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Original Article

Development of an oral suspension containing dry extract of *Aleurites moluccanus* with anti-inflammatory activity

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ABSTRACT

Aleurites moluccanus L. (Willd.), Euphorbiaceae, is a tree that is native to Indonesia and India. Various parts of this tree are commonly used in traditional medicine to treat pain, fever, inflammation, hepatitis, gastric ulcer and other ailments. An oral suspension containing dried extract of *A. moluccanus* was developed and *in vivo* anti-inflammatory activity was evaluated. Extract 100 and 50 mg/ml loaded oral suspensions were prepared using different suspending agents. The formulations were analysed by their appearance, pH, density, redispersion time, rate of settling, rheological behaviour, distribution of particle size and zeta potential. The dose uniformity was determined by measuring the content of total phenolic compounds expressed in swertisin by a validated HPLC method, as well as the dissolution profile. The stability of oral suspensions was analysed in accelerated studies (40 °C for 6 months). The anti-inflammatory activity was analysed using an *in vivo* paw oedema model. The taste and odour of the suspensions were shown to be characteristic of the extract. Carmellose sodium (CS; 0.5%) and microcrystalline cellulose and carmellose sodium mixture (MCCS; 1%) showed better physical behaviour. The content of total phenolic compounds was 1.6 mg/ml and approximately 100% of the total phenolic compounds dissolved within 10 min. During the stability study, the formulations were approved by their physical–chemical properties and were shown to lose 12–14% of total phenolic compounds at 40 °C after 6 months. Suspensions containing 50 mg/ml of standardised dried extract inhibited around 35 ± 7.6% of paw oedema. Formulations containing CS showed more anti-inflammatory activity. Suspensions containing dry extract of *A. moluccanus* were successfully obtained and showed physical and physical–chemistry properties that were appropriate and characteristic of this dosage form, suitable for administration in paediatric and elderly populations, making this an alternative to tablets.

Introduction

Aleurites moluccanus L. (Willd.), Euphorbiaceae, is a tree popularly known in Brazil as "nogueira-da-india" and as Kukui, Tuitui and Candlenut in other countries. This tree is originally from Indonesia and India and is widespread in tropical regions (Duke, 1991). Its use in traditional medicine is extensive. Various plant parts including seeds, leaves, flowers and bark are used in traditional medicine. There are reports of the use of the crushed seeds and toast for constipation, decoction of leaves in poultices for headache, fevers, ulcers, swollen joints, and gonorrhoea, the bark is used for bloody diarrhoea and flowers and the sap of branches in the treatment of oral Candidiasis (Dunfors et al., 2002).

Phytochemical studies of leaves allowed isolation of the compounds swertisin (Meyre-Silva et al., 1997), the mixture of α and β-amyrin, η-hentriacontane, the mixture of stigmasterol, β-sitosterol and campesterol (Meyre-Silva et al., 1998) and 2″-*O*-ramnosil swertisin (Meyre-Silva et al., 1999). Furthermore, three minor flavonoids, derived from swertisin (Cesca et al., 2012a), and five megastigmanes (Silva et al., 2012), which might contribute to the potent anti-nociceptive effect of this species, were described.

The analgesic effect of the plant may be associated with the GABAergic and oxidonitrergic system and not be influenced by adrenergic, cholinergic, dopaminergic and opioid systems (Quintão et al., 2012). The *A. moluccanus* dry extract showed a potential anti-nociceptive effect in the inflammatory sensitisation model,

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using different inducing agents such as carrageenan, CFA or PGE_2, in mice. This biological effect can be attributed to the 2″-*O*-ramnosilswertisin and swertisin flavonoids, since they also showed activity in the tested models (Quintão et al., 2011). Semisolid preparations containing 0.5 and 1.0% of *A. moluccanus* leaves dry extract were developed and showed potential anti-inflammatory, antinociceptive and wound healing activities in *in vivo* pharmacological models (Cesca et al., 2012b).

The leaves dry extract was obtained by spray-drying in the laboratory and at an industrial scale, as this is a reproducible process and technological transformations do not change the pharmacological properties of the medicinal plant (Quintão et al., 2011). The dry extract was standardised using HPLC methodology employing swertisin and 2″-*O*-ramnosilswertisin as markers (Cesca et al., 2012a).

Further to the use of the semisolid formulation for topical application (Cesca et al., 2012b), coated tablets containing dry extract for oral use have also been developed in our research group. As the 250 and 500 mg dried extract tablets are relatively large due to the required load carrier, their use in children or elderly patients is restricted. Thus, the present study aimed to developed oral suspensions in which the *A. moluccanus* dried extract has a slightly soluble fraction but, during the spray drying process, includes the addition of an insoluble excipient (colloidal silicon dioxide), which prevents incorporation into a solution dosage form. Also, instead of quantifying only the two markers 2″-*O*-ramnosilswertisin and swertisin, in the present work, we quantified the total phenolic compounds of the extract, expressed in swertisin in view of measuring more components of the herbal matrix (besides the two mentioned markers, also the previously detected minor flavonoids and other unknown phenolic compounds). The HPLC methodology was successfully validated and applied for content uniformity and dissolution of the formulations. The suspensions were characterised and the *in vivo* anti-inflammatory activity was performed to ensure that the technological process did not change the pharmacological properties of the extract.

Material and methods

Material

Microcrystalline cellulose and carmellose sodium mixture (Avicel® RC 591) was purchased from FMC Biopolymer (Philadelphia, USA), carmellose sodium was purchased from DEG (São Paulo, Brazil); methanol and acetonitrile (HPLC grade) were obtained from J.T. Baker (Phillipsburg, New Jersey, USA), and water was purified using Easy Pure equipment (Waltham, Massachusetts, USA). Sorbitol and sucralose, pharmaceutical grade, were purchased from Via Farma (São Paulo, Brazil). The swertisin isolated from *A. moluccanus* leaves with a purity of >95% (Quintão et al., 2011) was used as a reference substance for the development and validation of the HPLC method. The other reagents were of analytical grade and used as received, without any further purification.

Herbal material

A voucher specimen of *Aleurites moluccanus* L. (Willd.), Euphorbiaceae, 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, Florianópolis, Brazil), was deposited at the Barbosa Rodrigues Herbarium (Itajaí, Brazil), under number VC Filho 001.

Dry extract of *A. moluccanus* containing 2″-*O*-ramnosilswertisin and swertisin at concentrations of 3.0% and 0.4%, respectively (Cesca et al., 2012a), was prepared on an industrial scale (Centroflora, Botucatu, Brazil) from dry leaves. The extract was prepared by maceration with a 1:10 drug:solvent ratio and 7:3 ethanol:water as a solvent, for five days. After extraction, filtration and concentration, the extract was mixed with colloidal silicon dioxide and dried for the spray-drying method using inlet and outlet temperatures of 165–180 °C and 70–80 °C, respectively. This extract was previously characterised (Quintão et al., 2011).

Preparation of A. moluccanus *suspensions*

Suspensions containing 100 mg/ml and 50 mg/ml dry extract of *A. moluccanus* were prepared according to the formulations shown in Table 1. The dry extract used was prepared on an industrial scale and containing colloidal silicon dioxide (approx. 25%) as dry adjuvant. Initially, the suspensor vehicle was prepared using water or water/sorbitol as a vehicle, CS and MCCS as the suspending agent, sodium benzoate as the preservative and sucralose as a sweetener. The dry extract was wet and dispersed in the suspensor vehicle. The suspensions were stored in amber glass bottles for future analysis.

Analysis of the organoleptic characteristics and pH

In the organoleptic characteristics analysis, colour, physical aspects, odours and taste were observed by direct perception and/or sensory experimentation.

The pH value of the formulations was determined by direct reading in a potentiometer at 23 ± 2 °C, which was previously calibrated in pH 4 and 7. The analysis was performed in triplicate.

Analysis of settling behaviour

The suspensions were homogenised and transferred into a graduate cylinder and the volume of sediment was observed after 1, 2, 4, 6, 8 and 24 h. The rates of settling, *F*, which is defined as the ratio of the final settled volume V_u to the original volume V_o, was calculated using Eq. (1) (Florence and Attwood, 2006). The assay was performed in triplicate.

$$F = \frac{V_u}{V_o} \quad (1)$$

The redispersion time was determined by manual stirring according to Tagliari et al. (2009). Approximately 40 ml of suspension was stored in 60 ml glass bottles (approximately two-thirds

Table 1
Formulations of oral suspensions of *Aleurites moluccanus* dry extract.

Batches	I	II	III	IV	V	VI	VII	VIII	IX	X
Dry extract (mg/ml)	100	100	100	100	100	100	100	100	50	50
CS[a] (%)	1.0	1.0	0.5	0.5	–	–	–	–	0.5	–
MCCS[a] (%)	–	–	–	–	1.0	1.0	0.5	0.5	–	1.0
Sucralose (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Na benzoate (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sorbitol (%)		50	–	50	–	50	–	50	20	20
Water	qsp	qsp	qsp	qsp	qsp	qsp	qsp	qsp	qsp	qsp

[a] CS, carmellose sodium; MCCS, microcrystalline cellulose and carmellose sodium mixture.