

Influence of the preparation method on the low efficacy of multi-herb commercial products: the example of João da Costa e Associações®

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RESUMO: “Influência do método de preparação na baixa eficácia de produtos fitoterápicos: o exemplo do João da Costa e Associações®.” Plantas medicinais nativas do Brasil foram usadas por décadas pelas indústrias farmacêuticas nacionais para criar seus produtos. Neste estudo, foi investigado o produto *João da Costa e Associações®* (JCA) comercializado por mais de trinta anos para o tratamento de dismenorréia e outros problemas relacionados à saúde da mulher. JCA é preparado pela decocção de *Himatanthus lancifolius* (Muell. Arg.) Woodson (Apocynaceae), *Chondodendron platyphyllum* Miers (Menispermaceae), *Gossypium herbaceum* L. (Malvaceae), *Rosmarinus officinalis* L. (Lamiaceae) e *Echites peltata* (Apocynaceae), seguido de adição de açúcar. A eficácia de JCA foi verificada por meio da avaliação da atividade antinociceptiva. Já a composição química foi determinada por análises em HPLC/ DAD. Uma fraca inibição da segunda fase da nocicepção foi observada no teste da formalina, indicando uma ação semelhante aos anti-inflamatórios esteroidais e não esteroidais. Apesar de ser preparado pela decocção de cinco plantas, a análise no HPLC apresentou somente dois picos, e nenhum deles correspondeu aos componentes observados nos extratos etanólicos preparados com as mesmas plantas. Os resultados sugerem que o método de preparação de JCA promove a perda dos componentes químicos das plantas e interfere consideravelmente na eficácia do produto.

Unitermos: espécies nativas, produto comercial, *Himatanthus lancifolius*, *Chondodendron platyphyllum*, *Gossypium herbaceum*, *Echites peltata*.

ABSTRACT: Native medicinal plants have been used for decades by Brazilian pharmaceutical companies to create commercial products. In this study, we have investigated the herb-combined product *João da Costa e Associações®* (JCA) commercialized for thirty years to treat dysmenorrhoea. JCA is prepared by decoction of *Himatanthus lancifolius* (Muell. Arg.) Woodson (Apocynaceae), *Chondodendron platyphyllum* Miers (Menispermaceae), *Gossypium herbaceum* L. (Malvaceae), *Rosmarinus officinalis* L. (Lamiaceae) and *Echites peltata* (Apocynaceae), followed by addition of sugar. The efficacy of JCA was verified by antinociceptive studies. The chemical composition was determined by fingerprint analysis in HPLC/ DAD. A weak inhibition of the second phase of the nociceptive effect induced by formalin indicated an activity similar to those steroids and non-steroids anti-inflammatories. Despite being prepared by decoction of five plants, the fingerprint analysis showed only two peaks. None of them corresponds to the chemical compounds observed in ethanol extracts prepared with the same plant material. We argue that the methods of preparation of the formulas should be considered in studies of multi-herbs products, since they can be the responsible for inefficacy or low activity of such products.

Keywords: native species, commercial product, *Himatanthus lancifolius*, *Chondodendron platyphyllum*, *Gossypium herbaceum*, *Echites peltata*.

INTRODUCTION

Medicinal plants are widely used as home remedies by both rural and urban inhabitants of Brazil. However, the intense mixture of cultures (Native, African and European) during the last several centuries has led to a progressive substitution from native medicinal plants to other species from elsewhere in Latin America (Dean, 1996). The accelerating destruction of Brazil's botanically rich native ecosystems has also contributed to a gradual loss of knowledge about native plants used in traditional medicine, including those found in areas of the Atlantic Forest and the Amazon where accelerated occupation takes place (Begossi et al., 2002; Brandão et al., 2004; Shanley & Luz, 2003; Shanley & Rosa, 2005).

Native medicinal species have been used for decades by Brazilian pharmaceutical companies to create commercial products. These companies are represented by small laboratories that evaluate their products on the basis of traditional formulas (Ferreira, 1998; Brandão et al., 2010). However, very often the efficacy and safety of these products is not measured and they might not meet the minimal standard of the WHO recommendations for products for traditional use (WHO, 1998). In 1995, the Ministry of Health, following the recommendations of World Health Organization, established a set of herbal regulations in order to improve the quality of commercial herbal products (Ministério da Saúde 1995). According to these rules, the complete acceptance of an herbal medicine by Brazilian governmental agencies can occur only after the efficacy and safety of the product has been scientifically determined (Carvalho et al., 2008). Some effort has been made by the companies to develop standardized phyto-medicines from native species with proof of quality, safety and efficacy. In the present study, we have investigated the herb-combined product *João da Costa e Associações*® (JCA), commercialized for at least 30 years in Brazil to treat dysmenorrhoea, and discuss of the influence of preparation methods on the efficacy of these traditional commercial products.

MATERIAL AND METHODS

Drugs and products

Uleíne was isolated of *Himatanthus lancifolius* and kindly furnished by Professor Cid Aimbiré M. Santos, Universidade Federal do Paraná. The commercial formula (JCA) was prepared by Belém Jardim Laboratory by decoction of the five plants (*Himatanthus lancifolius* (Muell. Arg.) Woodson, Apocynaceae, *Chondodendron platyphyllum* Miers, Menispermaceae, *Gossypium herbaceum* L., Malvaceae, *Rosmarinus officinalis* L., Lamiaceae and *Echites peltata*, Apocynaceae), followed by addition of sugar. For the pharmacological experiments, we used the lyophilized decoction of the plants, without

addition of sugar (JCAws). Crude ethanol extracts (70%) were prepared by percolation, until exhaustion, with the same plant material used by preparation of JCA.

Animals

Swiss mice weighing 25-35g of both sexes were used in the experiments. All animals were housed in a room maintained at a constant temperature of 22±2 °C under a 12 h light/12 h dark cycle at 60-80% humidity with food and water available *ad libitum*. Animals were acclimatized to the laboratory for at least 1 h before the tests.

Percentage of dry residue

It was performed in order to verify the correlation between the concentration of the chemical compounds in commercial formula (JCA) and the lyophilized product (JCAws). 1 mL of each product was dried at 105 °C, for 4 h, and the percentage of dry residue was calculated. This analysis was performed in triplicate.

Antinociceptive activity

The efficacy of JCA to treat dysmenorrhoea was verified by antinociceptive studies. It was performed according the method described by Hunskaar & Hole (1987) modified by Vaz et al. (1996) for the formalin model of nociception. Briefly, 20 µL of 2.5% formalin solution (0.92% formaldehyde), made up in phosphate buffered saline (137 mM NaCl, 2.7 mM KCl and 10 mM phosphate buffer) was injected intraplantarly under the surface of the right hindpaw. This model provided evidence of two phases of painful sensitivity: an immediate early phase lasting for 5 min (pain of neurogenic origin) and a late phase, lasting from 15 to 30 min after the injection of formalin (pain of inflammatory origin) (Hunskaar & Hole, 1987). In the cited treatment, the mice were treated by oral route with JCAws or saline (0,01; 0,1 ou 1 g/kg, *p.o.*) 1 h before formalin injection. After intraplantar injection of formalin, the animals were placed immediately in a glass cylinder 20 cm in diameter, and the time spent licking and biting the injected paw was considered as indicative of nociception. In a second protocol, animals were treated with JCAws for seven days, two diaries dosis of 0,1 or 1g/kg, *p.o.* One hour after the last administration, formalin was injected. The results were analyzed by ANOVA one-way, followed by post-hoc de Newman Keuls. Statistic significance was define as $p < 0,05$.

The fingerprint of JCA and plant extracts established by HPLC

The HPLC analyses were carried out on an Agilent 1200 system (Palo Alto, CA, USA), composed of a quaternary pump, auto sampler, photodiode array detector

(DAD) and HP ChemStation software. The column used was a Zorbax reversed-phase C₁₈ (150 × 4.6 mm I.D.; 5 μm particle size) from Agilent (Palo Alto, CA, USA), maintained at 30 °C. The mobile phase was composed of (A) acetonitrile and (B) aqueous phosphoric acid (0.5%, v/v), using a gradient elution of 10-70% A at 0-30 min, at a flow rate of 1.0 mL/min. The re-equilibration time of the gradient elution was 10 min. UV-photodiode array detection was performed at 210 nm and UV spectra from 190 to 400 nm were on line recorded for peak identification. The injection volume was 20 μL. For the analyses, solutions were prepared by dissolving 50 mg of plant dry extracts in 5 mL of a diluent composed of acetonitrile and aqueous phosphoric acid 0.05% (1:1), in an ultrasonic bath for 10 min. JCA solution was prepared by dissolving 100 mg of the dry extract in 1 mL of diluent. All the solutions were filtered through a 0.45 μm membrane filter before injection.

RESULTS

Percentage of dry residue

The total percentage of JCA was of 10.83 g (±0.09) while for JCAws was of 0.43 (±0.04).

Evaluation of antinociceptive effect

The results presented in Figures 1 and 2 show that JCAws, administered by oral route, caused partial but significant inhibition of second phase of formalin-induced nociception.

Fingerprint of JCA, uleine and plant extracts

JCA showed one peak at Rt 11.28 min ($\lambda_{max} = 212$ e 248 nm), and UV spectrum similar as the conserving agent metilparabene, and other at Rt 18.33 min ($\lambda_{max} = 207$ e 256 nm) (Figure 3). The plant extracts shown several peaks between Rt 3 to 20 min, compatibles with the presence of

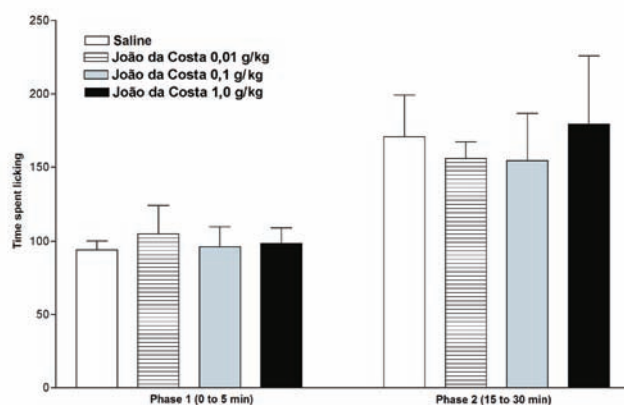


Figure 1. Effect of the acute treatment with JCAws (0,01; 0,1 ou 1 g/kg, *per os*, - 1 h) on nociceptive effect induced by formalin (0,92%, 20 μL) in mice (n=7).

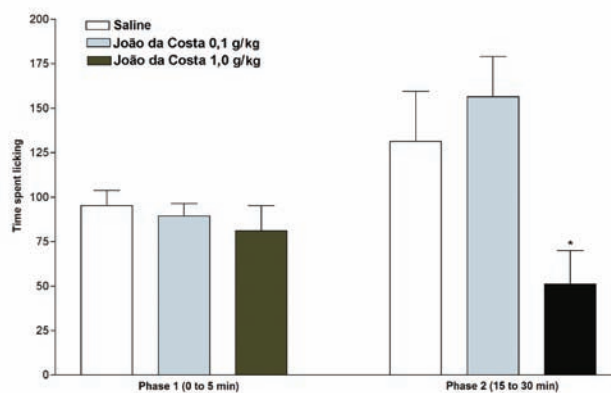


Figure 2. Effect of the treatment with JCAws (0,1 or 1 g/kg, *per os*, two dosis/ day, seven days) on nociceptive effect induced by formalin (0,92%, 20 μL) in mice (n=7). The last administration was 1 h before the test (* p<0,05).

compounds of high polarities. Standard of uleine showed a peak at Rt 11.66 min ($\lambda_{max} = 208, 239$ e 305 nm) and was detected in extract of *H. lancifolius* (Figure 4).

DISCUSSION

JCA is prepared by decoction of five medicinal plants, three of them (*H. lancifolius*, Apocynaceae; *C. platyphyllum*, Menispermaceae and *G. herbaceum*, Malvaceae) used for decades in traditional medicine of Brazil for the treatment of dysmenorrhoea and other troubles correlated with the health of women (Araujo & Lucas, 1930; Balbach, 1956; Cruz, 1982; Chernoviz, 1996). These same three species were included in the first Edition of Brazilian Official Pharmacopoeia, publicized in 1929, showing their importance also in official medicine (Brandão et al., 2006; Brandão et al., 2008a). *C. platyphyllum* and *G. herbaceum* have Amerindian origin and their use as emmenagogue and to treat dysmenorrhoea have been already registered by European naturalists who traveled in Brazil in 19th century (Brandão et al., 2008b). Other species used in the preparation of JCA are *Echites peltata*, native from Brazil and *Rosmarinus officinalis* L. from Europe.

Toxicological studies of JCA not showed any alteration in the biochemical and physiological parameters in mice and dogs (data not shown). This result can be, however, a consequence of the low concentration of plant chemical compounds in the product, since that 96.3% of its total weigh corresponds to sugar. An antinociceptive activity was observed with lyophilized product prepared without sugar (JCAws) in formalin test (Figure 1). The acute treatment does not change the nociceptive effect of the animals but in the second phase, the effect was significantly inhibited by the higher doses of the JCAws (Figure 2). The inhibition of the second phase of the nociceptive effect induced by formalin, and not the first, indicated that the activity of JCAws is similar those steroids and not-steroids anti-inflammatories and not with analgesics with central

actions, as the opioids (Hunnskaar & Hole, 1987; Tjølsen et al., 1992; Vaz et al., 1996).

Our first supposition was that the alkaloid uleine, present in *H. lancifolius*, was the responsible for the observed activity and the efficacy of JCA. Previous studies showed a significant activity of this alkaloid in smooth muscle and in production of nitric oxide (Souza, 2007; Franca et al., 2000; Rattmann et al., 2005). Recent study also shown a marked antinociceptive activity of extracts from *Rosmarinus officinalis*, including that induced by formalin (González-Trujano et al., 2007). However, the fingerprint analysis of JCA does not shown the presence of uleine or other chemical compound from *H. lancifolius* or *R. officinalis*. Despite to be prepared by decoction of five plants, the HPLC/DAD showed only two peaks, one of them (Rt 11.28 min) identified as the conserving metilparabene (Moffat, 1986; Figure 3). The other peak

shown a similar UV spectrum as standard of uleine, but it was detected in a different Rt (Rt 18.33; figure 2). Curiously, EtOH extracts prepared with the same plant material used in the preparation of JCA also not showed any peak at this Rt (Figure 4). These results suggested that other chemical substance, probably an artifact formed in the process of decoction of the plants, is present in JCA and is responsible by the antinociceptive activity of the product.

The Traditional Medicine Division of the WHO recognized recently the importance of the remedies prepared with century old plant species used in traditional medicine, and advises that their efficacies should be evaluated through pharmacological and toxicological assays (WHO, 2002). In recent works, the Chinese researches have beginning a series of studies in order to evaluate the efficacy of the multi-composition formulas

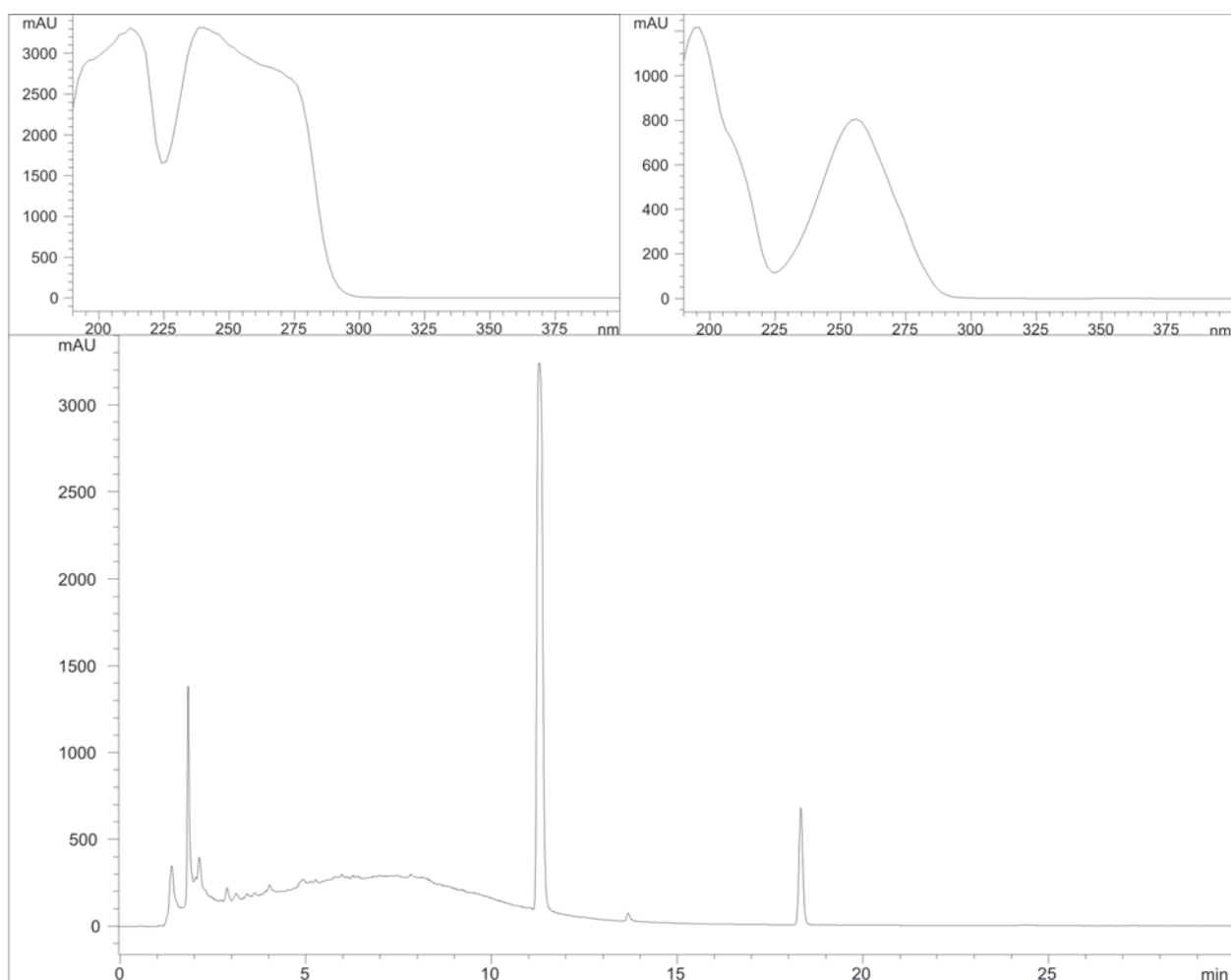


Figure 3. Chromatogram obtained by HPLC/DAD (210 nm) for JCA solution (100 mg/mL) and UV spectra of peaks Rt = 11,28 min and Rt = 18,33 min.

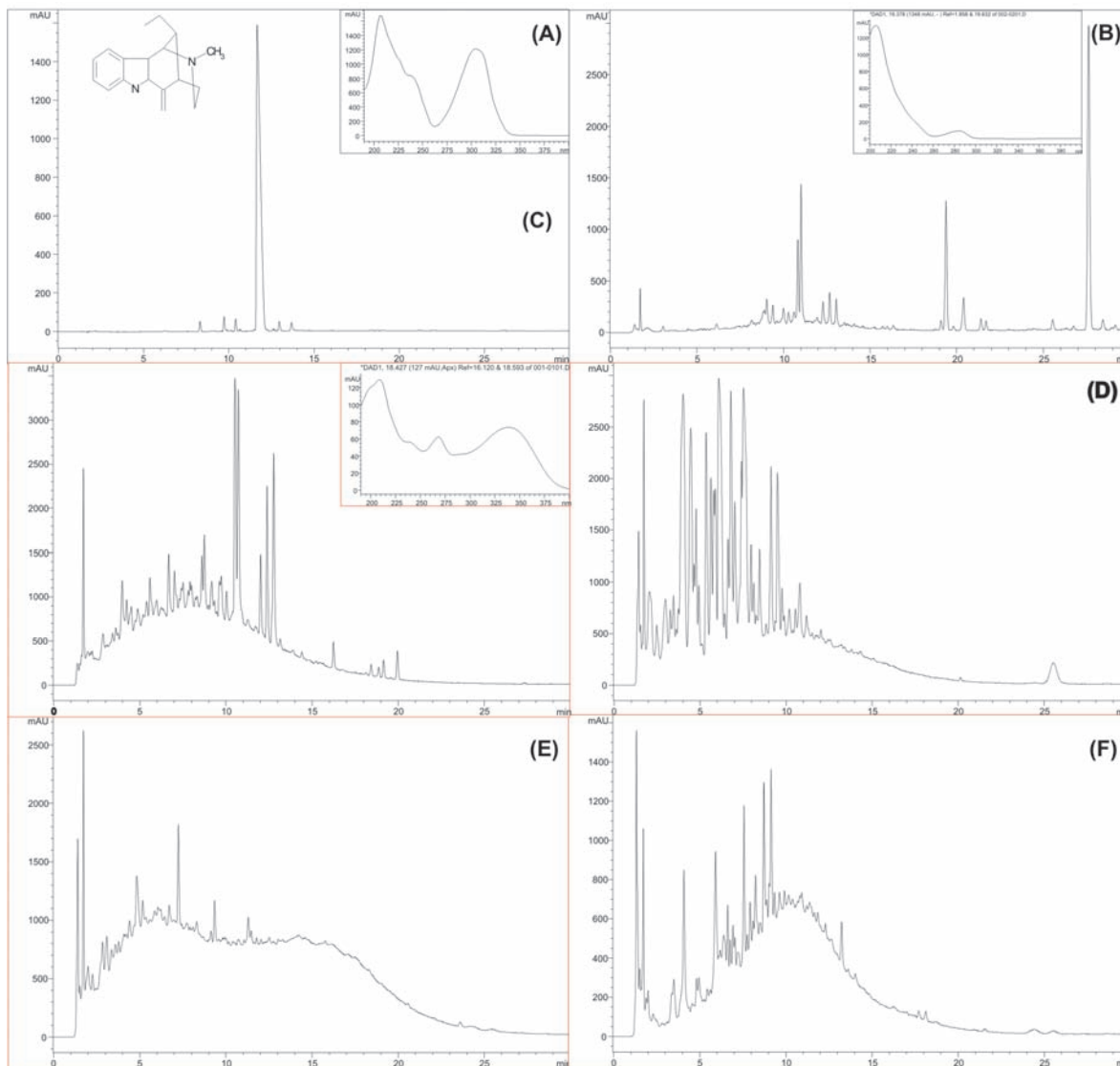


Figure 4. Chromatograms obtained by HPLC/DAD (210 nm) for uleine solution (250 µg/ml) and plant extract solutions (10 mg/mL). Chromatograms identification: (A) uleine standard with UV spectrum of the main peak; (B) *Rosmarinus officinalis* and UV spectra of the peak at 19.38 min; (C) *Himatanthus lancifolius* and UV spectra of the peak at 18.42 min; (D) *Chondodendron platyphyllum*; (E) *Gossypium herbaceum*; (F) *Echites peltata*.

from Chinese herbal medicine, as well as verify the synergism and compatibilities of the chemical components (Wei et al., 2007). JCA was created with Brazilian native plant species with historical use in traditional medicine and pharmacological potential. This study showed, however, that a inappropriate preparation method (decoction of the plants and addition of high amount of sugar) promotes the lost of chemical compounds and a low efficacy of the product. We argue that the methods of preparation of the Brazilian traditional commercial formulas should be evaluated in studies of multi-herb commercial formulas, since they can be the responsible for inefficacy or low activity of these products.

ACKNOWLEDGEMENTS

Grants from CNPq (521197/01-5) and FAPEMIG (PPM/2007). The authors thanks Laboratórios Belém Jardim for drugs and botanical material; L.T.S.Rocha and M.M. Coelho for antinociceptive studies and Cid A. M. Santos (UFPR) for standard of uleine.

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