Full Length Research Paper

Anti-inflammatory and antinociceptive activity of field-growth plants and tissue culture of *Cleome spinosa* (Jacq.) in mice

Norma Albarello[1]*, Claudia Simões-Gurgel[1], Tatiana Carvalho de Castro[1], Carlos Roberto M. Gayer[2], Marsen Garcia Pinto Coelho[2], Roberto Soares de Moura[3] and Elisabeth Mansur[4]

[1]Departamento de Biologia Vegetal - DBV/IBRAG. R. São Francisco Xavier, 524, PHLC, sala 509. Maracanã, RJ. Brasil. CEP: 20.550-013. Universidade do Estado do Rio de Janeiro (UERJ).
[2]Departamento de Bioquímica - DBq/IBRAG. Av. 28 de Setembro, 87 fundos. 4o. Andar. Vila Isabel, RJ. Brasil. CEP: 20.551-030. Universidade do Estado do Rio de Janeiro (UERJ).
[3]Departamento de Farmacologia e Psicobiologia - DFP/IBRAG. Av. 28 de Setembro, 87 fundos. 5o. Andar. Vila Isabel, RJ. Brasil. CEP: 20.551-030. Universidade do Estado do Rio de Janeiro (UERJ).
[4]Departamento de Biologia Celular - DBCel/IBRAG. R. São Francisco Xavier, 524, PHLC, sala 505. Maracanã, RJ. Brasil. CEP: 20.550-013. Universidade do Estado do Rio de Janeiro (UERJ).

Methanol extracts (ME) of *Cleome spinosa* obtained from plants of natural growing areas (NP) and acclimatized plants (AP), as well as from the lipophilic mass containing beta-carotene (LMBC) produced from callus cultures were evaluated for their anti-inflammatory and antinociceptive potentials. These properties were studied using carrageenan-induced paw edema and acetic acid-induced writhing animal models. The ME from leaves of NP and AP 10 mg/kg, intraperitoneally (i.p.) caused a significant inhibition of carrageenan-induced paw edema. Antinociceptive activity was observed in extracts from leaves of AP, leaves and stems of NP and LMBC, at 50 mg/kg (i.p.), corresponding to half concentration of the standard drug dipyrone (100 mg/kg). The extracts did not present toxicity as determined by *Artemia salina* bioassay. Our results demonstrated that *C. spinosa* has anti-inflammatory and antinociceptive properties, confirming its popular use, and also served to extend the use of plant biotechnology tools for investigations on the pharmacological potential and phytochemical exploitation of this species.

Key words: Cleomaceae, analgesic effect, anti-inflammation, plant tissue culture, beta carotene.

INTRODUCTION

Many species of the genus *Cleome* have been investigated for medicinal properties and some of them were evaluated for anti-inflammatory (Selloum et al., 1995; Nagaya et al., 1997; Fushiya et al., 1999; Bouriche et al., 2003, 2005; Simões et al., 2006; Sharma et al., 2010a) and analgesic (Singh and West, 1991; Parimaladevi et al., 2003; Bose et al., 2007) activities.

Cleome spinosa Jacq. is a shrub known in Brazil as "mussambê-de-espinho" or "sete-marias" used in Northeastern Brazil among herbal medicine practitioners for the treatment of diseases related to respiratory tract, and several inflammatory disorders. Leaf and flower infusions and syrups are usually prepared for treatment of fever, influenza, cough, bronchitis and asthma, while

*Corresponding author. E-mail: albarellopereira@yahoo.com.br. Tel: 55 021 23340273. Fax: 55 021 23340595.

the whole plant shows digestive and cicatrizing effects (Cabral and Agra, 1998; Agra et al., 2007).

There is little information about the medicinal properties of *C. spinosa* in the literature. Chemical studies led to the isolation of glucosinolates (glucocapparin and glucocleomin) and cembranes. Both groups are related to antineoplasic activity, and the latter is known to display several biological activities, including anti-human immunodeficiency virus (HIV) and neuroprotection (Ahmed et al., 1972; Germano et al., 2002; Collins et al., 2004).

During the last few years, many plants used in phytopharmaceutical preparations are being overexploited, and several species are disappearing at an alarming rate. In addition, medicinal plants can be associated with a broad variety of microbial contaminants and the main prerequisite for the development of high-quality phytopharmaceutical products is a consistent source of high-quality plant material.

Hence, advanced biotechnological methods for culturing plant cells and tissues should provide alternatives for propagating valuable and endangered medicinal plants and their metabolites (Murch and Saxena 2001; Kneifel et al., 2002; Nalawade and Tsay, 2004). In fact, these strategies contribute to the conservation and the sustainable use of biodiversity. Tissue culture technologies have been intensively used in afforestation and plantation programs, but also for plant improvement and *in vitro* production of metabolites. Considering the medicinal plants, the possibility of exploring bioactive compounds without commercial harvesting based on wild populations, represent the most efficient strategy to maintain the natural resources (Paunescu, 2009; Sharma et al., 2010b, 2011).

As part of our investigations on plant materials with medicinal interest obtained via tissue culture, the present study was undertaken to investigate the anti-inflammatory and antinociceptive potentials of *C. spinosa* cultured under *in vivo* and *in vitro* conditions and to support the traditional use of this species.

MATERIALS AND METHODS

Plant

Aerial parts of *C. spinosa* were collected after fructification at "Morro do Sampaio", Rio de Janeiro, Brazil. The species was identified by Dr. Maria Bernadete Costa e Silva (Herbarium of IPA - Pernambuco, Brazil), and a voucher specimen (HRJ 7639) is kept at the Herbarium of Rio de Janeiro State University.

Plant tissue culture

Production and acclimatization of in vitro plants

Shoots obtained through direct organogenesis induced on MS medium (Murashige and Skoog, 1962) supplemented with 1.0 mg/L BA were transferred to flasks containing MS medium devoid of growth regulators (MS0) for elongation and root induction (Albarello et al., 2006). Cultures were maintained at 26 ± 1°C and 16 h

photoperiod under an irradiance of 45 µmol m^{-2} s^{-1} supplied by cool white fluorescent and Grolux lamps (3:1). Whole plants were transferred to plastic pots containing a mixture of garden soil and sand (1:1) covered with plastic cloches for hardening, and were kept at 28 ± 3°C and 12 h photoperiod. During the second week, the cloches were gradually perforated with a style and were totally removed after the third week. Plants established under *ex vitro* conditions (acclimatized plants (AP)) were used for pharmacological assays eight weeks from planting.

Callus cultures

Stem segments (5 mm) excised from axenic plants kept on MS0 medium were used as explants. The cultures were incubated at 26 ± 2°C in a culture chamber with 16 h photoperiod. Callus cultures were established on MS medium supplemented with 1 mg/L 4-amino-3,5,6-trichloropicolinic acid (picloram, PIC) and sub-cultured to culture media with the same composition at 4-week intervals. Calluses produced a lipophilic mass containing β-carotene (LMBC) that was identified by the absorption spectrum, and through TLC and HPLC analyses using β-carotene standard (Albarello et al., 2007). LMBC were aseptically removed and transferred to Eppendorf tubes for the pharmacological tests.

Chemicals and drugs

Carrageenan, acetic acid, β-carotene, indomethacin, dipyrone, benzyladenine, and picloram (PIC) were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. Methanol and ethanol were obtained from Merck, SP, Brazil. All the chemicals and drugs used were of analytical grade.

Preparation of plant extracts

Samples of leaves (130 g) and stems (120 g) from plants of natural growing areas (NP) were fragmented and dried at 45°C for 48 h yielding 14.3 and 12.9 g, respectively. The same procedure was performed with leaves (73 g) and stems (82 g) from AP, yielding 8.6 and 8.9 g, respectively. The samples were extracted with MeOH p.a. for 14 days at room temperature, with agitation on a rotary shaker (90 rpm). The extracts were filtered and evaporated to dryness under vacuum to give a semi-solid residue, resulting in 1.0 g (NP leaf), 0.52 g (NP stem), 0.60 g (AP leaf), and 0.33 g (AP stem). The extracts were stored at 4°C and subsequently diluted in 15% ethanol for use in the pharmacological tests.

Pharmacological procedures

Animals

Experiments were conducted using Swiss Webster (SW) mice of either sex (25 to 35 g), housed at 26 ± 1°C under a 12 h light/dark cycle and with access to food and water *ad libitum* until 1 h before the experiments. Animals were maintained and handled according to standard procedures. All experiments were performed under the consent and surveillance of the Biomedical Center Ethics Committee of the Rio de Janeiro State University for the use of animals in research.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using λ-carrageenan-induced paw edema assay (Levy, 1969). Groups of 6 animals were

pre-treated intraperitoneally (i.p.) with methanol extracts (ME) from NP and AP leaves (1 - 50 mg/kg), NP and AP stems (10 mg/kg) and LMBC (10 mg/kg). Indomethacin in Na_2CO_3 0.2% (10 mg/kg, i.p.) was used as reference anti-inflammatory drug and the control group received only the vehicle (15% ethanol, 10 ml/kg, i.p). One hour after the treatments, acute inflammation was produced by subplantar administration of 50 µl of 0.6 g% (w/v) carrageenan suspension in physiological saline into the right hind paw of each mouse. The edema was measured immediately prior to the carrageenan injection and after 1, 3, and 4 h using a plethysmometer (7150 Ugo Basile) and was compared with the volume of the same paw at the time of carrageenan challenge (edema index) according to the following formula:

$$EI = \frac{t_1 - t_0}{t_0} \times 100$$

where t_0 = initial volume of the paw, and t_1 = final volume of the paw. The percentage of edema inhibition was calculated in comparison to the control animals.

Antinociceptive activity

The antinociceptive effect was evaluated by the writhing test as previously reported by Koster et al. (1959). Abdominal constrictions were induced by intraperitoneal injection of diluted acetic acid (10 ml/kg, 0.6% v/v). Animals were pre-treated i.p., thirty minutes before acetic acid injection, with the *C. spinosa* extracts at 50 mg/kg and with the standard analgesic drug dipyrone (100 mg/kg, s.c). Control groups were pre-treated i.p. with NaCl 0.9% (10 ml/kg) or with the vehicle (15% ethanol, 10 ml/kg). After acetic acid injections, mice were placed individually in polyethylene boxes (40 × 30 × 25 cm) and were maintained in a quiet and illuminated room. The number of resulting writhes was cumulatively counted over a period of 10 min, starting 5 min after the administration of the acetic acid solution. Antinociceptive activity was expressed as the reduction in the number of writhes.

Cytotoxicity assay

The evaluation of cytotoxicity was performed using the brine shrimp (*Artemia salina*) lethality assay according to Meyer et al. (1982) with small modifications. Eggs of *A. salina* were incubated in a hatching chamber with seawater, at temperatures from 25 ± 2°C and natural illumination. The eggs were hatched within 24 to 48 h producing a large number of larvae (*nauplii*). Ten *nauplii* were transferred with a Pasteur pipette into vials containing different concentrations of extracts (0.1, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/ml) in 5 ml of seawater. The experiments were carried out in triplicate and were repeated twice. Controls were prepared in vials containing only seawater and the number of survivors was counted 24 h later.

Statistical analysis

The results are reported as the mean ± standard error of mean (SEM). Statistical significance was determined by one-way analysis of variance (ANOVA) and treatments means were separated using Dunnett's test (Graph Pad in Stat, version 3.01), considering P ≤ 0.05.

RESULTS AND DISCUSSION

This is the first study related to anti-inflammatory and antinociceptive effects of extracts from *C. spinosa* using the material obtained from natural growing areas and from plant cell and tissue culture technology. Exploration of the biosynthetic capabilities of plant material obtained through *in vitro* cultures has been carried out for many scientific groups and promising findings have been reported for a variety of medicinally valuable substances, including anti-inflammatory and antinociceptive activities (Macedo et al., 1999; Catapan et al., 2000; Kuhlmann and Röhl, 2006; Gyurkovska et al., 2011). The possibility to control physical and chemical parameters under *in vitro* conditions can result in a standardized production of bioactive compounds (Rao and Ravishankar, 2002; Vanisree et al., 2004).

The antiedematogenic properties of *C. spinosa* extracts were evaluated during the first phase of the carrageenan-induced edema 2 to 4 h after injection. The time course of the inflammatory process showed the edema peak at 3 h. Reduction of carrageenan-induced edema by leaf extracts from both NP and AP was similar to that observed in response to the standard drug indomethacin (Figure 1). Then, leaf extracts from NP and AP were tested in order to determine the most effective concentration to reduce the inflammatory response. A significant decrease (P < 0.01) of edema was observed in extracts from both origins at concentrations between 10 and 50 mg/kg. This effect was not significantly different from that caused by indomethacin at 10 mg/kg (Figure 2). Based on these results, the concentration of 10 mg/kg was chosen for further studies on the anti-inflammatory activity. On the other hand, no significant reduction of the edema peak was observed when using stem extracts from both NP and AP or LMBC after the same period (Figure 3). Carrageenan-induced edema has been frequently used as an experimental model for acute inflammation. It evokes a potent local acute response (Levy, 1969) with a biphasic profile (Henriques et al., 1987) and is mainly mediated by histamine, serotonin, and prostaglandins (Perazzo et al., 2005).

In the present study, from the values observed during edema formation, it appears that *C. spinosa* leaves are more efficient in inhibiting the mediators involved in the anti-inflammatory process. This activity was also observed from leaf extracts in other species of the genus *Cleome*. Bouriche et al. (2003) reported the efficiency of leaf extracts from *Cleome arabica* to inhibited paw edema formation in a dose-dependent manner. Moreover, these authors found that the extract was beneficial for the treatment of inflammatory conditions, particularly those characterized by excessive leukotriene generation (Bouriche et al., 2005). Anti-inflammatory activity was also reported for the leaf extracts of *Cleome gynandra* (Narendhirakannan et al., 2005) and extracts of aerial parts of *C. rutidosperma* (Bose et al., 2007). However, leaf extracts from *C. spinosa* significantly inhibit the paw edema at low concentration (10 mg/kg) when compared with other *Cleome* species (Bouriche et al., 2003; Bose et
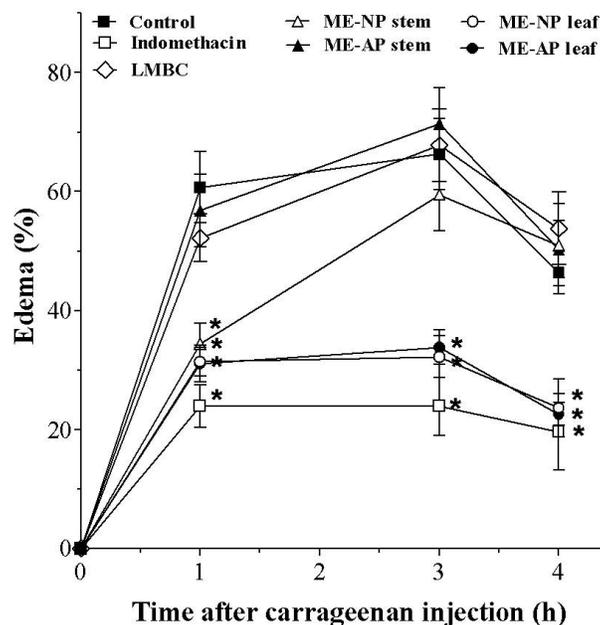
Figure 1. Time course effect of extracts from *C. spinosa* on carrageenan-induced mouse paw edema. ME-NP, ME-AP, and lipophilic mass containing beta-carotene produced from callus culture (LMBC) were evaluated at 10 mg/kg (i.p.). Control group received the vehicle (15% ethanol). Indomethacin (10 mg/kg, i.p.) was used as standard drug. Paw volumes were measured plethysmographically before and 1, 3, and 4 h after intraplantar carrageenan injection (50 µl of 0.6 g%). Each point represents the edema in relation to the same paw at t_0. *P < 0.01 versus control group.
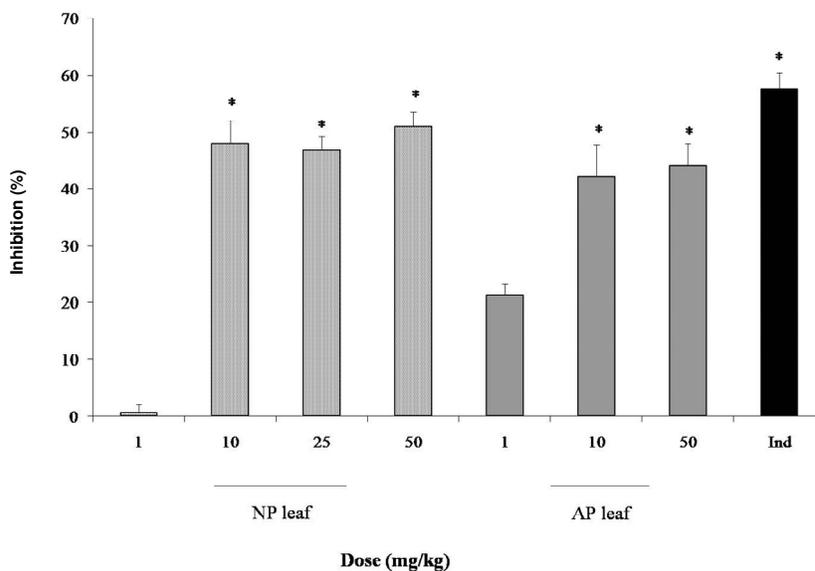

Figure 2. Effect of methanol extracts of leaves from *C. spinosa* on carrageenan-induced mouse paw edema. Extracts obtained from the natural growing areas (NP) and from acclimatized plants (AP) were tested at different doses (i.p). Indomethacin (Ind) at 10 mg/kg, i.p. was used as standard drug. Each point represents the inhibition index at 3 h in relation to control group (vehicle). *P < 0.01 (Dunnett's post-hoc test) was calculated from de edema of each group versus mean of control group.
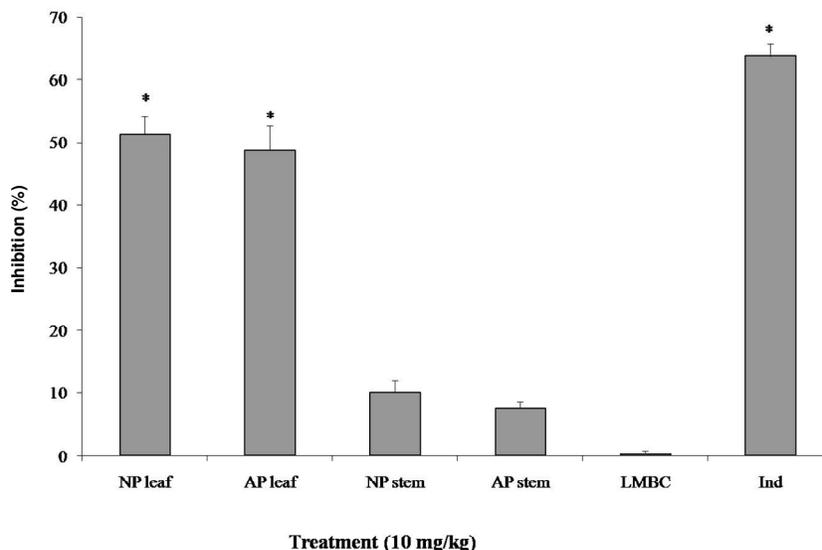
Figure 3. Comparison of the inhibition effect of extracts from *C. spinosa* on carrageenan-induced mouse paws edema. Methanol extracts obtained from plants of natural growing areas (NP) and from acclimatized plants (AP), as well as the lipophilic mass containing β-carotene produced from callus culture (LMBC) were evaluated at 10 mg/kg (i.p). Indomethacin (Ind) at 10 mg/kg, i.p. was used as standard drug. Each point represents the inhibition index in relation to control group (vehicle) at 3 h. *P < 0.01 versus control group (Dunnett's post-hoc test).

al., 2007).

The anti-inflammatory properties of plant extracts are associated to diverse bioactive substances, however, several phytochemical studies have shown that phenolic compounds present antioxidant properties and play an important role in the prevention of various pathological conditions and chronic diseases associated with oxidative stress, including inflammatory processes (Khansari et al., 2009; Oskoueian et al., 2011; Singab et al., 2011). Recent studies on the genus *Cleome* have reported anti-inflammatory activity of coumarino-lignoids from *Cleome viscosa* (Sharma et al., 2010a), and antioxidant properties were detected on *C. spinosa* extracts, which were considered a rich source of flavonoids (Leal et al., 2007).

A significant antinociceptive effect was observed in extracts from leaves and stems of NP, leaves of AP, and LMBC at 50 mg/kg. These materials were as effective as dipyrone at 100 mg/kg (Figure 4). Although, leaf extracts of AP presented similar effects when compared with dipyrone, these extracts were less effective to reduce the number of writhes, when compared with leaf extracts from NP, indicating that the micropropagation process might cause a reduction on the synthesis of antinociceptive compounds. The most significant reduction on the number of writhes (68%) was achieved in response to extracts from stem of NP, and to LMBC. The results obtained with LMBC are particularly important, since metabolites with medicinal interest can

be provided continuously on callus cultures.

Analgesic activity has also been reported for other species of *Cleome*. The effect of ME of *C. viscosa* in acetic acid-induced writhing test resulted in a significant and dose-dependent analgesic activity, showing 64% of writhing inhibition in the highest concentration tested (400 mg/kg) (Parimaladevi et al., 2003). Similar responses were observed with ethanol extracts of aerial parts of *Cleome rutidosperma* when animals were treated orally with 200 and 400 mg/kg (Bose et al., 2007). These results demonstrate that the extracts of *C. spinosa* presented a higher analgesic activity when compares with the other *Cleome* spp., once they reached similar wrighting inhibition at low concentration (50 mg/kg). The brine shrimp (*A. salina*) lethality assay was adopted for determining toxicity of *C. spinosa* extracts. This assay is a useful tool to evaluated toxicity of medicinal plants due to its quickness, simplicity, commercial availability, lack of animal use, and therefore, low cost (Meyer et al., 1982). The effectiveness of this method was studied by Parra et al. (2001) in comparison with *in vivo* methodologies in order to determine oral acute toxicity of 20 plant extracts, resulting in a good correlation (r = 0.85 P < 0.05) between them. The extracts of *C. spinosa* were evaluated at different concentrations until 3 mg/ml. The results showed that toxicity activity of the extracts was very low, since the rate of mortality reached by the highest extracts concentration tested against *A. salina* larvae ranged between 15 to 30%. According to Meyer et al. (1982),
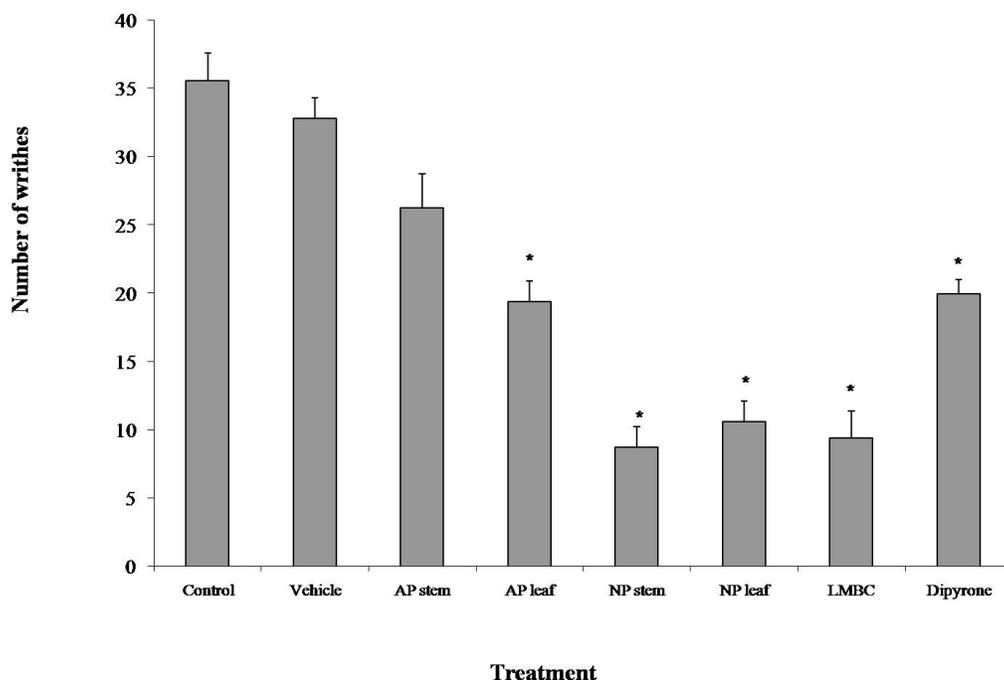
Figure 4. Antinociceptive effect of the *C. spinosa* extracts (50 mg/kg) propagated under *in vivo* (NP) and *in vitro* (AP) conditions on the acetic acid-induced writhing in mice. Dipyrone at 100 mg/kg, s.c. was used as standard drug. *$P \leq 0.05$ versus control (saline) or vehicle (ethanol 5%).

plant extracts that present median lethal concentration higher than 1 mg/ml are considered non-toxic. Therefore, they can be classified as biologically safety compounds with pharmaceutical properties. Some other studies have shown the low toxicity of bioactive plant extracts against *A. salina* (Lachumy et al., 2010; Taviano et al., 2011).

Conclusively, this work indicated that ME from leaves of field-grown and *in vitro* cultured plants have potent anti-inflammatory and analgesic activities. Stem extracts from field-grown plants and the lipophilic material containing beta carotene produced from callus cultures (LMBC) also displayed analgesic activity. These results demonstrate the significance of biotechnological methods in the study of medicinal plants, since pharmacological activities found on different materials obtained from *C. spinosa* validated the application of tissue culture technology for the production of plants and useful compounds of the species. Furthermore, our results scientifically support the therapeutic use of the species in folk medicine.

Further research would be of interest to elucidate the mechanism of anti-inflammatory, analgesic and protective effects found, and studies using fractionated samples must be carried out to determine the metabolites correlated with these pharmacological activities.

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ABBREVIATIONS

NP, plants from the natural growing areas; **AP**, acclimatized plants; **ME**, methanol extract; **MS**, Murashige and Skoog's medium; **MS0**, growth regulator-free MS medium; **BA**, benzyladenine; **PIC**, 4-amino-3,5,6-trichloropicolinic acid (picloram); **MeOH**, methanol; **LMBC**, lipophilic mass containing beta carotene.

REFERENCES

Agra MF, Freitas PF, Barbosa-Filho JM (2007). Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Braz. J. Pharmacog. 17: 114-140. doi: 10.1590/S0102-695X2007000100021.

Ahmed ZF, Riszk AM, Hammouda FM, Seif el-Nasr MM (1972). Naturally occurring glucosinolates with special reference to those of family Capparidaceae. Planta Med. 21:35-60. doi:10.1055/s-0028-1099522.

Albarello N, Ribeiro IG, Simões C, Castro TC, Gianfaldoni MG, Callado CH, Kuster RM, Coelho MGP, Mansur E (2007). Histological

analysis of calluses from *in vitro* propagated plants of *Cleome spinosa* Jacq. Braz. J. Biosci. 5:699-701.

Albarello N, Simões C, Rosas PGF, Castro TC, Gianfaldoni MG, Callado CH, Mansur E (2006). *In vitro* propagation of *Cleome spinosa* (Capparaceae) using explants from nursery-grown seedlings and axenic plants. *In vitro* Cell. Dev. Biol. Plant 42:601-606. doi:10.1079/IVP2006828.

Bose A, Mondal S, Gupta JK, Ghosh T, Dash GK, Si S (2007). Analgesic, anti-inflammatory and antipyretic activities of the ethanolic extract and its fractions of *Cleome rutidosperma*. Fitoterapia 78:515-520. doi:10.1016/j.fitote.2007.05.002.

Bouriche H, Miles EA, Selloum L, Calder PC (2005). Effect of *Cleome arabica* leaf extract, rutin and quercetin on soybean lipoxygenase activity and on generation of inflammatory eicosanoids by human neutrophils. Prostaglandins Leukot. Essent. Fatty Acids 72:195-201. doi: 10.1016/j.plefa.2004.10.018

Bouriche H, Selloum L, Tigrine C, Boudoukha C (2003). Effect of *Cleome arabica* leaf extract on rat paw edema and human neutrophil migration. Pharm. Biol. 41:10-15.

Cabral SCM, Agra MF (1998). Etnomedicina e farmacobotânica das Capparaceae da caatinga paraibana, Brasil. Braz. J. Pharm. 79:2-6.

Catapan E, Otuki MF, Viana AM, Yunes RA, Bresciani LFV, Ferreira J, Santos ARS, Calixto JB, Cechinel V (2000). Pharmacological activity and chemical composition of callus culture extracts from selected species of *Phyllanthus*. Pharmazie 55:945-946.

Collins DO, Reynolds, WF, Reese PB (2004). New Cembranes from *Cleome spinosa*. J. Nat. Prod. 67:179-183. doi: 10.1021/np0303299.

Fushiya S, Kishi Y, Hattori K, Batkhuu J, Takano F, Singad ANB, Okuyama T (1999). Flavonoids from *Cleome droserifolia* suppress NO production in activated macrophages *in vitro*. Planta Med. 65:404-407.doi: 10.1055/s-1999-14084.

Germano MP, Pasquale R, D'angelo V, Catania S, Silvari V, Costa C (2002). Evaluation of extracts and isolated fraction from *Capparis spinosa* L. buds as an antioxidante source. J. Agric. Food Chem. 50:1168-1171. doi: 10.1021/jf010678d

Gyurkovska V, Alipieva K, Maciuk A, Dimitrova P, Ivanovska N, Haas C, Bley T, Georgiev M (2011). Anti-inflammatory activity of Devil's claw *in vitro* systems and their active constituents. Food Chem. 125:171-178. doi:10.1016/j.foodchem.2010.08.056.

Henriques MGMO, Silva PMR, Martins MA, Flores CA, Cunha FQ, Assreury-Filho F, Cordeiro RSB (1987). Mouse paw edema: A new model for inflammation? Braz. J. Med. Biol. Res. 20:243-249.

Khansari N, Shakiba Y, Mahmoudi M (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Recent. Pat. Inflamm. Allergy Drug Discov. 3:73-80. doi:10.2174/187221309787158371.

Kneifel W, Czech E, Kopp B (2002). Microbial Contamination of Medicinal Plants - A Review. Planta Med. 68:5-15. doi:10.1055/s-2002-20060.

Koster R, Anderson M, de Beer EJ (1959). Acetic acid for analgesic screening. Fed. Proc. 18:412.

Kuhlmann A, Röhl C (2006). Phenolic antioxidant compounds produced by *in vitro* cultures of Rosemary (*Rosmarinus officinalis*) and their anti-inflammatory effect on lipopolysaccharide-activated microglia. Pharm. Biol. 44:401- 410.

Lachumy SJT, Sasidharan S, Sumathy V, Zuraini Z (2010) Pharmacological activity, phytochemical analysis and toxicity of methanol extract of *Etlingera elatior* (torch ginger) flowers. Asian Pac. J. Trop. Med. 3:769-774.

Leal RS, Maciel MAM, Dantas TNC, Melo MD, Pissinate K, Echevarria A (2007). Perfil etnobotânico e atividade antioxidante de *Cleome spinosa* (Brassicaceae) e *Pavonia varians* (Malvaceae). Rev. Fitos. 3:25-31.

Levy L (1969). Carrageenan paw edema in the mouse. Life Sci. 8: 601-606. doi:10.1016/0024-3205(69)90021-6.

Macedo AF, Barbosa, NC, Esquibel, MA, Souza, MM, Cechinel, V (1999). Pharmacological and phytochemical studies of callus culture extracts from *Alternanthera brasiliana*. Pharmazie 54:776-777.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982). Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med. 45:31-4.

Murashige T, Skoog F (1962). A revised medium for rapid growth and biossays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Murch SJ, Saxena PK (2001). Molecular fate of thidiazuron and its effects on auxin transport in hypocotyls tissues of elargonuim x hortorum Bailey. Plant Growth Regul. 35:269-275.

Nagaya H, Tobita Y, Nagae T, Itokawa H, Takeya K, Halim AF, Abd el-Halim O (1997). Cytotoxic triterpenes from *Cleome africana*, Phytochemistry 44:1115-1119. doi:10.1016/S0031-9422(96)00681-4.

Nalawade SM, Tsay HS (2004). *In vitro* propagation of some important Chinese medicinal plants and their sustainable usage. *In vitro* Cell. Dev. Biol.-Plant 40:143-154. doi: 10.1079 /IVP2003504

Narendhirakannan RT, Subramanian S, Kandaswamy M (2005). Anti-inflammatory activity of *Cleome gynandra* L. on hematological and cellular constituents in adjuvant induced arthritic rats. J. Med. Food 8:93-99. doi:10.1089/jmf.2005.8.93.

Oskoueian E, Abdullah N, Saad WZ, Omar AR, Ahmad S, Kuan WB, Zolkifli NA, Hendra R, Ho YW (2011). Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas* Linn. J. Med. Plant Res. 5:49-57.

Parimaladevi B, Boominathan R, Mandal SC (2003). Studies on analgesic activity of *Cleome viscosa* in mice. Fitoterapia 74:262-266. doi: 10.1016/S0367-326X(03)00020-0.

Parra AL, Yhebra RS, Sardiñas IG, LI Buela (2001). Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD_{50} value) in mice, to determine oral acute toxicity of plant extracts. Phytomedicine 8:395-400.

Paunescu A (2009). Biotechnology for endangered plant conservation: A critical overview. Rom. Biotechnol. Lett. 14:4095-4103.

Perazzo FF, Souza GHB, Lopes W (2005). Anti-inflammatory and analgesic properties of water-ethanolic extract from *Pothomorphe umbellata* (Piperaceae) aerial parts. J. Ethnopharmacol. 99:215-220. doi: 10.1016/j.jep.2005.02.023.

Rao SR, Ravishankar GA (2002). Plant cell cultures: Chemical factories of secondary metabolites. Biotechnol. Adv. 20:101-153.

Selloum L, Arrar L, Medani B, Khenchouche A, Bisker H (1995). Effect of *Cleome arabica* leaves extract on inflammatory cell response in rat. Biochem. Soc. Trans. 23:609.

Sharma K, Dubey S (2011). Biotechnology and conservation of medicinal plants. J. Exp. Sci. 2:60-61.

Sharma S, Rathi N, Kamal B, Pundir D, Kaur B, Arya S (2010b). Conservation of biodiversity of highly important medicinal plants of India through tissue culture technology - A review. Agric. Biol. J. N. Am. 1:827-833. doi:10.5251/abjna.2010.1.5.827.833.

Sharma S, Chattopadhyay SK, Priyanka T, Bawankule DU (2010a). Synthesis and anti-inflammatory activity of derivatives of coumarino-lignoid, cleomiscosin A and its methyl ether. Eur. J. Med. Chem. 45:5150-5156. doi: 10.1016/j.ejmech.2010.08.027.

Simões C, Mattos JCP, Sabino KCC, Caldeira-de-Araújo A, Coelho MGP, Albarello N, Figueiredo SFL (2006). Medicinal potential from *in vivo* and acclimatized plants of *Cleome rosea* Vahl ex DC. (Capparaceae). Fitoterapia 77:94-99.

Singab A, Ayoub N, Al-Sayed E, Martiskainen O, Sinkkonen J, Pihlaja K (2011). Phenolic constituents of *Eucalyptus camaldulensis* Dehnh, with potential antioxidant and cytotoxic activities. Rec. Nat. Prod. 5:271-280.

Singh PDA, West ME (1991). Pharmacological investigations of sticky viscome extract (*Cleome viscosa* L.) in rats, mice and guinea-pigs. Phytother. Res. 5:82-84.

Taviano MF, Marino A, Trovato A, Bellinghieri V, La Barbera TM, Guvenc A, Hurkul MM, Pasquale R, Miceli N (2011). Antioxidant and antimicrobial activities of branches extracts of five *Juniperus* species from Turkey. Pharm. Biol. 49:1014-1022. doi: 10.3109/13880209.2011.560161.

Vanisree M, Lee CY, Lo SF, Nalawade SM, Lin CY, Tsay HS (2004). Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures. Bot. Bull. Acad. Sin. 45:1-22.