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AVALIAÇÃO DA ATIVIDADE ANTI-INFLAMATÓRIA DE EXTRATOS DE *Indigofera suffruticosa* Mill EM MODELOS DE INFLAMAÇÃO EM CAMUNDONGOS

JANAINA KARIN DE LIMA CAMPOS

Recife (PE) - Brasil

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Dissertação de Mestrado apresentada para o cumprimento parcial das exigências para obtenção do título de Mestre em Ciências Biológicas pela Universidade Federal de Pernambuco.

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À D'us pelo seu amor e minha existência .

À minha família, principalmente meus pais pela dedicação e pelo apoio.

À meu amor pelo incentivo, carinho e respeito .

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“Nunca tenha medo de tentar algo novo.

Lembre-se de que um amador solitário construiu a Arca.

Um grande grupo de profissionais construiu o Titanic”

(Luiz Fernando Veríssimo)

RESUMO

Indigofera suffruticosa, conhecida popularmente como “anil” , é empregada na medicina popular como analgésico e anti-inflamatório, porém não há registros científicos que confirmem suas atividades farmacológicas. Com isto, o presente estudo objetivou avaliar as possíveis ações anti-inflamatórias e antinociceptivas de extratos fracionados com solventes orgânicos (Éter, Clorofórmio, Acetona e Metanol) de sementes secas de *I. suffruticosa.* No modelo de nocicepção induzida por ácido acético todos os extratos fracionados de *I. suffruticosa* foram capazes de inibir as contorções abdominais, assim como no modelo de nocicepção de placa quente, os extratos Clorofórmio, Acetona e Metanol foram capazes de aumentar o tempo de latência, com ação duradoura de até três horas. No modelo de edema de pata induzida por carragenina observou-se uma diminuição significativa do edema nos animais tratados com os extratos Acetônicos e Metanólicos (60,1 e 58,7%, respectivamente) de *I. suffruticosa* num período de 3 horas. Na indução da inflamação no modelo de peritonite induzida por carragenina, apenas o extrato clorofórmico foI capaz de diminuir significativamente o acúmulo de neutrófilos na cavidade peritoneal dos camundongos no tempo de 4 horas. O conjunto de resultados sustenta a hipótese popular que a *Indigofera suffruticosa* possui ações antinociceptivas e anti-inflamatórias, além de indicar a existência de diferentes substâncias biologicamente ativas obtidas a partir das diferentes solventes, de maneira que torna a *I. suffruticosa* uma potencial fonte de substâncias promissoras para obtenção de novos fármacos.

Palavras-chave: *Indigofera suffruticosa*, inflamação, nocicepção

ABSTRACT

Indigofera suffruticosa, popularly known as "indigo" is used in folk medicine as an analgesic and anti-inflammatory, but no scientific records to confirm this account. With this, the present study aimed to evaluate possible antiinflammatory and antinociceptive extracts fractionated with organic solvents (ether, chloroform, acetone and methanol) of dry seeds of *I. suffruticosa*. In the model of nociception induced by acetic acid extracts all fractionated *I. suffruticosa* were able to inhibit writhing, as well as nociception model hot plate, both extracts were able to increase the latency time with sustained action up to three hours. In the model of carrageenan-induced inflammation in the paws of mice, we observed a significant reduction of edema in acetonic extract and methanol (60.1 and 58.7%, respectively) of *I. suffruticosa* a period of 3 hours. For the induction of inflammation model in carrageenan-induced peritonitis, only the chloroform extract was able to significantly decrease the accumulation of neutrophils into the peritoneal cavity of mice at the time of 4 hours. The set of results support the hypothesis that the popular Indigofera suffruticosa have antinociceptive actions and anti-inflammatory, and indicates the existence of different biologically active substances obtained from the various extractions of the plant, so that makes *I. suffruticosa* a potential source of promising substances for obtaining new drugs.

Keywords: *Indigofera suffruticosa*, inflammation, nociception.

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LISTA DE ABREVIATURAS

AAS	**Ácido acetilsalicilico**
AINES	**Anti-inflamatórios não esteroidais**
COX 1	**Cicloxigenase 1**
COX 2-	**Cicloxigenase 2**
CSF	**Fator estimulador de colônias**
EETs	**Epoxieicosatetranóicos**
HETEs	**Ácido hidroxieicosatetranóicos**
HPETEs	**Hidroperoxieicosatetranóicos**
IFN	**Interferon**
IL	**Interleucina**
IPG	**Isopropilenoglicol**
OMS	**Organização mundial de saúde**
PAG	**Substância cinzenta perioquedutal**
TGF	**Fator de transferência de crescimento**
TNF	**Fator de necrose tumoral**

SUMÁRIO

1 INTRODUÇÃO

A dor é uma sensação extremamente importante para a sobrevivência, pois têm a função de proteger o organismo, como também evitar comportamentos perigosos (LE BARS, GOZARIU, CADDEN, 2001). A nocicepção diferente da dor, é um processo fisiológico que utiliza estruturas neurais com campos receptivos específicos para codificar e processar os estímulos. A dor pode ser influenciada por diversos mecanismos e mediadores, levando ao seu desaparecimento ou agravamento. A percepção dolorosa pode ser detectada após um trauma ou lesão tecidual, devido a ativação de nociceptores e sistema imunológico, desencadeando reações pela liberação de substâncias pró-inflamatórias (MILITZER, 1975).

A inflamação, resposta imunológica em células e/ou tecidos decorrente de um agente agressor, é caracterizada pelo aumento da permeabilidade vascular, do recrutamento de leucócitos e da liberação de mediadores químicos. Essa resposta também é essencial para a proteção do organismo afetado, pois tem a finalidade de eliminar o agente agressor e manter a homeostase (SCHMID-SCHONBEIN, 2006). A reação inflamatória pode ser originada de fonte endógena, proveniente da degeneração ou necrose tissular, ou de fonte exógena, causada por agentes físicos, químicos ou biológicos.

Terapias analgésicas e anti-inflamatórias têm sido relacionadas com a utilização de alguns fármacos, tais como: anti-inflamatórios não esteroidais, corticosteróides, opóides e fármacos com funções diversas, conhecidos com adjuvantes (SAWYNOK, 2003). Porém a utilização dessas drogas, também está associada ao surgimento de efeitos adversos, que incluem transtornos gastrointestinais e alterações cardiovasculares (CARVALHO, CARVALHO e RIOS-SANTOS, 2004), o que incentiva a busca por novos agentes bioativos que possam ser eficientes, trazendo mais benefícios, menos danos e mas acessibilidade a população (SULEYMAN et al., 2010).

As plantas sintetizam produtos através do seu metabolismo secundário, os quais têm sido frequentemente utilizados na investigação de novos fármacos por apresentarem excelentes atividades biológicas. Estima-se que 25% dos medicamentos prescritos em todo o mundo são provenientes de plantas medicinais (SAHOO, MANCHIKANTI,

DEY, 2010). No Brasil, a diversidade da flora propicia, o uso de plantas medicinais de maneira significativa.

A *Indigofera suffruticosa*, pertencente a família fabaceae, conhecida como “anil” vem se destacando como fitoterápico por apresentar propriedades antiespasmódica, sedativa e diurética (LORENZI, 1982). Alguns estudos científicos realizados com esta espécie demonstraram efeitos benéficos nas seguintes atividades biológicas: anticonvulsivante, antiepiléptica e antigenotóxica utilizando extratos aquosos de partes aéreas (ALEJO et al, 1996, BADELL, et al 1998, ROIG E MESA, 1974, respectivamente), antimicrobiana, antifúngica e anti-tumoral de extratos fracionados com Hexano, Acetato de Etila, Metanol e água (LEITE et al., 2006 ,VIEIRA et al., 2007), e anti-inflamatória com extratos aquosos de partes áreas (LEITE et al., 2003).

No entanto, não há estudos científicos que relatem as propriedades presentes nas sementes desta espécie, demonstrando a necessidade ainda de se estudar e avaliar os potenciais efeitos biológicos presentes na *I. suffruticosa* para que suas atividades sejam validadas e que tanto a planta quantos seus derivados possam ser utilizados como fitoterápicos seguros e eficazes.

2 REVISÃO DE LITERATURA

2.1 Nocicepção

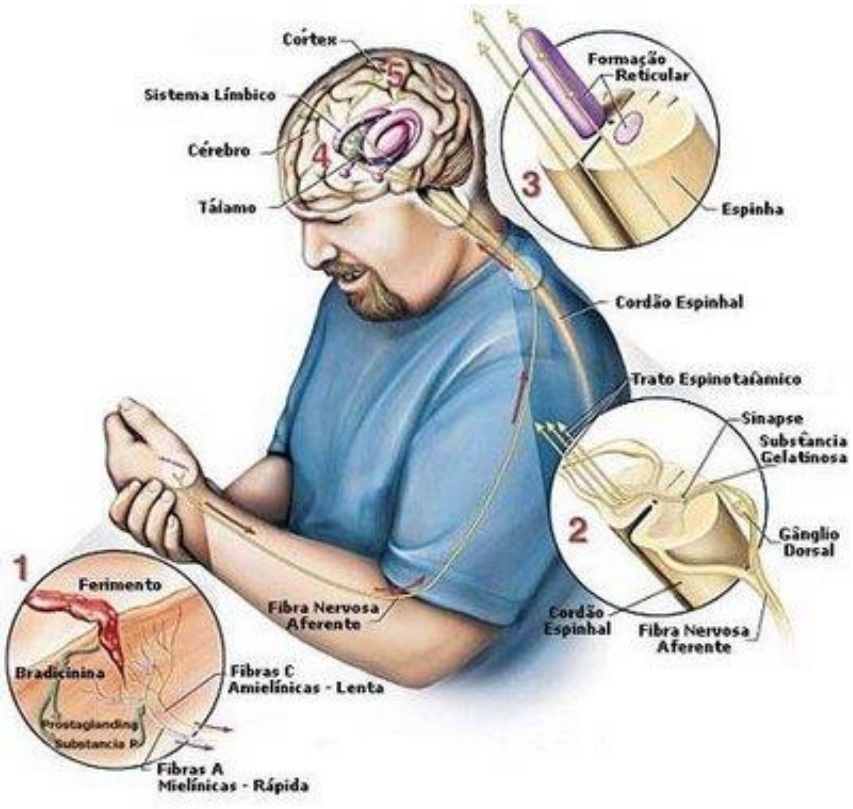
Termo derivado do latim, “nocicepção”, que significa “danificar” e “prejudicar”, foi introduzido por Sherrington em 1910, no qual propôs a existência de neurônios primários com características somatosensoriais, e que são ativados mediante estímulos capazes de causar lesões (JULIUS E BASBAUM, 2001). Sherrington nomeou esses neurônios primários com capacidade de serem evocados frente a estímulos nocivos de “nociceptores”, os quais possuem campos específicos receptivos para ativar diferentes fibras nervosas sensoriais. Os receptores nociceptivos são representados pelas terminações livres presentes nas fibras amielínicas C e mielínicas finas Aδ. As fibras do tipo C, sem a presença da mielina, respondem a estímulos nocivos de origem térmica, mecânica e química, e por isto são chamados de nociceptores polimodais (LAWSON, 2002; COUTAUX et al., 2005). A fibra mielínica fina Aδ propaga o sinal de forma mais rápida que a fibra do tipo C, estando apenas relacionada com estímulos de origem térmica e mecânica (MELZACK, 1999). Os nociceptores podem ser sensibilizados e ativados pela ação de diversas substâncias, denominadas algiogênicas, que inclui a acetilcolina, bradicinina, histamina, leucotrieno, substância P, fator de ativação plaquetário, prostaglandinas, tromboxano, interleucinas, fator de necrose tumoral (TNF-α), entre outras (BEDBROOK, 1976, PIOTROWSKI, 1986).

2.1.1 Vias e a Transmissão da Nocicepção

Frente a estimulos nocivos o potencial de ação é propagado nos neurônios nociceptivos principalmente pela ação de canais de sódio e potássio dependentes de voltagem (MCCLESKEY et al., 1999). Esses neurônios de primeira ordem enviam projeções em diferentes lâminas espinhais para região do corno dorsal na medula espinhal, e através da liberação de neurotransmissores realizam sinapses (MILLAN, 1999).

Os neurônios primários chegam a médula de duas formas: 1º) As fibras Aβ responsáveis pelas sensações de mecanocepções e propriocepção terminam em lâminas mas profundas; 2º) As fibras C e Aδ propagam informações nociceptivas de todo o corpo, projetam-se para lâminas mais superficiais, onde realizam mono e poli-sinapses com neurônios de segunda ordem que se elevam para regiões superiores (CRAIG, 2003). Esses neurônios também chamados de neurônios de projeção sobem por diferentes tratos, como os espinotalâmicos, trigemial, espinoparabraquial e espinomesencefálico, sendo os mais importantes para a condução nociceptiva. A via mas utilizada para estudo relacionada com a transmissão de sinal nociceptivo é a via espinotalâmica (MELZACK, 1999). Nesta via, os axônios são direcionados para o tálamo sem sofrer sinapses no decorrer do caminho, porém ao chegar, realizam sinapses em diferentes núcleos e posteriormente liberam neurônios de terceira ordem que são responsáveis por levar informações para várias regiões do córtex cerebral, onde ocorre o processamento que resulta em consciência da dor (CRAIG, 2003) (Figura 1).

Figura1. Processo de nocicepção



Fonte: http://www.google.com.br/imgres?q=nocicep%.

O organismo possui várias regiões e estruturas que são responsáveis em modular mecanismos intrínsecos da sensação dolorosa, como por exemplo a substância cinzentada periaquedutal (PAG) que está localizada no tronco cerebral, e atua recebendo e emitindo projeções para medula, córtex frontal, tálamo, hipotálamo, entre outros (COFFIELD et al., 1992; BANDLER et al., 1996). Uma estimulação elétrica da PAG pode causar analgesia intensa, ocorrendo principalmente pela liberação da serotonina (neurotransmissor) que ativa interneurônios, inibindo assim a transmissão da via espinotalâmica.

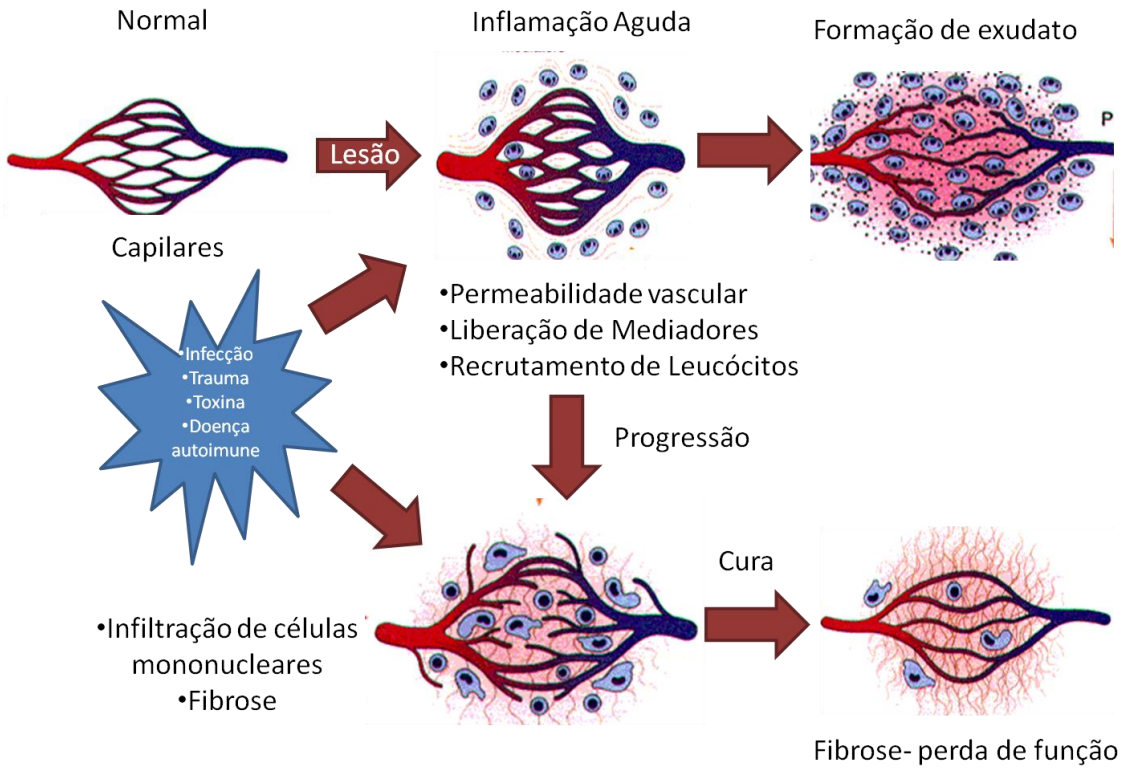
2.2 Inflamação

A inflamação é um processo pelo qual o organismo utiliza para se defender contra uma lesão causada por agentes físicos (calor, frio, trauma), químicos (substâncias irritantes) e/ou biológicos (microorganismos) (CALIXTO et al., 2004). Neste processo, células competentes são ativadas e atuam no sentido de inativar ou destruir o agente agressor, além de iniciar a reparação tecidual. Basicamente, a inflamação (fig. 2) tem sido classificada em aguda ou crônica, dependendo da persistência da lesão e dos seus sintomas clínicos. A reação inflamatória aguda se caracteriza por ser de curta duração e apresentar dilatação arteriolar, aumento da permeabilidade vascular, acúmulos de leucócitos, produção de diversos hormônios e proteínas de fase aguda e dor (LEVINE et al., 1999). Quando este processo perdura (agressor ainda ativo), ocorre a inflamação crônica, ou seja, a destruição tecidual e a tentativa de reparo. Neste caso, as células acionadas também serão os mononucleares (macrófagos, linfócitos e plasmócitos) que aderem aos vasos sanguíneos, provocando a vasodilatação e também migram para tecidos resultanto em exsudatos e granulomas. O processo inflamatório chegará ao fim quando o agente agressor for eliminado e os mediadores secretados disseminados ou detruídos.

Estas reações inflamatórias são mediadas por fatores químicos derivados de proteínas ou de células plasmáticas. As citocinas, classificadas por pró-inflamatórias e anti-inflamatórias, são moléculas de origem proteica, com a capacidade de sinalizar diferentes células de defesa e regular a resposta inflamatória (CALIXTO et al., 2004).

Citocinas pró-inflamatórias estão envolvidas na iniciação e amplificação do processo inflamatório, enquanto que as citocinas antiinflamatórias modula negativamente esses eventos (CALIXTO et al., 2004). Essas moléculas são produzidas por células residentes e migratórias, tais como, macrófagos e neutrófilos e quando liberadas podem atuar especificamente ou sistematicamente (CALIXTO et al., 2004). Podem ser enquadradas em diversas categorias: Interferon (IFN), Interleucinas (IL), Fator estimulador de colônias (CSF), Fator de necrose tumoral (TNF) α e β e Fator de transformação de crescimento (TGF β). Dentre essas, as que destacam no processo inflamatório tanto agudo como crônico, bem como no processo de reparo, são a Il-1, Il-6 e a TNF-α. Outros mediadores químicos importantes também podem ser as aminas (histamina), os lipídios (prostaglandinas) e os pequenos peptídeos (brandicinina).

Figura 2. Evolução da inflamação aguda.

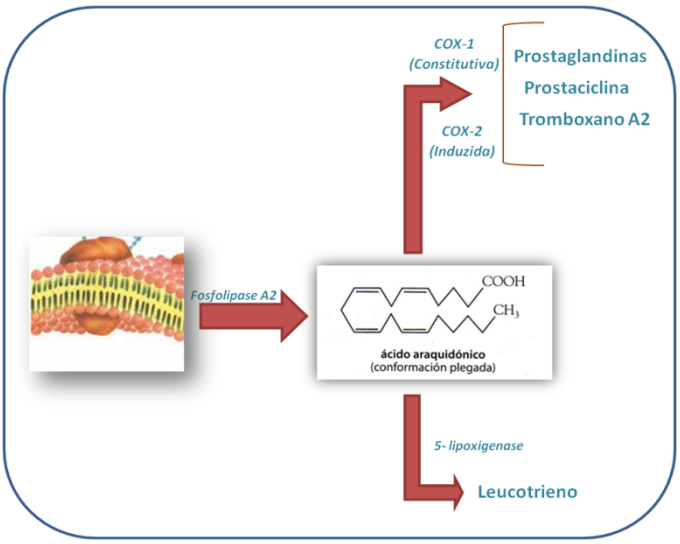


Fonte:Campos, J. K. L. (2012)

O processo inflamatório é desencadeado pela liberação desses mediadores originados no tecido lesado e em células migratórias provocando danos nas membranas celulares e a ativação da fosfolipase A2, consequentemente a liberação de ácido araquidônico, fator ativador de plaquetas e enzimas lisossômicas (SHERWOOD & TOLIVER-KINSKY, 2004). Este processo metabólico do ácido araquidônico dá origem a substâncias com um importante papel na fisiopatologia da inflamação, que podem ser as prostaglandinas, os tromboxanos, os leucotrienos, as lipoxinas e ácidos epoxieicosatetraenóicos (EETs), o ácido hidroxieicosatetraenóicos (HETEs) e hidroperoxieicosatetraenóicos (HPETEs).

O mecanismo de ação de muitos medicamentos anti-inflamatórios é a inibição da síntese das prostaglandinas, que são eicosanóides produzidos em mínima quantidade nos tecidos. Existem duas principais vias de síntese das prostaglandinas (fig. 3) a partir do ácido araquidônico: i) via da cicloxigenase, dada pela ação catalitica das enzimas cicloxigenase, a cicloxigenase 1 (COX-1), chamada de constitutiva por ser produzida constantemente e distribuida amplamente nos tecidos e a cicloxigenase 2 (COX-2), denominada de induzida, pois só é vista em estimulos inflamatórios; e via da lipoxigenase, atuando na formação dos ácidos hidroperoxieicosatetraenóicos ou leucotrienos ou lipoxinas (CARVALHO, CARVALHO & RIOS-SANTOS, 2004). As prostaglandinas estão envolvidas particularmente no desenvolvimento de dor e da febre durate a inflamação. E os leucotrienos estão relacionados com a migração dos leucócitos para o local afetado. Quando ligados aos seus respectivos sitios promovem a desgranulação das células leucocitárias e a produção de superóxidos, que contribuem para o dano tecidual.

Figura 3. Via da síntese Prostaglandinas, Prostaciclinas, Troboxano A2 e Leucotrienos.



Fonte: Campos, J. K. L. (2012).

2.3 Fármacos Utilizados na Inflamação e Nocicepção

Existem atualmente diversos tipos de medicamentos utilizados como analgésicos e/ou anti-inflamatórios, como os anti-inflamatórios não-esteroidais (AINES), e os corticosteróides. Os AINEs possuem atividades antipirética, analgésica, anti-inflamatória, e inibem também a síntese de protrombina e agregação plaquetária, especialmente o ácido acetil salicilico (ASS), introduzido em 1899 que acetila irreversivelmente a cicloxigenage (GANGREIRO et al., 2008). Os corticoesteróides são substâncias endógenas, originalmente da glândula adrenal e são classificados em três classes gerais com funções características, os glicocorticóides, os mineralocorticóides e os estrogênios. Os glicocorticóides possuem diversas atuações farmacológicas, dentre elas, a redução da resposta inflamatória e a supressão da imunidade. Apesar do excelente potencial farmacológico presente nestes fármacos, a sua utilização produz efeitos colaterais, tais como transtornos gastrointestinais (provocados pelos AINES diversos) e alterações cardiovasculares (associados por AINES seletivos para COX-2) (CARVALHO, CARVALHO & RIOS-SANTOS, 2004), o que estimula a busca por

substâncias que sejam menos prejudiciais e possuam maior especificidade na ação analgésica e anti-inflamatória.

Moléculas de origem vegetal estão sendo fortemente citadas na literatura por apresentarem ações antinociceptiva e anti-inflamatórias, que são relacionadas com o bloqueio de canais de cálcio ou inibindo a calmodulina; com a atuação em receptores de mediadores inflamatórios; atuação com falsos substratos; com a inibição da síntese ou ação de citocinas, quimiocinas, moléculas de adesão, vias do ácido araquidônico e óxido nitrico (CALIXTO et al., 2004; WERZ, 2007). As plantas, frequentemente quando utilizadas de diversas formas inibem mais de uma via de ação, intensificando os efeitos antinociceptivos, anti-inflamatórios e reduzindo os efeitos adversos (SCHMITZ & BACHER, 2005). Esse fato pode ser atribuído por haver mais de um componete que atua sinergicamente ou antagonicamente, através de diferentes mecanismos de ação. De outra forma, também sugere que estes compostos isolados de plantas possam atuar como monopreparados ou associados a outros fármacos, para intensificar a sua eficácia e reduzir o seu custo (CALIXTO et al., 2004). Desta forma, tem crescido os estudos utilizando plantas de uso popular em tratamentos de nocicepção e inflamação.

2.4 Plantas Medicinais

As plantas são usadas como substância para cura de doenças por diversos povos desde os tempos pré-históricos. Elas sintetizam produtos químicos essenciais para o seu desenvolvimento e sua proteção. Muitos destes compostos, tais como, flavanóides, alcalóides, triterpenos, taninos, saponinas entre outros, tem pronunciado efeitos no organismo humano, e são utilizados como agentes medicinais no tratamento de patologias. Vários bio-produtos são extraídos de plantas em larga escala para comercialização e muitos deles têm sido utilizados como protótipos para síntese ou semi-síntese de drogas com um perfil farmacológico. Segundo a OMS, 80% da população depende dos fitoterápicos como ferramenta chave para cuidados básicos de saúde (CASTARDO et al. 2008). No entanto, há uma grande escassez de estudos que comprove os componentes fitoquímicos e potenciais toxicológicos destas plantas.

O Brasil é considerado um dos países mais ricos do mundo em biodiversidade, ocupando o primeiro lugar dentre os 17 selecionados (RATES, 2001), o que revela a sua atração pelas indústrias famacêuticas para a produção de fitoterápicos. Dentro dessa biodiversidade, encontra-se a família *fabaceae*, que possui cerca de 18.000 espécies (OLIVEIRA & PAIVA, 2005), e mostra uma característica comum em quase todas, de apresentar frutos semelhantes a legumes, conhecidos como vagens (WATSON & DALLWITZ, 1992).

O gênero *Indigofera* pertencente a essa familia se destaca por ser usado como forrageira (SHERMAN, 1982), adubo verde e cobertura de solo (FROMAN, 1975). Esta planta é conhecida popularmente como "anil", "anileira", ou "índigo", cujo nome provém do alemão, devido a produção de um pigmento azul extraído, que é obtido por infusão quente a partir da fermentação de suas folhas, e utilizados comumente para tingimento de fios (PESAVENTO, 2005). Até o início do nosso século as indústrias utilizavam este pigmento como fonte de coloração, até ser substituído pela anilina sintética. Há também relatos que esse pigmento foi bastante utilizado em rutuais, como tinta para os templos Maias, Toltecas e Olmecas, e como matéria-prima para desenvolvimento do corante conhecido pelo nome "azul de maia" (MATADAMAS-ORTIZ, 2002).

Este gênero possui aproximadamente 700 espécies distribuídas na Ásia, África Tropical, Austrália, América do Norte e Sul. No Brasil é possível encontrar três espécies: *Indigofera truxillensis*, *I. hirsuta*, e *I. suffruticosa*. (PESAVENTO, 2005). São consideradas plantas silvestres que crescem em todos os tipos de solos, tolerando secas, inundações e elevadas salinidades.

2.4.1 Aplicações Terapêuticas de *Indigofera*

As espécies do gênero *Indigofera* têm sido utilizadas para tratamento de patologias desde a antiguidade por diversos povos. As folhas de *I. dendroides* têm sido usadas no tratamento de infecções de pele (AMOS et al., 2003) e de garganta (ESIMONE et al, 1999). Alguns estudos também relatam que esta espécie também

possui propriedades antibacterianas e antifúngica contra *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Candida albicans e Aspergillus niger* (ESIMONE et al., 1999). Outra espécie, *I. oblongifolia*, também apresenta propriedades antimicrobianas em bactérias gram positivas e gram negativas (AWADH et al., 2001). A *I. asphalatoides*¸ predominante da Índia, foi utilizada em células cancerígenas de camundongos, e se mostrou potencialmente protetora contra alguns tipos de câncer (CRISTINA et al., 2003).

A espécie *I. suffruticosa* é utilizada como um fitoterápico com propriedades antiespasmódicas, sedativas, diuréticas, purgativas, odontálgicas (LORENZI, 1982; BRAGA, 1985). Alguns estudos farmacológicos realizados com esta espécie mostram também as atividades anticonvulsivante (ALEJO et al, 1996), antigenotóxica (BADELL, et al 1998) e antiepiléptica (ROIG & MESA, 1974). Há relatos que compostos presentes em outro gênero desta planta, chamado o indirrubin, possui ação de inibir a produção de interferon-gama e interleucina-6, por mecanismos ainda desconhecidos (KUNIKATA et al., 2000).

Entre as aplicações clínicas da *I. suffruticosa*, estudos científicos utilizando partes aéreas e de folhas destacam também as atividades: citotóxica para células embrionárias em ratos (LEITE et al., 2004), antimicrobiana contra a bactéria gram-positiva *Staphylococcus aureus* (LEITE et al., 2006), antifúngica contra dermatófitos *Microsporium canis e Trichophyton rubrum* (LEITE et al., 2006), anti- tumoral (VIEIRA et al., 2007), e anti-inflamatória na redução de edema de pata de camundongos (LEITE et al., 2003).

2.4.2 *Indigofera suffruticosa* Mill.

A *Indigofera suffruticosa* Mill.(figura 4) é uma espécie originária da Antilha e América Central (ALMEIDA, 1993) mais predominante por toda a América Tropical. No Brasil, encontra-se distribuídas nos estados do Mato Grosso (FERNANDES, 1987), Alagoas (RIBEIRO, 1984), Paraíba (RIET-CORREA, 2000), Ceará, Rio Grande do Norte, Pará e Pernambuco (NETO et al., 2001). É caracterizada como uma planta

arbustiva, medindo 1m a 2 m de altura, com ramos pubescentes, caule anguloso, de cor acizentada, folhas pinadas compostas por 7 a 15 folíolos oblongos ou ovais, glabros na face e no verso, apresentando flores pequenas, numerosas, albo-roseas ou amareladas, em racemos axilares, e seu fruto é uma pequena vagem falciforme com 6 a 10 sementes medindo 25 mm de comprimento (BRAGA, 1976).

A *I. suffruticosa* pode ser conhecida também por jiquilite, tzitzupu, anil do campos, anileira-da-índia, anileira verdadeira, caá-chica, caá-chira, timbó-mrim, timbozinho, e indigueira. As primeiras investigações dos componentes químicos de *I. suffruticosa* foram realizados por Miller e Smith, 1973, utilizando extrato de sementes. O isolamento de esteres de glicose de ácido 3-nitropropanóico desta espécie é destacado por possuir efeitos tóxicos, devido a sua conversão para o ácido 3-nitropropanóico, uma toxina respiratória que inibe enzimas mitocondriais. Além deste isolado, Kamal e Mangla (1993), identificaram, caracterizaram e quantificaram seis rotenóides de diferentes partes de *I. suffruticosa*, com atividade biológica eficaz contra larvas de *Anopheles* e *Callosobruchus chinensis* adultos. Estudos preliminares de folhas, sementes e caules de *I. suffruticosa* demostram a presença de alcalóides, polifenóis e flavanóides, triterpenoides, carboidratos e índigo (LEITE et al, 2003).

Figura 4. *Indigofera suffruticosa*.

Fonte: http://www.google.com.br/imgres?q=INDIGOFERA+SUFFRUTICOSA.

Os compostos naturais isolados têm contribuído para o desenvolvimento de novas drogas com perfil farmacológico. Um número crescente de estudos no país tem buscado verificar a extensão do uso de plantas, além de identificar e desenvolver novos agentes terapêuticos, provenientes de espécies comuns e exóticas, que possuam eficácia comprovada e baixa ou nenhuma toxicidade. Deste modo, com base em estudos científicos que relatam as ações anti-tumoral, antimicrobiana, entre outras, de *I. suffruticosa*, justifica-se a necessidade de avaliar as outras possíveis atividades anti-inflamatória e antinociceptiva desta espécie, que possam contribuir com o arsenal terapêutico.

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4 ARTIGO

Artigo: Anti-inflammatory and Antinociceptive Activity of Organic Extracts of Seeds of *Indigofera suffruticosa*.

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Anti-inflammatory and Antinociceptive Activity of Organic Extracts of Seeds of *Indigofera suffruticosa*

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Anti-inflammatory, Antinociceptive and Antioxidant Activity of Organic Extracts of Seeds of *Indigofera suffruticosa*

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ABSTRACT

Ethnopharmacological relevance: Indigofera suffruticosa is a medicinal plant commonly used in traditional medicine to relieve pain in inflammatory processes.

Aim of the study: The present study assessed the possible anti-inflammatory and antinociceptive action of the organic extracts obtained from seeds of *Indigofera suffruticosa* (Fabaceae) in models of chemical inflammation and nociception in mice.

Materials and methods: Anti-inflammatory activity was evaluated through oedema paw and peritonitis test in mice. Antinociceptive activity was evaluated through writhing and hot plate tests in mice. The antioxidant activity of *I. suffruticosa* extracts was evaluated and compared with α-Tocopherol using a 2,2—diphenyl-β-picrylhydrazyl (DPPH) assay.

Results: The acetone and methanol extracts of *I. suffruticosa* (400mg/kg, i.p.) produced significant inhibition on inflammation induced by carrageenan for oedema paw (60.1 and 58.7%, respectively). The Chloroform, acetone and methanol (400mg/kg, i.p.) extracts produced significant inhibition on inflammation for peritonitis (59, 46 and 49%, respectively) test. All organics extracts of *I. suffruticosa* (400mg/kg, i.p.) produced significant inhibition on nociception induced by acetic acid and hot-plate test. The Acetone and Methanol extracts presented too antioxidant activities.

Conclusions: Thus, this set of results supports the popular hypothesis of *I. suffruticosa* actions have antinociceptive, anti-inflammatory and antioxidants, and indicates that different extracts of this plant, it becomes a potential source of promising substances for obtain new drugs.

Keywords: anti-inflammatory, antinociceptive, antioxidant, *Indigofera suffruticosa*

1. Introduction

Historically, human have used medicinal plants as a traditional way of providing healing of several diseases.Currently, based onethnopharmacological data many pharmaceutical agents have been found at random by screening natural products coming plants (Salis-Lagoudakis, et al., 2012). It is well know that many this compounds derived of plants present several significant properties, such as anti-inflammatory and analgesic.

Indigofera suffruticosa Mill, known in Brazil as ‘‘anil’’ and‘‘anileira’’ is a plant of Antilles and Central America origin, frequently found in Brazil Northeast country side. It has been indicated in folk medicine for treating infection, throes, inflammation, and other diseases (Leite et al., 2003, Leite et al., 2006). Phytochemical investigations of this kind show that the extract has several biologically active chemical constituents including proteins, carbohydrates, steroids, phenols, alkaloids, flavonoids and indigo (Barros and Teixeira, 2008).

Assuming that the popular use of *I. suffruticosa* demonstrates anti-inflammatory effect was investigated in this study their biological actions using other biologic models. Few studies were performed with the species *I. suffruticosa*, thus, this study aimed to establish in vivo the antinociceptive and anti-inflammatory potential of fractionated extract with solvents of dry seeds of *Indigofera suffruticosa*.

2. Materials and methods

2.1 Plants materials

The seeds of *I. suffruticosa* were collected in the municipality of São Caetano, the arid region of Pernambuco State. Plant samples were indentified and authenticated by the Biologist Marlene Barbosa from the Botany Department, Federal University of Pernambuco (UFPE), Brazil, where a voucher specimen has been deposited at the Herbarium of the Botany department and registered with the number 45 217.

2.2 Preparations of plants extracts

The extracts were prepared from the dried finely ground seeds (100g) and extracted 3 x 200 mL with increasing polarity solvents (Ether, Chloroform, Acetone and Methanol), homogenized for two hours in a mechanical stirrer, kept under refrigeration (4 – 10º) overnight and filtered with Whatman filter paper (Nº 1). The solvents were removed by rotary evaporation (BUCHLER INSTRUMENTS, Fort Lee, NJ, USA).

2.3 Drugs admnistration

All the extracts (Ether, Chloroform, Acetone and Methanol) were dissolved in a vehicle Isopropilenoglicol (IPG). The vehicle (IPG) alone served as negative control. Acetyl salicylic Acid (ASA- 100 mg/kg-) served as positive control was also dissolved with IPG. Acetyl Salicylic Acid, IPG, Lambda (λ) - carrageenan was purchased from Sigma Chemicals Co. (St. Louis, MO, USA) and Acetic Acid from Merck (Damstadt, Germany). Solvents used for preparation in the extracts were purchased from Vetec (Rio de Janeiro, RJ, Brazil).

2.4 Animals

Male Swiss Albino mice (25 - 30g) were purchased for this study from researchers at the Keizo Asami Laboratory of immunopathology (LIKA). The animals were separated into groups (n=6, each) and were housed in cages and kept in light cycle (12h) and dark (12h). They were kept with free access to food (Labina) and water *ad libitum*. The experimental procedures were performed in accordance with the animal ethics committee rules and regulations followed by the university (case nº 0144113/2007-78). Studies of all extracts of *I. suffruticosa* in doses of 100, 200 e 400 mg/kg were tested in animals for paw oedema.

2.5 Preliminary Phytochemical Screening

A simple qualitative and semiquantitative phytochemical analysis was performed by screening tests according to Wagner et al. (1984), Markham (1982), Sharma and Bakhashi (1991). The phytochemical profile of extracts of *I. suffruticosa* were evaluated on thin layer chromatography plates in front of the mobile phase solvents containing different proportions and different polarities, and revealing, using as stationary phase, pre-activated GEK silica GF 254 plates (Merck). For identification

and differentiation of the disclosed compounds The following parameters were used: staining band and luminescence in UV lamps.

2.6 Anti-inflammatory Activity

2.6.1 Carrageenan-induced paw edema

The paw edema was induced from a subplantar injection of 0.1 ml carrageenan (1%) in saline half hour before the administration of the extracts (Winter et al., 1962). Dose of 400 mg / kg (i.p) of organic extracts of *I. suffruticosa* (Ether, Chloroform, Acetone and Methanol) was chosen because it has best results. The volume of the paw was measured by one caliper rule (Kanon- Staineless Mardened), at the time 0 and intervals of 1, 2, 3 and 4 h immediately after the subplantar injection of carrageenan. For the positive control group, animals received a dose 100mg/kg Acetylsalicylic acid (ASA). The negative control animals received just vehicle (IPG). The data obtained for the various groups were reported as mean ± S.D. and expressed in millimeter. The percentage inhibition was calculated using the formula given below, that represents the period of peak edema (3h). Percentage inhibition (%) = [(Vf-Vi) Control group mean – (Vf-Vi) Test group mean / (Vf-Vi) Control group mean] x 100, where Vf and Vi represent the volume of the initial and final paw.

2.6.2 Carrageenan-induced peritonitis

Peritonitis was conducted as described by Foster et al. (1986). Male Swiss mice (6 animals per group) were pre-treated with vehicle (Negative Control – IPG, i.p.), Acetylsalicylic acid (Positive Control - ASA, 100 mg/kg, *I.*p.), and different extracts of *I. suffruticosa* (400 mg/kg, i.p.), and 1h later, the animals received an injection of 1% carrageenan (i.p.). After 4 h, the animals were sacrificed. After, saline containing EDTA (1mM, i.p.) was injected, immediately a brief massage was done for further fluid collection and used for leukocyte (mainly neutrophils) counting in a Cell Counter (ABX MICROS 60). The results were expressed as the number of Leukocytes x $10^3/mm^3$. The percentage of the leukocyte inhibition = (1 – T/C) x 100, where T represents the treated groups leukocyte counts and C represents the control group leukocyte counts.

2.7 Antinociceptive Activity

2.7.1 Acetic acid-induced abdominal writhing

Abdominal writhing based of a contraction of the abdominal muscle together with a stretching of the hind limbs, induced by agent nociceptive (0.8% Acetic acid) with intraperitoneal injection (Koster et al., 1959). The animals received 400 mg / kg of organic extracts of *I. suffruticosa* (test group), ASA (100 mg / kg, positive control group) and vehicle (negative control group) 1 hour before administration of acetic acid (0.8%, i.p). The number of writhing reflexes was counted during the following 20 min. The percentage inhibition of the writhing response was calculated from the formula: % inhibition = (*D*0 – *D*t) / *D*0×100 where *D*0 was the average writhing response of the control group while *D*t was the average writhing response in the treated rats. A significant reduction of writhes in tested animals compared to those in the control group was considered as an antinociceptive response.

2.7.2 Hot plate test

The central analgesic activity of *I. suffruticosa* against thermal stimuli was studied in male mice using the hot plate test (MacDonald et al., 1946). Mice were individually placed in a hot plate heated at fixed temperature (55 ± 1.0 ° C) and response time to the stimulus was marked by a timer. Measurements were performed at time 0, 30, 60, 90, and 120 min after the first thermal stimulus. The maximum stay of the animal was 60 s to avoid damage. The control group was treated with vehicle and the test group (n = 6 each) with 400 mg / kg (i.p.) of different organic extracts of *I. suffruticosa* 1 hour before performing the experiments. ASA (100mg/kg, i.p.) was administered to control positive.

2.8 Acute Toxicity studies

Acute Toxicity study performed as per OECD Guidelines (OECD, 2004). Swiss Albino mice of either sex were used. The animals were fasted for 4h, but allowed free access to tap water throughout. Dose maximum of the extracts of *I. suffruticosa* was from 1000 mg/kg 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 drug administration.

2.9 Determination of antioxidant activity by the DPPH radical scavenging method

This methodology was performed using an ELX 800 Universal Microplate Reader (Biotek instruments, Inc., Winooski, VT). The reaction mixture in each one of the 96 well consisted of one of the different concentrations (50, 100, 200 and 500 µg/mL) of the extracts (100 µL) and methanolic solution(100 µL) containing DPPH radicals (150 µM). The mixture was kept for 30 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 495nm. The samples were compared with standard samples of α-tocopherol (50, 100, 200, 500 µg/mL). The radical scavenging activity was calculated as a percentage of DPPH discoloration using the equation: % AA = [*(Adpph - Aa*) / *Adpph*] ×100 where *Aa* was the sample absorbance, and *Adpph* was the DPPH solution absorbance.

3.0 Statistical analysis

The results of activities are presented as the mean ± standard deviation (S.D.). Statistical significance was determined by analysis of variance (ANOVA) followed by Bonferroni's test and Tukey test, with $p<0.05$ considered significant.

3. Results

3.1 Preliminary Phytochemical Screening

The brief phytochemical analysis of organic extracts of I. suffruticosa were described in the table 1.

3.2 The anti-inflammatory profiles of I. suffruticosa

3.2.1 Oedema paw

The injection of carrageenan in the sub-plantar tissue of the right hind paw of mice in negative control group caused a swelling that ranged from 5.27 to 4.48 mm thick. The positive control group which received a dose of 100 mg of aspirin (ASA) has produced a 60.3% inhibition in paw edema (Table 2). Groups that received doses of 100 and 200 mg/kg of *I. suffruticosa* showed no inhibition of inflammation. All extracts (400 mg/kg) showed an inhibition of the inflammation 35.2, 27.0, 60.1, 58.7% (Table 1) (ether, chloroform, acetone and methanol, repectively). However, we observed statistically significant only in groups acetone and methanol at all times evaluated (1 to 5 hours) compared with the negative control group. The effect

shown by acetone and methanol extracts, as shown in Table 1 was similar to aspirin, but not significantly different ($p > 0.05$) (Table 2).

3.2.2 Carrageenan-induced peritonitis

The results presented in Fig. 1, showed that administration of carrageenan in the peritoneal cavity resulted in the migration of leukocytes cells. The treatment of the chloroform, acetone and methanol extracts of the *I. suffruticosa* reduced the migration of leukocytes, with the percentage of inhibition, 59, 46, 49 and 60%, respectively, when compared with negative control, $p<0.0001$. The ether extract showed no significant difference compared to control. The chloroform extract had an effect comparable to ASA.

3.3 The anti-nociceptive profiles of I. suffruticosa

3.3.1 Acetic Acid-induced writing in mice

In the writhing test, intraperitoneal injection of 0.8% acetic acid evidently resulted in writhing reflex in mice. Figure 2 demonstrates the anti-nociceptive profile of *I. suffruticosa* assessed using the writhing test. The results showed that all extracts of *I. suffruticosa* (Ether, Chloroform, Acetone and Methanol), reduced significantly the numbers of abdominal construction acid acetic-induced in the mice with inhibition of 85.3, 88.7, 97 and 100%, respectively. Thus, the organic extracts of the *I. suffruticosa* were potent in inhibiting nociception when compared with the negative control (vehicle), and also with the positive control (ASA, 100mg/kg), with the percentage of inhibition 86.5(%).

3.3.2 Hot-plate test

The anti-nociceptive profile of *I. suffruticosa* assessed using the hot-plate test is shown in Fig. 3. Results of hot-plate test were very similar to that of the acetic acid-induced writing in mice. The all extracts of *I. suffruticosa* (400 mg/kg) also significantly ($p<0.05$) increased the mean of latency time to discomfort reaction when compared with positive control (ASA, 100 mg/kg).

3.4 Acute Toxicity studies

Finally, we evaluated the acute toxicity of extracts (máximum dose 1000mg/kg) which was administrated by intraperitoneal injection. Although the mice were given *I. suffruticosa*, no mortality was observed, but was noted some symptoms acute with administration of all extracts of *I. suffruticosa* included minor noticeable pilo-erection and hyperventilation during the assessment period (Data not shown). Further study on the chronic toxicity and physiological changes induced by *I. suffruticosa* will be carried out.

3.5 Determination of antioxidant activity by the DPPH radical scavenging method

The percentage of antioxidant activities by *I. suffruticosa* extracts due to hydrogen donation from the antioxidant for free radical DPPH was showed in Fig.4. The order of antioxidant activity of *I. suffruticosa* extract was: Acetone extract > Methanol extract with in the increase concentrations but not presented statistic significance among themselves. At 50 a 500 µg/mL, *I. suffruticosa* showed similar scavenging activity to α- tocopherol, but the standard was better when compared with all extracts. Further noted decreased antioxidant activity of the Chloroform fraction of with increasing its concentration, and the ether fraction had a little reduction and remained stable.

4 **Discussion**

Considering that the use of commercially analgesic and anti-inflammatory drugs exert a wide range of side effects (Vane & Botting, 1990), there is currently a strong interest in developing new therapeutic agents from plants (Iwalewa et al., 2007). The present study reported here demonstrated that *I. suffruticosa*, had significant anti-inflammatory and anti-anociceptive effects when assessed in models of chemical inflammation and nociception in mice.

We report here that the acetone and methanol extracts, but not the ether and chloroformic extracts obtained from *I. suffruticosa* reduced significantly the paw oedema induced by carregeenan. The carrageenam-induced inflammatory response was described in 1969 in the models of mice's paw (Levy,1969). Since the, for the development new anti-inflammatory drug this test has been more than used. According to Gemache et al. (1986), the carrageenan-induced paw oedema test controlled with the

arachidonate cycloxygenase (COX) inhibitors due to its Cox- dependent mechanism, thus, it is suggested that the acetonic and metanolic extracts of *I. suffruticosa* has compounds that may be acting in decreased vascular permeability, mediators, such as histamine, serotonin and prostaglandins. A similar effect was observed in study of methanol extracts of *I. oblongifolia* at a dose of 500mg/kg, wich decreased approximately 40% of paw oedema in rats (Upwar et al., 2011).

Cell recruitment during inflammation depends on the release of local mediators which is responsible for tissue changes as well as for the recruitment of host defense cells. These mediators are able to recruit leukocytes in the inflammation induced with carrageenan, such as neutrophilis. The tretatment with chloroform, acetone and methanol extracts of *I. suffruticosa* inhibited leukocyte migration induced by carrageenan ($p<0.0001$). This mechanism associated with this activity may be inhibition of the synthesis of inflammatory cytokines whose involvement in the cell migration is well- established. Approximately result was seen in a study with another plant, *Piptadenia stipulacea*, of the same family (*Fabaceae)* that *Indigofera suffruticosa* a decrease os approximately 36% of leukocyte migration (Queiroz et al., 2010).

We mention here that all extracts (ether, chloroform, acetonic and methanol) of *I. suffruticosa* produced a effect significant antinociceptive in models nociception in mice. Moreover, the methanolic extract showed more potent in inhibiting (100%) the acetic acid –induced nociceptive response. The acetic acid-induced has been used to confirm the peripheral anti-nociceptive activity and is considered non-specific (Chan et al., 1995), because this model test reflexes the direct interaction of the compounds with the various peripheral receptors within the peritoneal cavity (Bentley et al., 1983). The method involve the liberation of mediators, such as histamine, serotonin, cytokines and eicosanoids with an increase in peritoneal fluid that stimulate the nociceptive neurons (Zhang et al., 2005). This analgesic effect of the *I. suffruticosa* could be attributed, at least in part, to its anti-inflammatory effect as, in the pain model, the processor releases arachidonic acid via cyclooxygenase and prostaglandin. Santos et al. (2013) also obtained similar results to ours with inhibition of nociception of 82.3% at the dose of 400 mg / kg of aqueous extract of *Anadenanthera colubrina.*

Other test such as the hot-plate test, it's a central pain model, particularly the strong sensitivity to pain and limited tissue damage (Deraedt et al., 1980). The method induces an effect of termonociceptive skin and the integration of stimulus is due to stimulation of myelinated C fibers not driving slow (Hendry et al., 1999). The point is that the all extracts of *I. suffruticosa* increase the latency time of response in animal model of hot plate, and could be associated activity of supra-spinal analgesia. This effect anti-nociceptive, it has been reported with other study of the *Sutherlandia frutescens*, also family, *Fabaceae*, using aqueous extracts at doses of 5-800 mg/kg in mice (Ojewole, 2004). Therefore, from the methods of nociception analyzed, the antinociceptive effect central of all extracts of *I. suffruticosa* can be attributed to the presence of opioid molecules that may be acts as an antagonist that binds to receptor sites stereo specific and saturable in the brain, spinal cord and other tissues, providing the relieving pain.

In addition, we also evaluated the acute oral toxicity of all extracts of *I. suffruticosa*, which did not cause any death of mice at the dose 1000 mg/kg, although showing some clinical signs, can be considered safe phytotherapic when administered at lower doses. The increase of use of medicinal plants as oppose to the scarcity of scientific evidences on the safety of these have raised concerns regarding toxicity and detrimental effects. *Indigofera suffruticosa*, just as other plants, contains several bioactive principles which have the potential to cause beneficial and/or detrimental effects. These clinical abnormalities can be attributed to the presence of theses constituents. Long-term studies of tocixidade should be conducted to better explanations.

The metodology of DPPH based in the reduction of radical DPPH (2,2-diphenyl-1- picrilhidrazil), which change your colour of purple to yellow when receive one eletron or one hydrogen radical remained stable (RoginskyLissi, 2005). Quantitative evaluation of the antioxidant activity of extracts from *I. suffruticosa* suggested in the existence of substance with antioxidant acitivity.

The results of biological tests reveal the importance of furthering scientific knowledge of this specie, because suggest possible effects similar to that presented by the drug trade ASA.

5 Conclusion

In summary, this study the previous results on the anti-inflamatory and anti-nociceptive effect of seeds *Indigofera suffruticosa*, also scientifically justifies the use of this plant in the inflammation and pain in folk medicine. Futher investigations are need to identify the active constituints responsible for the anti-inflammatory and anti-nociceptive property of *I. suffruticosa*. These properties showed in this study, make this plant a potential target for the development of new compounds that can be explored as alternatives to drugs that are already in use.

Appendices

Table 1. Preliminary phytochemical Screening of organics extracts of *I. suffruticosa*.

Tests	Results of the extracts of *I. suffruticosa*			
	Ether	Chloroform	Acetone	Methanol
Alkaloids Dragendorf	++	++	++	++
Flavanoids Neu	+++	+++	+++	+++
Mono, sesqui, diterpene Vanilin sulfuric 2%	++	-	-	-
Steroids and triterpenoids Lieberman Buchard	++	-	+	+
Iridoids Vanilin sulfuric	-	-	-	+++
Coumarins UV	-	-	++	++
Cinnamic derivates Neu	-	-	-	-
Phenylpropanoglycosides UV	+++	+++	+++	+++
Proanthocyanidins Vanilin hydrochloric	-	-	-	+++

Table 2. Effect of organics extracts of *I. suffruticosa* (400mg/kg) in the thickness (mm) of the right posterior paw oedema of mice.

	Time				
Treatment	T0	T1	T2	T3	T4
Control	4.16 ± 0.114	5.27 ± 0.321	5.04 ± 0.281	4.82 ± 0.064	4.71 ± 0064
Acetyl salicylic acid	4.16 ± 0.182	4.90 ± 0.145*	4.55 ± 0.154*	4.42 ± 0.108*	4.35 ± 0.143*
Éther	4.32 ± 0.07	4.41 ± 0.079	4.51 ± 0.094	4.75 ± 0.161	4.82 ± 0.07
Chloroform	4.26 ± 0.1	4.70 ± 0.188	4.75 ± 0.136	4.74 ± 0.037	4.74 ± 0.037
Acetone	4.13 ± 0.072	4.6 ± 0.121*	4.5 ± 0.121*	4.4 ± 0.108*	4.26 ± 0.075*
Methanol	4.02 ± 0.115	4.43 ± 0.146*	4.39 ± 0.158*	4.29 ± 0.185*	4.2 ± 0.199*

Values are expressed as Mean ± S. D. ANOVA: * $p<0.001$, in relation to control group.

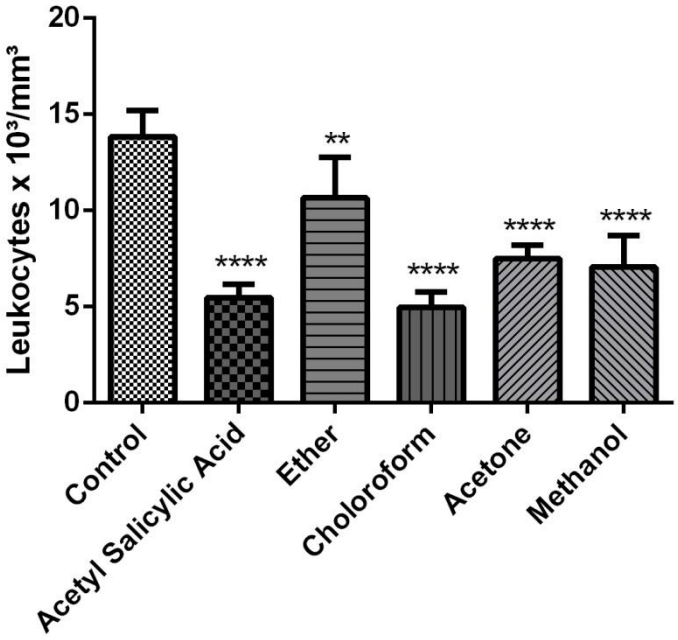

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Fig 1. Effect of the diferent extracts of *I. suffruticosa* in the leucocyte migration in the model of carrageenan-induced peritonitis. Groups of mice were pre-treated with vehicle (Control), Acetyl Salicylic Acid (100 mg/kg), and extracts of *I. suffruticosa* (400mg/kg) 60 min before carrageenan (1%) induced peritonitis. Cell couters were performed at the time 4 h after the injection of carrageenan. Each value representes the mean ± S.D (n=6, group). ** denote statistical significance, $p<0.05$ and ****$p<0.0001$, in relation to control group. ANOVA followed by Bonferroni's test.

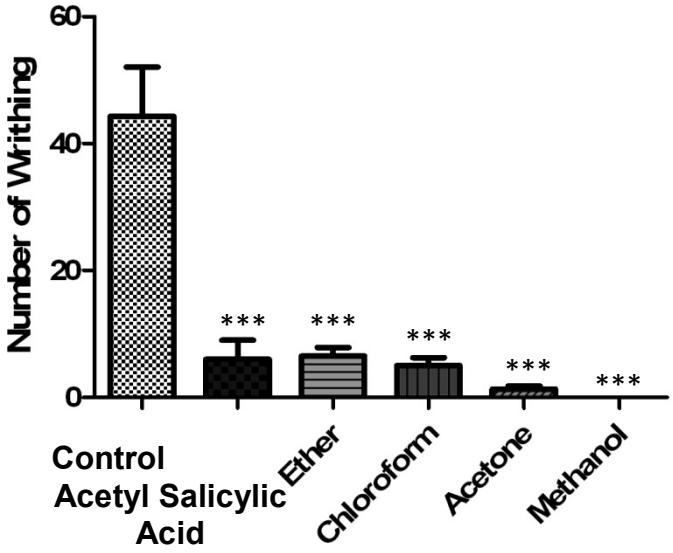

Fig 2. Effect of the different extracts of *I. suffruticosa* in the model of nociception induced by acetic acid. Nociception was registered by the number of writhes, wich the animal presented 20 min following i.p. acetic acid injection. Groups of mice were pre-treated with vehicle (Control), Acetyl Salicylic Acid (100 mg/kg), and extracts of *I. suffruticosa* (400mg/kg) 60 min before nociception agent. Each column represents the mean ± S.D. *** denote statistical significance, $p<0.001$ in relation to control group. ANOVA followed by Bonferronis test.

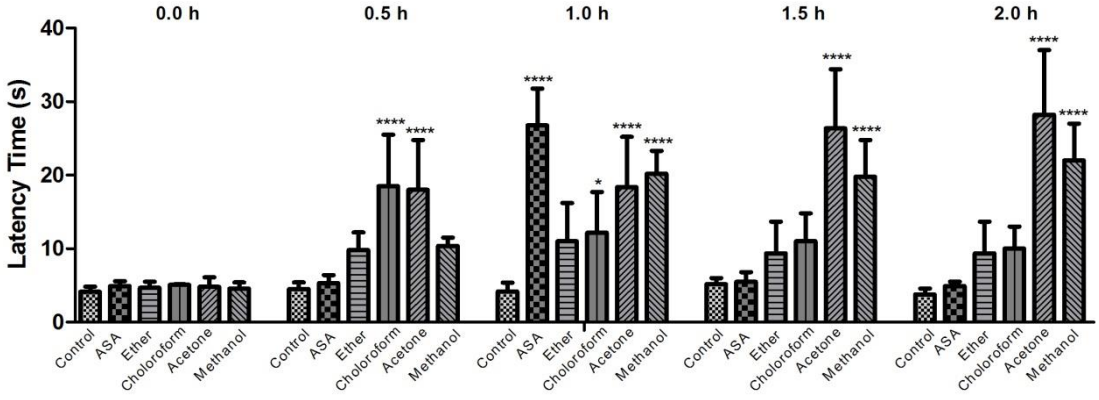

Fig 3. Analgesic effect of the diferent organics extracts of *I. suffruticosa* and Acetyl Salicylic Acid on hot plate test on mice. Groups of mice were pre-treated with vehicle (Control), Acetyl Salicylic Acid (100 mg/kg), and extracts of *I. suffruticosa* (400mg/kg) 60 min before nociception agent. Each column represents the mean ± S.D. * denote statistical significance, $p<0.05$, and **** $p<0.0001$ in relation to control group. Values are expressed as Mean ± S. D. ANOVA.

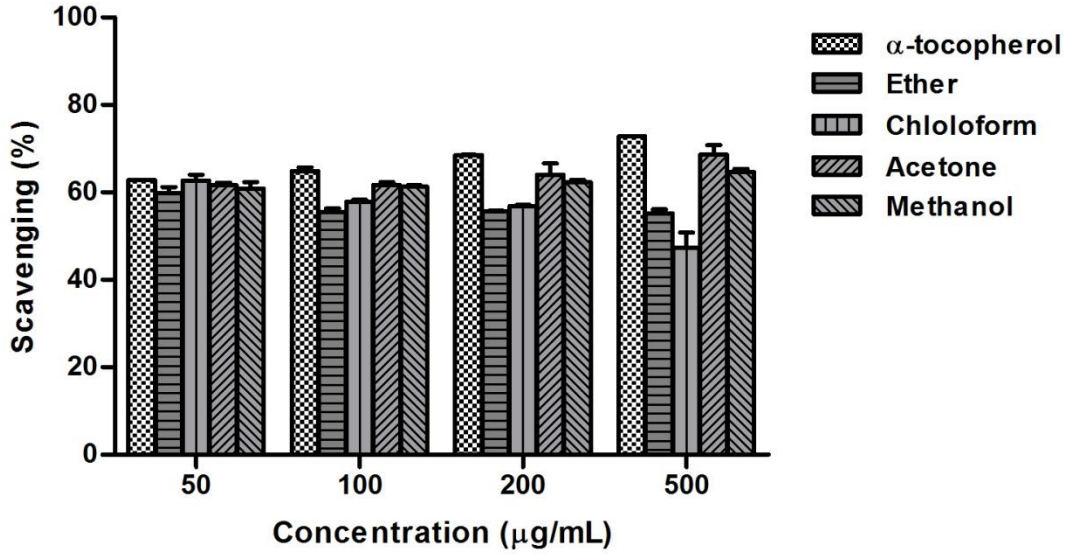

Fig 4. Effect of the different extracts of *I. suffruticosa* in the determination of antioxidant activity by the DPPH radical sacavenging method. The standard used was α-Tocopherol. Each column represents the mean ± S.D. ANOVA followed by Tukey test was used..

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5 CONCLUSÕES

- Os extratos orgânicos de *Indigofera suffruticosa* apresentaram ações anti-inflamatórias, especificamente o Acetônico e Metanólico para o modelo de inflamação aguda (edema de pata induzido com carragenina) em diferentes intervalos de tempos, e o Clorofórmico apenas para o modelo de inflamação com peritonite induzida com carragenina, sugerindo a ação de compostos presentes nas sementes desta espécie que tenha ação sobre diferentes mediadores e quimiotaxia.

- Com os modelos de nocicepção utilizados nos camundongos foi possível evidenciar que todos os extratos de *Indigofera suffruticosa* possuem compostos com ações anti-nociceptivas, e seus mecanismos de ação podem estar associados com as suas propriedades anti-inflamatórias como também a modulação de receptores de dor.

- Os resultados apresentados contribui para ampliação dos conhecimentos das ações biológicas e apontam o potencial farmacológico de *Indigofera suffruticosa* e também identifica a necessidade de realizar novos estudos sobre seus compostos bioativos e seus mecanismos de ação, para que possam ser promissores para obtenção de novas drogas.

6 PERSPECTIVAS

A busca por medicamentos derivados de plantas, também conhecidos como fitoterápicos, tem crescido expressivamente em países em desenvolvimento. Os compostos derivados de plantas são atualmente utilizados na terapêutica moderna, além de terem um papel relevante para síntese de algumas moléculas mais complexas. Diante dos resultados obtidos sobre as atividades antiinflamatórias e antinociceptivas presentes nos extratos de *I. suffruticosa*, observa-se que essa espécie conhecida na medicina popular constitue uma fonte biologicamente ativa promissora para exploração e desenvolvimento de novos fármacos com menos efeitos adversos e baixo custo.

ANEXOS

ANEXO 01

Carta de aprovação do Comitê de Ética

Universidade Federal de Pernambuco
Centro de Ciências Biológicas
Av. Prof. Nelson Chaves, s/n
50670-420 Recife - PE - Brasil
Fone: (55 81) 2126 8340 | 2126 8351
Fax: (55 81) 2126 8350
www.cb.ufpe.br

Oficio nº 08/08

Recife, 04 de janeiro de 2008

Da Comissão de Ética em Experimentação Animal (CEEA) da UFPE
Para: **Profa. Vera Lúcia de Menezes Lima**
Departamento de Bioquimica - UFPE
Processo nº 014413/2007-78

Os membros da Comissão de Ética em Experimentação Animal do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEEA-UFPE) avaliaram a resposta de V. Sa. referente ao primeiro parecer da CEEA sobre o projeto de pesquisa intitulado **"Avaliação de atividade biológica em extratos de *Indigofera suffruticosa* Mill. (LEG. PAPILIONOIDEAE)"**

Concluimos 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 CEEA-UFPE.

Encontra-se de acordo com as normas vigentes no Brasil, especialmente a Lei 9.605 – art. 32 e Decreto 3.179-art 17, de 21/09/1999, que trata da questão do uso de animais para fins cientificos.

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

Atenciosamente,

Prof. Silene Carneiro do Nascimento
Presidente CEEA
UFPE

ANEXO 02

GUIDE FOR AUTHORS

(*Journal of Ethnopharmacology*)

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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2. Ethnopharmacological communications (formerly 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.
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Strunk Jr., W., White, E.B., 1979. The Elements of Style, third ed. Macmillan, New York.
Reference to a chapter in an edited book:
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