Antinociceptive, anti-inflammatory and wound healing features in animal models treated with a semisolid herbal medicine based on *Aleurites moluccana* L. Wild. Euforbiaceae standardized leaf extract

Semisolid Herbal

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**A R T I C L E   I N F O**

**A B S T R A C T**

**Ethnopharmacological relevance:** *Aleurites moluccana* (L. Wild) Euforbiaceae is a native tree of Indonesia and India that has become acclimatized and well-adapted to the South and Southwest of Brazil. It is commonly used in traditional medicine to treat pain, fever, inflammation, asthma, hepatitis, headache, gastric ulcer, cuts, skin sores and other ailments. The oral antinociceptive effects of standardized 70:30 \((v/v)\) ethanol:water spray dried extract of *A. moluccana* leaf, as well as its flavonoids 2\(^{\prime}\)-\(\beta\)-rhamnosylswertisin (I) and swertisin (II), have previously been reported.

**Aim:** The aim of this study was to develop a stable and effective semisolid herbal medicine for topical use in the treatment of pain, inflammation and wound healing, containing 0.5 and 1.0% of standardized dried extract of *A. moluccana*.

**Materials and methods:** The chemical markers I and II were assayed by HPLC-UV analysis after extraction by matrix solid dispersion phase (MSDP) followed analytical validation as ICH Guidelines. The semisolid preparations of Hostacerin CG\(^\circledR\) vehicle containing 0.5 and 1.0% of dried extract of *A. moluccana* were submitted to stability studies (180 day of accelerated and long-term studies). The phytomedicine semisolid was analysed in croton oil-induced ear oedema model in mice, in the healing process, using the excisional wound model in rats, and to prevent mechanical sensitization following plantar incision in rats in the postoperative model of pain.

**Results:** The MSDP method showed average recovery of 101.6 and 105.7% for I and II, respectively, with good precision \((RSD < 2.0\%)\) and selectivity, without interference of the excipients. The formulations were approved in the stability studies, maintaining conformity after 180 day of accelerated and long-term studies, with variation < 10% in the analytical parameters. The phytomedicine reduced the ear oedema in 37.6 ± 5.7% and 64.8 ± 6.2%, for 0.5 and 1.0% of dried extract, respectively. The formulation also accelerated the healing process by up to 50.8 ± 4.1% and 46.0 ± 4.0% at 0.5 and 1.0% of extract, respectively, and both amounts were capable of preventing the development of mechanical sensitization following plantar incision in rats.

**Conclusions:** The MSDP followed by HPLC-UV analytical method was appropriate for the quality control of the topical phytomedicine based on *A. moluccana*. The formulation developed at 0.5 and 1.0% of *A. moluccana* dried extract proved to be effective as an analgesic, anti-inflammatory and wound healing in the pre-clinical studies, which is in agreement with the ethnopharmacological data.

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1. **Introduction**

Leaves of *Aleurites moluccana*, popularly known as “Nogueira da India”, are used for the treatment of pain and other ailments. It is commonly known as Kukui ("Light") in Hawaii, candle nut in the USA, tuitui in the Cook Islands and Tonga, lama in Samoa, ti’i’i in Tahiti, ‘ama in Marquesas, tutu’i in the Austral Islands, shi li in China, lauci in Fiji and kurup in Papua New Guinea (Leonard, 2012). The antinociceptive effect of *A. moluccana* was previously demonstrated by Meyre-Silva et al. (1998, 1999) and mechanical anti-hypersensitivity of the orally dosed standardized spray dried extract of the plant was recently reported, with 3% of the chemical marker, the flavonoid 2\(^{\prime}\)-\(\beta\)-rhamnosoyswertisin (Quintão et al., 2011). At least in part, the antinociceptive effect...
of both A. moluccana spray dried and 2’’-O-rhamnolsylswertisin is due to its activity on the peripheral and central pathways of pain (Quintão et al., 2012). Additionally, the sap which wells up at the stem attachment just after harvesting young kukui fruits is traditionally used by Hawaiians to treat cuts and skin sores (Elevitch and Manner, 2006). Malayans apply boiled leaves on the temples to relieve headache and on the groin to treat gonorrhoea. Furthermore, Filipinos use oiled leaves to treat rheumatism (Duke, 1991). Previous studies with extracts and fractions from leaves and bark of A. moluccana have shown biological effects, such as antiviral properties of dichloromethane fraction (Locher et al., 1996), and antibacterial effects against Staphylococcus aureus and Pseudomonas aeruginosa (Locher et al., 1995). The methanol extract from the leaves of A. moluccana also presented hypolipidemic effects in rats (Pedrosa et al., 2002). It is important to mention that this plant has been the subject of extensive investigation by our group, producing a patent aimed at the development of an analgesic and anti-inflammatory phyto-medicine product (Cechinel et al., 2007). Moreover, to the best of our knowledge, the topical effects of the referred extract in pain, inflammation and wound healing have not yet been explored in the literature. The standardization of phytomedicines is important for safety and efficacy reasons, due to the geographical and seasonal variation of the concentration of active compounds in the plant material. Also, the concentration of compound markers in the raw material is essential for adjusting the composition of the final product. However, the extraction of active components from a semisolid formulation usually presents difficulties due to the interference of vehicle components in the analytical methods. Therefore, the analytical methods previously applied and validated for the spray dried extract of A. moluccana leaves (Cesca et al., 2012) must to be adapted to the final formulation, in order to achieve a reasonable recovery of markers I and II from the semisolid formulations. Matrix solid-phase dispersion (MSPD) has been shown to be a good alternative to liquid–liquid extraction due to its simplicity and robustness. It allows complete fractionation of the sample matrix components and has the ability to selectively isolate a single compound or several classes of compounds from the sample. MSPD involves direct mechanical blending of a sample with a SPE sorbent (mainly octadecyl-modified silica). In this process, the sorbent acts both as an abrasive material, disrupting the sample architecture, and as a ‘bound’ solvent that assists in accomplishing sample disruption (Dawidowicz and Rado, 2010).

The present work concerns the production of a stable topical semisolid containing standardized spray dried extract of A. moluccana, the validation of MSPD application for qualitative and quantitative analysis of the phytopharmaceutical product, and the evaluation of the new topical phytomedicine in inflammatory, postoperative pain and wound healing in animal models.

2. Material and methods

2.1. Reagents and samples

Methanol and acetonitrile (LC grade) was obtained from J.T. Baker (Phillipsburg, New Jersey, USA). Water was purified using Easy Pure equipment (Waltham, Massachusetts, USA). Silica gel (0.063–0.2 mesh, Merck), methylparaben and propylparaben were obtained from Vetec (Duque de Caxias, Rio de Janeiro, Brazil). Hostacerin CG (batch BRAC161037), EDTA and butylated hydroxytoluene were purchased from PharmaSpecial (Santana de Parnaiba, São Paulo, Brazil). Polymol® (triglycerides of caprylic and capric acid) was purchased from All Chemistry (São Paulo, Brazil). Croton oil was purchased from Sigma Aldrich (St Louis, MO, U.S.A.). Ketamine and xylazine were purchased from Anadán® (Miramar, FL, USA) and Dexamethasone from Galena (Campinas, SP, Brazil). The chemical markers swertisin and 2’’-O-rhamnolsylswertisin were isolated from A. moluccana leaves with HPLC purity of > 95% (Quintão et al., 2011) by our research group. All the other solvents and reagents were analytical grade, purchased from national industry and commercially available.

A voucher specimen of A. moluccana, collected in July 2007 in Tijucas (State of Santa Catarina, Brazil) and identified by Prof. Dr. Ademir Reis (Department of Botany/Santa Catarina Federal University-UFSC, Florianópolis, Brazil), was deposited at the Barbosa Rodrigues Herbarium (Itajaí, Brazil) under number VC Filho 001.

The dried extracts composed by 1:10 dried leaves:solvent (ethanol 70% v/v), submitted to static maceration for 5 day at room temperature, were prepared at Centroflora (Botucatu-SP, Brazil) on a pilot scale (5 kg) composed of 25% (w/w total solids of concentrate extract) of colloidal silicon dioxide as describe in Quintão et al. (2011). This dried extract was standardized to 3.0 and 0.4% of I and II, respectively (Cesca et al., 2012).

2.2. Topical semisolid preparations

Semisolid formulations were prepared containing 0.5% and 1% of A. moluccana standardized dried extract. The extract was assayed by the HPLC methodology, previously validated by Cesca et al. (2012). The dried extract was leviگated with propylene glycol (5%) before being incorporated into the formulations. The anionic vehicle Hostacerin CG® (12%), the Polymol® (3%), the preservatives methyl and propylparaben (0.1% each), the butylated hydroxytoluene (0.01%) and EDTA (0.1%) were prepared in single phase without heating. The formulations were packed in aluminium tubes, with seal and polypropylene cover.

2.3. Instrumentation and chromatographic conditions

A Shimadzu LC-10AD LC system (Shimadzu, Tokyo, Japan) was used, consisting of a binary pump and a Shimadzu SPD-M10A photo diode array detector. The samples were monitored at 338 nm and 254 nm. A SIL-10 A auto-sampler and software Class VP (version 5.33) were used. The injections (20 μl) were carried out on a C8 X-Bridge 150 × 4.6 mm (5 μm) (Waters, Tauton, Massachusetts, USA) and conditioned in a Shimadzu CTO-10 A column oven, equilibrated at 30 °C. The mobile phase consisted of a gradient A (acidified water pH 3.5 with acetic acid) and B (acetonitrile) of 90:10 (A:B) (0 min) to 75:25 (20 min), to 90:10 (30 min), maintaining this composition for 40 min at a flow rate of 0.5 ml/min (Cesca et al., 2012). All solvents were degassed in an ultrasonic bath (Unique, São Paulo, Brazil) and all solutions were filtered through 0.45 μm Millipore Millex PTFE membrane (Maidstone, Kent, UK) before injection.

This methodology, previously validated for spray dried extract from A. moluccana leaves, proved to be linear over a range of 5.89–117.8 and 1.38–27.68 μg/ml for I and II, respectively, accurate (recovery for I and II of 100.3 and 102.8%, respectively), and precise, with RSD% < 1.0% (intra-day) and < 3.5% (inter-days) for area, and less than 0.5% for retention time (Cesca et al., 2012).

2.4. Matrix solid phase dispersion (MSPD) extraction method

The semisolid (2.5 g of placebo or phytomedicine) was mixed with 10 g of silica (0.063–0.2 mesh) and triturated in a mortar. This mixture was packed in a 60 ml polypropylene syringe over a cotton portion previously placed in the bottom of the syringe. This system was eluted with a single addition of 100 ml of methanol:dichloromethane 65:35 (v/v). Each experiment was carried out in triplicate. The elution solvent was evaporated at
room temperature. The residue, containing the major components of the extract of *A. moluccana* previously incorporated into semisolid formulation, was dissolved in methanol portions and transferred to a 10 ml volumetric flask, and the volume was made up with methanol. This solution was filtered and injected in triplicate into the LC system. The major markers, I and II, were quantified comparing the corresponding area with those measured in the reference solution for calculation of recovery. A blank was performed using the semisolid components without the dried extract, to analyze the influence of the matrix on the recovery of the markers.

The reference solutions were prepared with the same quantity as the previous standard (Cesca et al., 2012) dried extract contained in the aliquot of semisolid used in the extraction process above. The corresponding quantity of dried extract was dissolved in a 10 ml volumetric flask with methanol. After 15 min of ultrasonic, the volume was made up to the mark with methanol, filtered and injected as described for the samples. The reference solution was freshly prepared each day, and used to analyse the system suitability of the chromatographic system, by injection of six replicates and analysis of the retention time, resolution and RSD% of the corresponding marker areas.

2.5. Analytical validation

The extraction method by MSDP was submitted to an analytical validation process following the ICH guideline (2005), using the semisolid, with 1% and 0.5% of dried extract of *A. moluccana*. The selectivity was assessed by FIDA detector analysing the purity of the major peaks and the chromatographic profile at 254 and 338 nm, with the aim of detecting possible interference of the semisolid components in the marker assay.

The accuracy of the method was evaluated using the semisolid with 1% of dried extract of *A. moluccana*, due to the higher marker concentration, and performing a recovery experiment. An increased quantity of standardized dried extract (5, 10 and 15 mg) was added to 2.5 g of the semisolid in triplicate. The sample was homogenised and submitted to the extraction process described above. The recovery of the markers was calculated in relation to the freshly prepared reference solution.

The precision of the method was evaluated by repeating the extraction method as described in Section 2.4, with the semisolid formulation at 0.5% and 1.0% of dried extract of *A. moluccana*, in sextuplicate, on the same day and by the same analyst. These studies were repeated on two additional days. The average marker recovery and RSD% were calculated.

2.6. Stability study

The semisolid formulations with 0.5% and 1.0% of *A. moluccana* dried extract and the blank (placebo) were submitted to a stability study by storage at room temperature (25 ± 2 °C) and accelerated conditions in an oven (40 ± 2 °C) for 6 months. The samples were analysed in relation to appearance, pH, homogeneity and viscosity, at zero time and after 15, 30, 60, 90 and 180 day. The markers (I and II) were assayed by HPLC at zero time and after 90 and 180 day, after clean up the formulations by MSDP.

2.7. Pharmacological study

2.7.1. Animals

Male Swiss mice (25–30 g) and Wistar rats (180–250 g), obtained from the Universidade do Vale do Itajaí (UNIVALI, Itajaí, Brazil), were used in this study. The animals were housed under conditions of optimum light, temperature and humidity (12 h light-dark cycle, 22 ± 1 °C, 60 to 80% humidity), with food and water provided ad libitum. All procedures used in the present study followed the “Principles of Laboratory Animal Care” in NIH publication No. 85-23, and were approved by the Animal Ethics Committee of UNIVALI (Protocol numbers 358/2009 UNIVALI). Every effort was made to minimise animal suffering and reduce the number of animals used.

2.7.2. Croton oil-induced ear oedema

To evaluate the topical anti-inflammatory effect, the semisolid containing standardized spray dried extract of *A. moluccana* (0.5% or 1%), dexamethasone (0.5%) or placebo (Hostacerin CG®, 12%) was applied topically to the inside of the right ear of the mice, 30 min before the application of 20 µl of croton oil (2.5% diluted in acetone) on the outside of the right ear. The thickness of the ears was measured before and 4 h after induction of inflammation. Oedema was expressed as the increase in ear thickness due to the inflammatory challenge. Ear thickness was measured before and after induction of the inflammatory response, using a micrometer (Mitutoyo Series 293). The micrometer was applied near the tip of the ear, slightly distal to the cartilaginous ridges, and the thickness was recorded in μm (Calixto et al., 1991).

2.7.3. Excisional wound model

Wistar male rats were weighed and anesthetized with xylazine (20 mg/kg, i.p.) and ketamine (100 mg/kg, i.p.). The animals were then placed in the prone position and underwent manual trichotomy and antisepsis with 0.1% iodine alcohol along the dorsal midline of the cervical region. The incision area was marked using a surgical pen and an acetate template (225 mm²). To remove the skin, subcutaneous tissue, fascia, and fleshy panniculus, a scalpel and straight scissors and Adson forceps were used (Cross et al., 1995). After the incision, the layout of each wound was designed, and approximately 0.7 g of semisolid containing spray dried extract of *A. moluccana* (0.5% or 1%), Cicatrena® (neomycin sulphate and bacitracin zinc), a semisolid commonly used in the clinic to treat wound healing, or placebo (Hostacerin CC®, 12%) were applied. The groups of animals received the semisolids once a day for up to 15 day and the layout of each wound was performed on days 1, 2, 4, 6, 8, 11 and 15, before the first daily treatment, until the wounds had healed completely.

The layout of each wound was calculated in triplicate and the arithmetic media for each evaluation was calculated. The wound areas (cm²) were calculated with Scion Image software (Scion Corp®). The healing percentages of the treated groups were calculated based on the area under curve (AUC).

2.7.4. Postoperative model of pain

Paw incision was performed as described by Brennan et al. (1996). In brief, Wistar male rats were anesthetized with xylazine (20 mg/kg, i.p.) and ketamine (100 mg/kg, i.p.), and after sterile preparation with 70% ethanol, a 1-cm-long incision was made in the plantar region of the left hind paw, starting 0.5 cm from the edge of the heel toward the toe. The plantar muscle was elevated and incised longitudinally. The wound was closed with two mattress 5.0 silk sutures. To evaluate the mechanical nociception, rats were individually placed in clear Plexiglass boxes (18 × 18 × 18 cm) on raised wire mesh platforms to allow access to the ventral surface of the hindpaws. The frequency of withdrawal response was measured following 10 applications of a von Frey hair (VFH, Stoeling, Chicago, USA). Pilot studies carried out in our laboratory have indicated that the 8.0 g VFH produces a mean withdrawal frequency of about 15%, which was considered an adequate value for the measurement of mechanical nociception. Therefore, only the 8.0 g VFH was used in these experiments.
The semisolid containing spray dried extract of *A. moluccana* (0.5% or 1.0%) or placebo (Hostacerin CG, 12%) was applied twice a day for 8 uninterrupted days. The evaluation of mechanical thresholds was carried out 6 h after the first daily application. All the animal-groups were submitted to previous evaluation to determine the basal mechanical threshold, and re-evaluated after

Fig. 1. Chromatograms of: (A) dried extract *A. moluccana* at 338 nm; (B) semisolid formulation of 1% dried extract of *A. moluccana* at 338 nm submitted to MSDP; (C) semisolid formulation of 1% dried extract of *A. moluccana* at 254 nm submitted to MSDP; D) placebo of semisolid formulation at 254 nm submitted to MSDP.
the surgery. A non-operated group was used as experimental control.

2.7.5. Statistical analysis

The results are presented as the mean ± S.E.M. of 5 to 7 animals. The ID50 values were determined by the least squares method. The percentages of inhibition are reported as the mean ± S.E.M. of inhibitions obtained for each individual experiment. For the wound healing method, the values for percentage of inhibition were based on the AUC (area under curve). Statistical comparisons of the data were performed by one-way analysis of variance (ANOVA), followed by Dunnett’s test, or by a two-way ANOVA, followed by Bonferroni’s test. P-values of less than 0.05 ($P < 0.05$ or less) were considered significant.

3. Results and discussion

Generally, to determine the amount of an active component or a marker compound present in a crude extract or its derivative preparations, the use of a chromatographic technique such as HPLC and a reference material is required. For the analysis of *A. moluccana* preparations, the standardized dried extract was selected as reference material with known assay of the major markers containing 3.0 and 0.4% of I and II, respectively (Cesca et al., 2012). This reference material was used to validate the extraction process (by MSDP) of semisolid formulations containing 0.5% and 1.0% of standardized dried extract, using the commercially available anionic vehicle Hostacerin CG®.

3.1. MSDP analytical validation

After extraction of semisolid formulation containing 1.0% of standardized *A. moluccana* dried extract by MSDP (Fig. 1b) a similar chromatographic profile was observed at 338 nm, when compared to the dried extract (Fig. 1a), applying the previously validated HPLC method described by Cesca et al. (2012) to this latter sample, with acceptable resolution between the major markers (I and II). However, an expressive peak was observed at 254 nm, eluted at 31 min (Fig. 1c), which was absent in the dried extract sample at this wavelength (data not shown). It was attributed to the semisolid components, as shown for the placebo (Fig. 1d) at the same wavelength, absent at 338 nm (Fig. 1b). The same result was observed with 0.5% dried extract semisolid formulation (data not shown). Thus, the selectivity of the method was demonstrated by the resolution of the interfering components of the matrix semisolid formulation in relation to the markers of *A. moluccana* extract, which eluted early in the chromatogram, with no co-elution components of the matrix semisolid formulation.

The advantages of MSDP include the absence of general drawbacks associated with liquid–liquid extraction, such as the use of large amounts of solvent, the occurrence of troublesome emulsions, and its slowness. MSDP can be carried out simultaneously with sample homogenization, extraction and clean-up (Barker, 2000). MSDP has been used to perform the extraction of a variety of matrices for a number of compounds, e.g.; caffeine in green tea leaves (Dawidowicz and Wianowska, 2005), rutin in *Sambucus nigra* L. (Dawidowicz and Wianowska, 2009), polybrominated diphenyl ethers and polychlorinated biphenyls in biota samples (Martinez et al., 2005) phenolic compounds in fruit green tea (Karasova and Lehotay, 2004), pesticides in fruits (Barker, 2000; Blasco et al., 2002; Fernandez et al., 2000; Soler et al., 2004; Suli et al., 2007; Wang et al., 2007), essential oil components in herbs (Dawidowicz and Rado, 2010), and umckalin in commercial tincture and syrup of *Pelargonium sidoides* (Franco and Oliveira, 2010).

The accuracy of the present MSDP developed method for analysis of *A. moluccana* based phytopharmaceuticals, followed by HPLC assay, was evaluated by spiking the sample (semisolid formulation with 1% of dried extract) prior to the extraction process, with 3 doses of reference material, within the previous determined linearity of the HPLC method. The recovery of the markers was between 90% and 110%, as recommended for the level of analytes in the sample (Huber, 2001) and shown in Table 1. The average recovery for the marker I was 101.6% and 105.7% for II, with greater bias for the less concentrated analyte.

The MSDP application in intra-day analysis showed good precision with RSD% values of 1.9 and 2.0% for I and II, respectively, in 1% dried extract semisolid formulation and 2.2 and 3.7% for I and II, respectively, in 0.5% dried extract semisolid formulation. Also, in the inter-day determination the RSD% were 1.7 and 1.1% for I and II, respectively, in 1% dried extract formulation and 3.8 and 3.3% for I and II, respectively, in 0.5% dried extract formulation. In both case (intra and inter-day assay) the precision was higher for 1.0% dried extract formulations, compared with the 0.5% concentration.

Thus the MSDP extraction method developed proved to be selective, accurate and precise for the selected markers assayed in the semisolid Hostacerin CG® formulations, and was applied to the stability study of the 0.5 and 1.0% dried extract formulations.

3.2. Stability study

The physical characteristics (colour, odour, homogeneity, clarity and consistency) of semisolid formulations were maintained during all the storage conditions. The initial pH of 5.6–6.0 did not show significant variation during the study. The initial apparent viscosity of 500 and 1.200 mPa.s (at 80/s, 25 °C) for 0.5% and 1.0% of dried extract semisolid formulation, respectively, showed an increase only for the 0.5% dried extract semisolid formulation after 180 day of storage, in both conditions (room temperature and 40 °C).

In relation to the marker assay, for the 1.0% dried extract semisolid formulation, a decrease < 5% was observed at room temperature and < 10% at 40 °C, after 180 day, showing a high stability of this formulation. On the other hand, the 0.5% dried extract semisolid formulation showed a variation > 10% in both storage conditions, after the same period. These results suggest adequate stability of 1.0% dried extract semisolid formulation, and greater liability of the major flavonoids of the dried extract in the less concentrated semisolid formulation.

3.3. Pharmacological studies

Fig. 2 demonstrates that the semisolids containing 0.5% or 1.0% *A. moluccana* dried extract were capable of significantly reducing ear oedema induced by croton oil when compared with placebo group (Hostacerin CG®, 12%), with inhibition of 37.6 ± 5.7% and 64.8 ± 6.2%, respectively. The results obtained with dexamethasone

<table>
<thead>
<tr>
<th>Amount of reference material (mg) added to sample</th>
<th>2′-O-rhamnosylswertisin (I) recovery (average and RSD%)</th>
<th>swertisin (II) recovery (average and RSD%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>104.3 (1.7)</td>
<td>102.0 (1.7)</td>
</tr>
<tr>
<td>10</td>
<td>101.3 (3.5)</td>
<td>102.5 (3.1)</td>
</tr>
<tr>
<td>15</td>
<td>104.9 (3.0)</td>
<td>112.4 (3.2)</td>
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(0.5%) presented similar inhibition values (79.8 ± 2.7%). Croton oil is a viscous liquid obtained from Croton tiglium (Euphorbiaceae) seeds. The croton oil-induced ear oedema model is mainly used to investigate topical anti-inflammatory activity (Tonelli et al., 1965). The inflammatory response induced by topical application of croton produces oedema (which peaks 6 h after the application), cell infiltration and proliferation, production of arachidonic acid metabolites (prostanoids and leukotrienes), cytokines and other pro-inflammatory mediators (Patrick et al., 1987). The anti-inflammatory effect of topical treatment with A. moluccana is probably due to the presence of flavonoids (Tall and Raja, 2004; Meotti et al., 2007; Filho et al., 2008; Lapa et al., 2009).

With the aim of evaluating the topical healing activity of A. moluccana dried extract, rats submitted to the wound healing model were treated daily with semisolid formulations. The results, presented in Fig. 3 (A and B), show that the topical application of semisolids containing Hostacerin CG® containing A. moluccana dried extract (0.5% and 1.0%) was able to accelerate the healing process by up to 50.8 ± 4.1% and 46.0 ± 4.0%, respectively, based on area under curve (AUC) data. Based on these results, we suggest that the 0.5% of dried extract in the semisolid probably achieved the maximum activity in this protocol since it was administered on the wound, without a skin barrier. It is important to note that the rats treated with Cicatren®, the commercial semisolid commonly used for wound healing, did not present any difference when compared with the placebo group. Locher et al. (1995) demonstrated the antimicrobial activity of A. moluccana extract against Staphylococcus aureus and Pseudomonas aeruginosa. This activity, together with the anti-inflammatory effects presented above, may represent primordial factors for the wound contraction and healing process acceleration evidenced in this study. Furthermore, Said and co-authors (Said et al., 2007, 2009) recently demonstrated that A. moluccana essential oil was capable of conferring ocular cytoprotection, with corneal epithelium regeneration and reduction of inflammatory cell infiltration.

Finally, the antinociceptive activity of topical application of A. moluccana was investigated using the postoperative pain model in rats. This pain model is characterized as acute pain that develops while the patient is at rest (Brennan et al., 1996). Currently, acute postoperative pain remains a major clinical problem, and it is known that effective treatment can facilitate patient recovery (Kehlet and Holte, 2001; Brennan, 2011). The identification of pre-operative intervention methods requires an understanding of the molecular mechanisms involved (Pogatzki and Raja, 2003). Studies performed by Weber et al., 2005 in rats demonstrated that primary mechanical sensitization persisted for up to 6 day after surgery, whereas secondary mechanical sensitization was maintained for up to 1 day after surgery (Zahn and Brennan, 1999). It was demonstrated that after plantar incision in rats, there is an increase of Aβ (Hämäläinen et al., 2002) and/or Aδ (Pogatzki et al., 2002) fibers, otherwise the responsiveness of the C fibers remained unchanged. Considering that the existing drugs for the treatment of post-operative pain present major side effects, such as nausea, vomiting, ileus, respiratory depression, and sedation (Dahl and Kehlet, 2006), several pharmaceutical companies have sought to develop new effective and safe therapy (topical or systemic) for the prevention and treatment of post-operative pain. We have previously demonstrated the antinociceptive activity of A. moluccana spray dried extract and the flavonoids swertisin and 2’-O-rhamnosylswertisin, when administered orally, in different models of mechanical hypersensitivity in mice (Quintão et al., 2011). Recently, Quintão et al. (2012) suggest that the compounds present in the A. moluccana dried extract, mainly 2’-O-rhamnosylswertisin, showed antinociceptive effect by interacting with (1) opioid system enhancing the

**Fig. 2.** Effects of semisolids containing A. moluccana dried extract (0.5% to 1.0%), dexamethasone (0.5%) or placebo (Hostacerin CG®) on ear oedema induced by croton oil (2.5%) in mice. Each group represents the mean of 5 to 7 animals, and the vertical lines indicate the SEM. Significantly different from control values: **p < 0.01 (one-way ANOVA followed by Dunnett’s post hoc test).**

**Fig. 3.** (A and B) Effects of semisolids containing A. moluccana dried extract (0.5% to 1.0%), Cicatren® or placebo (Hostacerin CG®) on wound healing in rats. Each group represents the mean of 5 to 7 animals, and the vertical lines indicate the SEM. Significantly different from control values: *p < 0.05 (one-way ANOVA followed by Dunnett’s post hoc test).
Fig. 4. Effects of semisolids containing A. moluccana dried extract (0.5% to 1.0%) or placebo (Hostacerin CG®) on the post-operative pain induced by plantar incision in rats. Each group represents the mean of 5 to 7 animals, and the vertical lines indicate the SEM. Significantly different from control values: ***p < 0.001; significantly different from basal naive group mechanical threshold: # p < 0.001 (Two-way ANOVA followed by Bonferroni's post hoc test).

descendent-control of pain; (2) dopaminergic and oxidonitrergic system reducing the central sensitization resulted from glutamate activity; and (3) the inflammatory components such as neutrophil migration and cytokine release (IL-1β).

In this study, the repeated topical application (2 x day, for up to 6 consecutive days) of semisolids containing A. moluccana dried extract (0.5% or 1.0%), prevented the development of mechanical sensitization after plantar incision in rats, as observed in Fig. 4. The absence of an efficient commercial topical drug for treating postoperative pain emphasizes the importance of the results obtained, which may accelerate the patient’s recovery, potentially reducing adverse effects and increasing the adherence to treatment.

4. Conclusions

The semisolid formulations containing dried extract of A. moluccana showed important topical pharmacological results in inflammation, pain and healing process, in agreement with ethnopharmacological data (Duke, 1991). The polar components of dried extract were successfully extracted by MSDP followed HPLC-UV. This method was validated and showed adequate selectivity, with average recovery of 101.6% for 2α-O-rhamnosylswertisin (I) and 105.7% for swertisin (II), and with appropriate intra-day and inter-day precision (RSD below 2.0%). The marker content for the semisolids was 98.5% and 99.2% for 2α-O-rhamnosylswertisin and 103.2% and 112.5% for swertisin, when evaluated in 0.5% and 1.0% of dried extract of A. moluccana semisolid formulations, respectively.

The novelty of the present work is the development of a stable semisolid phytomedicine for topical use, based on ethnopharmacological data; the validation of an analytical method for quality control of the phytomedicine and the demonstration of its topical biological activities in the treatment of the post-operative pain, would healing and inflammation, have not yet been described.

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References


**Glossary**

- **LC**: Liquid chromatography;
- **HPLC**: High pressure liquid chromatography;
- **EDTA**: Ethylenediaminetetraacetic acid;
- **RSD**: Relative standard deviation;
- **MSPD**: Matrix solid phase dispersion;
- **ICH**: International conference on harmonisation;
- **PDA**: Photodiode array;
- **UNIVALI**: University of vale of Itajaí;
- **AUC**: Area under curve;
- **ANOVA**: Analysis of variance;
- **pH**: Potential of hydrogen;
- **UV**: Ultraviolet.