicos bruto e fracionado de partes aéreas de *C. urens* (MeOHC.urens e MeOHFrac) demonstrou ser eficiente contra *S. zeamais* em baixas concentrações.

- Ambos os extratos metanólicos (0,18- 0,5 mg/g) foi eficiente em induzir efeito deterrente sobre *S. zeamais.*
- Alterações no perfil nutricional de insetos, pode ser verificado quando foram expostos a uma dieta impreguinada por MeOHC.urens e MeOHFrac.
- MeOHC.urens e MeOHFrac é capaz de induzir letalidade de 50% da população de *S. zeamais* em baixíssimas concentrações.

ANEXOS

ANEXO A - *Carta de aprovação do comitê de ética (01)*

Universidade Federal de Pernambuco
Centro de Ciências Biológicas
Av. Prof. Nelson Chaves, s/n
50670-420 / Recife - PE - Brasil
fones: (55 81) 2126 8840 | 2126 8351
fax: (55 81) 2126 8350
www.ccb.ufpe.br

Recife, 06 de agosto de 2012.

Ofício nº 458/12

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: **Profª. Noêmia Pereira da Silva Santos**
Centro Acadêmico de Vitória
Universidade Federal de Pernambuco
Processo nº 23076.041082/2011-25

Os membros da Comissão de Ética no Uso de Animais do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEUA-UFPE) avaliaram seu projeto de pesquisa intitulado, **"Avaliação da ação antitumoral de *Cnidoscolus urens* sobre tumores sólidos experimentais em camundongos *Swiss*".**

Concluímos que os procedimentos descritos para a utilização experimental dos animais encontram-se de acordo com as normas sugeridas pelo Colégio Brasileiro para Experimentação Animal e com as normas internacionais estabelecidas pelo National Institute of Health Guide for Care and Use of Laboratory Animals as quais são adotadas como critérios de avaliação e julgamento pela CEUA-UFPE.

Encontra-se de acordo com as normas vigentes no Brasil, especialmente a Lei 11.794 de 08 de outubro de 2008, que trata da questão do uso de animais para fins científicos e didáticos.

Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais a serem realizados.

Origem dos animais: Biotério do LIKA - UFPE; Animais: Camundongos; Linhagem: Swiss; Sexo: machos; Peso: 30g; Idade: 40 a 60 dias; Número de animais previsto no protocolo: 100

Atenciosamente,

Presidente do CEEA

CCB: Integrar para desenvolver

ANEXO B - ***Carta de aprovação do comitê de ética (02)***

Universidade Federal de Pernambuco
Centro de Biociências
Av. Prof. Nelson Chaves, s/n
50670-420 / Recife - PE - Brasil
fones: (55 81) 2126 8840 | 2126 8351
fax: (55 81) 2126 8350
www.ccb.ufpe.br

Recife, 07 de julho de 2016.

Ofício nº 65/16

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: **Prof. Nicácio Henrique da Silva**
Departamento de Bioquímica
Centro de Biociências
Universidade Federal de Pernambuco
Processo nº **23076.012693/2016-71**

Certificamos que a proposta intitulada **"*Cnidoscolus sp* da caatinga: fitoquímica e atividades biológicas"**, registrada com o nº **23076.012693/2016-71**, sob a responsabilidade de Prof. **Nicácio Henrique da Silva** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo CONSELHO NACIONAL DE CONTROLE DE EXPERIMENTAÇÃO ANIMAL (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA UNIVERSIDADE FEDERAL DE PERNAMBUCO (UFPE), em reunião de 28/06/2016.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da autorização	Até 27/02/2020
Espécie/linhagem/raça	Camundongo albino *Swiss Mus musculus*
Nº de animais	138
Peso/Idade	30-35g/ 30-60 dias
Sexo	machos
Origem	Biotério do LIKA

Atenciosamente,

Prof. Dr. Pedro V. Carelli
Presidente da CEUA / CCB - UFPE
SIAPE 1801584
UFPE

Anexo C - Guide for authors – JOURNAL OF ETHNOPHARMACOLOGY

JOURNAL OF ETHNOPHARMACOLOGY

An Interdisciplinary Journal Devoted to Indigenous Drugs

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 0378-8741

DESCRIPTION

The *Journal of Ethnopharmacology* is dedicated to the exchange of information and understandings about people's use of plants, fungi, animals, microorganisms and minerals and their **biological** and **pharmacological effects** based on the principles established through international conventions. Early people confronted with illness and disease, discovered a wealth of useful **therapeutic agents** in the plant and animal kingdoms. The empirical knowledge of these **medicinal substances** and their toxic potential was passed on by oral tradition and sometimes recorded in herbals and other texts on *materia medica*. Many valuable drugs of today (e.g., atropine, ephedrine, tubocurarine, digoxin, reserpine) came into use through the study of **indigenous remedies**. Chemists continue to use **plant-derived drugs** (e.g., morphine, taxol, physostigmine, quinidine, emetine) as prototypes in their attempts to develop more effective and less toxic medicinals.

In recent years the preservation of local knowledge, the promotion of indigenous medical systems in primary health care, and the conservation of biodiversity have become even more of a concern to all scientists working at the interface of social and natural sciences but especially to ethnopharmacologists. Recognizing the sovereign rights of States over their natural resources, ethnopharmacologists are particularly concerned with local people's rights to further use and develop their autochthonous resources.

Accordingly, today's ethnopharmacological research embraces the multidisciplinary effort in the:

- documentation of **indigenous medical knowledge**,
- scientific study of **indigenous medicines** in order to contribute in the long-run to improved health care in the regions of study, as well as
- search for pharmacologically unique principles from existing indigenous remedies.

The *Journal of Ethnopharmacology* publishes original articles concerned with the observation and experimental investigation of the biological activities of plant and animal substances used in the traditional medicine of past and present cultures. The journal will particularly welcome interdisciplinary papers with an **ethnopharmacological**, an **ethnobotanical** or an **ethnochemical** approach to the study of indigenous drugs. Reports of **anthropological** and **ethnobotanical** field studies fall within the journal's scope. Studies involving **pharmacological** and **toxicological** mechanisms of action are especially welcome. Clinical studies on efficacy will be considered if contributing to the understanding of specific ethnopharmacological problems. The journal welcomes review articles in the above mentioned fields especially those highlighting the multi-disciplinary nature of ethnopharmacology. Commentaries are by invitation only.

AUDIENCE

Ethnopharmacologists, Medicinal Chemists, Pharmacologists, Toxicologists, Anthropologists,

Pharmacognosists, Ethnobotanists, Economic Botanists, Ethnobiologists

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INTRODUCTION

The *Journal of Ethnopharmacology* is dedicated to the exchange of information and understandings about people's use of plants, fungi, animals, microorganisms and minerals and their biological and pharmacological effects based on the principles established through international conventions. Early people, confronted with illness and disease, discovered a wealth of useful therapeutic agents in the plant and animal kingdoms. The empirical knowledge of these medicinal

substances and their toxic potential was passed on by oral tradition and sometimes recorded in herbals and other texts on *materia medica*. Many valuable drugs of today (e.g., atropine, ephedrine, tubocurarine, digoxin, reserpine) came into use through the study of indigenous remedies. Chemists continue to use plant-derived drugs (e.g., morphine, taxol, physostigmine, quinidine, emetine) as prototypes in their attempts to develop more effective and less toxic medicinals.

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Types of paper

The *Journal of Ethnopharmacology* will accept the following contributions:

1. Original research articles - whose length is not limited and should include Title, Abstract, Methodsand Materials, Results, Discussion, Conclusions, Acknowledgements and References. As a guideline, a full length paper normally occupies no more than 10 printed pages of the journal, including tables and illustrations.
2. Short Communications - whose average length is not more than 4 pages in print (approx.2000-2300 words, including abstract and references). A maximum of 2 illustrations (figures or tables) is allowed. See paragraph below for description and format.
3. Letters to the Editors.
4. Reviews - Authors intending to write review articles should consult and send an outline to theReviews Editor (see inside front cover for contact information) before preparing their manuscripts. The organization and subdivision of review articles can be arranged at the author's discretion. Authors should keep in mind that a good review sets the trend and direction of future research on the subject matter being reviewed. Tables, figures and references are to be arranged in the same way as research articles in the journal. Reviews on topics that address cutting-edge problems are particularly welcome. Outlines for potential reviews need to include: A detailed

abstract using the structure provided in the guidelines An annotated table of contents A short CV of the lead author 5. Book reviews - Books for review should be sent to the Reviews Editor.

6. Commentaries - *invited*, peer-reviewed, critical discussion about crucial aspects of the field but most importantly methodological and conceptual-theoretical developments in the field and should also provide a standard, for example, for pharmacological methods to be used in papers in the *Journal of Ethnopharmacology*. The scientific dialogue differs greatly in the social / cultural and natural sciences, the discussions about the common foundations of the field are ongoing and the papers published should contribute to a transdisciplinary and multidisciplinary discussion. The length should be a maximum of 2-3 printed pages or 2500 words. Please contact the Reviews Editor j.ethnopharmacol@pharmacy.ac.uk with an outline.
7. Conference announcements and news.

BEFORE YOU BEGIN

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Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Policy and ethics

In the covering letter, the author must also declare that the study was performed according to the international, national and institutional rules considering animal experiments, clinical studies and biodiversity rights. See below for further information. The ethnopharmacological importance of the study must also be explained in the cover letter.

Animal and clinical studies - Investigations using experimental animals must state in the Methods section that the research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in for example the European Community guidelines (EEC Directive of 1986; 86/609/EEC) or the US guidelines (NIH publication #85-23, revised in 1985). Investigations with human subjects must state in the Methods section that the research followed guidelines of the Declaration of Helsinki and Tokyo for humans, and was approved by the institutional human experimentation committee or equivalent, and that informed consent was obtained. The Editors will reject papers if there is any doubt about the suitability of the animal or human procedures used.

Biodiversity rights - Each country has its own rights on its biodiversity. Consequently for studying plants one needs to follow the international, national and institutional rules concerning the biodiversity rights.

Author contributions

For each author the contribution to the publication should be mentioned.

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Matteucci, E., Giampietro, O. (2008) Proposal open for discussion: defining agreed diagnostic procedures in experimental diabetes research. Journal of Ethnopharmacology,115: 163-172.

Froede, T.SA. and Y.S. Medeiros, Y.S. (2008) Animal models to test drugs with potential antidiabetic activity. Journal of Ethnopharmacology 115: 173-183. Gertsch J. (2009) How scientific is the science in ethnopharmacology? Historical perspectives and epistemological problems. Journal of Ethnopharmacology, 122: 177-183.

Chan K., et al. (2012) Good practice in reviewing and publishing studies on herbal medicine, with special emphasis on traditional Chinese medicine and Chinese Materia Medica. Journal of Ethnopharmacology 140: 469-475.

Heinrich, M., Edwards. S., Moerman. D.E.. and Leonti. M. (2009), Ethnopharmacological field studies:

a critical assessment of their conceptual basis and methods. J. Ethnopharmacol, 124: 1-17.

PREPARATION

Use of word processing software

It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.

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Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Glossary

Please supply, as a separate list, the definitions of field-specific terms used in your article.

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If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

- ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lowercase superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
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A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

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PESTICIDE BIOCHEMISTRY AND PHYSIOLOGY

AUTHOR INFORMATION PACK

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[2] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000.
Reference to a chapter in an edited book:

[3] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z.Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304.
Reference to a website:

[4] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13.03.03).
Reference to a dataset:

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Nº	Família	Nº IPA	Nome Científico
01	Euphorbiaceae.	84.190	*Cnidoscolus urens* (L.) Arthur

Material identificado por A.G.Silva.

Drª. Rita de Cássia Pereira
Curadora do Herbário IPA

Procedência: PE – Caruaru.
Determinada em: Setembro de 2012.

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