In vivo anti-inflammatory activity of Alchornea cordifolia (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae)

H. Mavar Manga a,∗, D. Brkic a, D.E.P. Marie a,b, J. Quetin-Leclercq a

a Laboratoire de Pharmacognosie, Unité CHAM 72.30, Ecole de Pharmacie, UCL, Av. E. Mounier 72, 1200 Brussels, Belgium
b Mauritius Oceanography Institut, France Centre, Victoria avenue, Quatres Bornes, Maurice, Belgium

Received 25 June 2003; received in revised form 27 January 2004; accepted 17 February 2004

Abstract

Alchornea cordifolia (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae) is a widely distributed plant in Africa. It is used in the traditional medicine of many African countries for the treatment of bacterial, fungal, parasitic and inflammatory disorders. Aqueous decoction and methanol leaf extracts were tested for their ability to reduce Croton oil-induced oedema in the mouse ear, after topical application. The methanol leaf extract dose-dependently inhibited the Croton oil-induced ear oedema in mice (ID50 < 500 μg/cm²).

A bio-assay guided liquid–liquid fractionation of this methanol extract gave four active fractions: water insoluble (F1), hexane (F2), ethyl acetate (F3) and water (F4).

The hexane fraction showed a very high activity (42% inhibition at 0.7 μg/cm²) as compared to the control. The other fractions were less active (F1: 56% at 506.2 μg/cm²; F3: 57% at 289.3 μg/cm²; F4: 32% for 203.8 μg/cm²) while indomethacin gave 49% of inhibition at 90 μg/cm².

The activity of F1 and F3 may be at least in part explained by the presence of anti-inflammatory flavonoids (hyperoside and quercitrin, quercitrin being identified in the plant for the first time) while the activity was not correlated to the tannin contents. None of these compounds were detected in the most active F2 fraction.

These results support the reported traditional use of this plant against topical inflammatory disorders.

Keywords: Alchornea cordifolia; Anti-inflammatory; Mouse ear oedema; Quercitrin; Plant extract; Traditional medicine

1. Introduction

Alchornea cordifolia leaves are widely used in African traditional medicine from Senegal to Uganda. There is many convergence in its traditional use throughout tropical Africa as topical anti-inflammatory: chancres, yaws (Neuwinger, 2000), wounds, cicatrisation, ulcers (Bouquet and Debray, 1974; Kerharo and Adam, 1974; Adjanohoun et al., 1984, 1988, 1990; Neuwinger, 2000), caries, toothache (Delaude et al., 1971; Adjanohoun et al., 1988; Akendengue and Louis, 1994; Neuwinger, 2000), gum inflammation (Bouquet and Debray, 1974; Kerharo and Adam, 1974; Adjanohoun et al., 1988; Neuwinger, 2000) and conjunctivitis (Bouquet and Debray, 1974; Neuwinger, 2000).

Different research teams analysed the chemical constituents of Alchornea cordifolia leaves and identified: tannins, phenolic acids: gallic acid, ellagic acid, protocatechic acid (Ogungbamila and Samuelsson, 1990; Lamikanra et al., 1990; Banzouzi et al., 2002), flavonoids: quercetin, hyperoside and guaijaverin (Lamikanra et al., 1990; Ogungbamila and Samuelsson, 1990; Ajali, 2000) and an alkaloid: trisopentenylguanidine (Lamikanra et al., 1990).

This plant has also been shown to possess antibacterial (Ogunlana and Ramstad, 1975; Kambo, 1990; Lamikanra et al., 1990, 1999; Ebi, 2001; Okeke et al., 1999, 2000), anti-fungal (Neuwinger, 2000) and antiparasitic (Tona et al., 1998; Banzouzi et al., 2002) and spasmyloytic properties (Ogungbamila and Samuelsson, 1990; Tona et al., 2000).

Recently, Osadebe and Okoye (2003) have shown that a methanolic extract of Alchornea cordifolia leaves possess anti-inflammatory activity, when given by intraperitoneal injection in the egg-albumen-induced rat paw oedema test (inhibition of 68.25% for 50 mg/kg).

However, to our knowledge, the topical anti-inflammatory activity of the plant has never been tested.

The aim of this study was to evaluate the anti-inflammatory activity of different extracts of Alchornea
cordifolia on Croton oil-induced ear oedema and subsequently to identify the active molecules.

2. Materials and methods

2.1. Plant material

The fresh green leaves of *Alchornea cordifolia* were collected in Kinshasa and identified at Institut National d’Etudes et de Recherches Agronomique of University of Kinshasa (INERA). A voucher specimen is deposited at the National Botanic Garden of Belgium (BR) bearing the number SP 848103.

2.2. Extraction

Leaves were sun dried and pulverised. Three extracts were prepared from the powdered leaves: decoction 20 min (H₂O-D), maceration 24 h in methanol (MeOH-M) and Soxhlet extraction for 4 h with methanol (MeOH-S).

The obtained extracts were dried under vacuo using a rotary evaporator or lyophilised (H₂O-D).

2.3. Fractionation

The MeOH-S extract was dissolved in boiling water and kept for 24 h at room temperature. Filtration gave a residue (F1) and a water soluble fraction.

This aqueous soluble part was then successively extracted (50 ml, four times) with hexane and ethyl acetate. Afforded organic fractions were dried over anhydrous sodium sulphate, filtered and concentrated. The residual aqueous extract was lyophilised.

This gave four fractions: water insoluble (F1), hexane (F2), ethyl acetate (F3) and residual aqueous fractions (F4) (Fig. 1).

---

**Fig. 1. Recapitulative extraction scheme.**

- **A. cordifolia** Leaves 25 g
  - MeOH 300 ml Soxhlet, 4h
  - Dried MeOH-S extract 6.080 g
    - (1000 µg/cm² – 88.4 % inh)
    - H₂O, 250 ml
      - F1 tannins (12.3%), flavonoids (2.8%) (H₂O), EA, terpenoids, 3.080 g - 50.62 % (506.2 µg/cm² – 56.1 % inh)
      - H₂O phase
        - Hexane 4 X 50 ml
          - F2 0.0042 g - 0.07 % (0.7 µg/cm² – 42.1 % inh)
          - AcOEt 4 X 50 ml
            - F3 tannins (10.5%), flavonoids (4.9%) (H₂O), EA, alkaloids, 1.7597 g – 28.93 % (289.3 µg/cm² – 57.3 % inh)
            - H₂O phase (F4)
              - F flavonoids (0.6%)... 1.2399 g - 20.38 % (203.8 µg/cm² – 31.9 % inh)

H = hyperoside  Q = quercitrin  EA = ellagic acid  inh = inhibition
2.4. Animals

Twenty-eight to thirty-three grams of male albino Swiss mice (Cd-1) were used (Charles River Company, Iffa-Credo, Belgium). Animal quarters were maintained at 22°C and 60% humidity with a 12-h light/12-h dark cycle.

2.5. Chemicals

Croton oil, indomethacin, pyrogallol and ellagic acid were Sigma-Aldrich products (Steinheim, Germany); ketamine hydrochloride was Ketalar® (Pfizer); hyperoside and quercitrin were purchased from Extrasynthese (Genay, France); hide powder CRS was from European Pharmacopoeia (Council of Europe, Strasbourg, France).

2.6. Anti-inflammatory activity (AIA)

Cutaneous inflammation was induced to the inner surface of the right ear (surface: about 1 cm²) of anaesthetised mice (145 mg/kg ketamine hydrochloride, intraperitoneally) by application of 15 μl of acetone or acetone-water (1:1) solutions containing 80 μg of Croton oil as irritant (control animals). For treated animals, appropriate amounts of indomethacin (reference drug) or of the tested extracts were dissolved in the Croton oil containing solution and applied as for controls. After 6 h, mice were sacrificed and a punch (5 mm of diameter) was excised from both ears. Inflammation was measured as oedema formation and quantified by the weight difference between the treated and the untreated (opposite) ear samples (Tobara et al., 1985). The anti-inflammatory activity was expressed as a percentage of the oedema reduction in treated mice compared to the control mice. At least two experimental groups of five animals were tested for each dose level.

The experimental design was approved by the ethical committee for animal experimentation of Faculty of Medicine (Catholic University of Louvain) bearing the number 2003/03/FMD/UCL/013.

2.7. Dosage of tannins and flavonoids

Dosage of tannins was realised as per the European Pharmacopoeia (Pharmacopéie Européenne, 2002a) and expressed in pyrogallol. Total flavonoids were quantified following the method described for hawthorn (Crataegus spp.) leaves and flowers and expressed in hyperoside (Pharmacopéie Européenne, 2002b).

2.8. TLC analysis

TLC on silica gel 60 (Merck plates 0.25 mm) was performed in EtOAc–HCOOH–CH₃COOH–H₂O (100:11:11:26, v/v) (A), toluene–EtOAc–HCOOH (8:2:1, v/v) (B) or 30 ml of B with 5 ml of MeOH (C) as mobile phases, at room temperature. Developed TLCs were examined under UV 254 nm and UV 366 nm prior to spraying.

Flavonoids were detected using aminoethanol diphenylborate–PEG 400 mixture and visualised at 365 nm (Brasseur and Angenot, 1986). Dragendorff reagent (Wagner and Bladt, 1996a) was used for alkaloid detection. Anisaldehyde/H₂SO₄ reagent was used to reveal the presence of terpenoids (Wagner and Bladt, 1996b). For polyphenolic compounds, Iron (III) chloride reagent R3 (Pharmacopéie Européenne, 2002c) was used.

For each samples, 10 μl of 5 mg/ml solutions in appropriate solvent were spotted and allowed to migrate over 10 cm. Ellagic acid (Rₖ 0.30 in system C), hyperoside (Rₖ 0.13 in system C) and quercitrin (Rₖ 0.22 in system C) were used as references at a concentration of 1 mg/ml.
2.9. Statistical analysis

The pharmacological data were analysed by Student’s t-test and significance was assumed for P-values lower than 0.05.

### 3. Results

#### 3.1. Extraction yield of plant material

Extraction yields (w/w) were 11.46% for aqueous decoction (H2O-D), 13.08% for methanol macerate (MeOH-M) and 24.34% for the Soxhlet extract (MeOH-S) in term of dry weight.

Among fractions from MeOH-S, fraction F1 (water insoluble, 50.62%) had the highest yield followed by the ethyl acetate fraction (F3, 28.93%) and residual aqueous one (F4, 20.38%). The F2 (hexane fraction) yield (0.07%) was far lower than the others (Fig. 1).

#### 3.2. Anti-inflammatory test

In the first set of tests, H2O-D, MeOH-M and MeOH-S were tested at 2000 μg/cm². At this concentration, the MeOH-S extract showed the highest activity while the water decoction demonstrated only a mild effect (Table 1).

These first experimental results were confirmed by a second set of tests on the MeOH-S extract, giving a higher yield than the macerate, which exhibited dose-dependent activity (Table 2).

#### 3.3. TLC analysis

TLC analysis revealed the presence of tannins, flavonoids, terpenoids and alkaloids in both crude methanolic extracts as reported before (Toma et al., 1998). These extracts have the same chromatographic profiles.

Tannins were present in F1 and F3, while all extracts except F2 contained flavonoids. In the methanol extracts, the most abundant flavonoids were hyperoside and quercitrin. These molecules were also found in F1 and F3 fractions, while ellagic acid was also identified in these two fractions.

All alkaloids were concentrated in F3 fraction and, apparently, only F1 fraction contained terpenoids (Fig. 1).

#### 3.4. Dosage of tannins and flavonoids

MeOH-M extract contained more tannins (11.9%) than MeOH-S (8.2%) and H2O-D extracts (9.6%), while the concentrations of flavonoids in MeOH-M and MeOH-S were in the same range (2.7 and 2.9%, respectively). The decoction contained less flavonoids (1.8%) (Table 3).

The most active fraction (F2) contained neither tannins nor flavonoids.

### 3.5. Dosage of tannins and flavonoids

<table>
<thead>
<tr>
<th>Substance</th>
<th>Tannins (%)</th>
<th>Flavonoids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O-D</td>
<td>9.6</td>
<td>1.8</td>
</tr>
<tr>
<td>MeOH-M</td>
<td>11.9</td>
<td>2.7</td>
</tr>
<tr>
<td>MeOH-S</td>
<td>8.2</td>
<td>2.9</td>
</tr>
</tbody>
</table>

The activity of a quantity of each fraction corresponding to what was present in 1000 μg of MeOH-S was then analysed. At these doses, all fractions induced a significant reduction of the oedematous response (Table 3).

From these results, it can be seen that the hexane fraction (F2) was the most active, giving 42% oedema inhibition at 0.7 μg/cm² (0.07% of 1000 μg/cm²) (Fig. 1).
4. Discussion

MeOH-S extract produced dose-dependent reduction of the Croton oil-induced ear oedema with an IC50 of < 500 μg/cm².

All fractions of the MeOH-S extract had some activities and contribute to the activity of the total extract. However, the hexane fraction (F2) showed the highest activity (0.7 μg/cm² gave 42.2% of inhibition).

This lipophilic fraction (F2) can be regarded as a potential source of highly active anti-inflammatory compounds with a higher potency than the pure non-steroidal anti-inflammatory drug (NSAID) indomethacin. This is quite unusual for a crude extract since their active compounds are often diluted by other bulk substances. However, synergistic or additive effects will be investigated.

In fraction F3, which also has an interesting activity, we identified the presence of ellagic acid, hyperoside and quercetin. These three compounds were also present in F1 fraction but in lower quantity.

There is a need to emphasise that this is the first time that the presence of quercetin in Alchornea cordifolia is reported. Lee et al. (1993) showed that hyperoside had a significant activity on the mice ear oedema test after oral administration.

Guided bio-assay by the inhibitory activity on TPA-induced ear oedema in mice on Erythroseptenum monticulum identified quercetin among the two active compounds (Recio et al., 1995). Quercetin was also identified in an anti-inflammatory fraction of Solanum melongena leaves (Barnabas and Nagarajan, 1989); furthermore, Sanchez de Medina et al. (1996, 2002) the inhibitory effect of quercetin on inflammation.

Previous reports about Alchornea cordifolia showed that ellagic acid was responsible for anti-plasmodial activity in vitro (Banzouzi et al., 2002). Ellagic acid was also successfully tested for its protective activity against colitis inflammation induced by dextran sulphate sodium (Ogawa et al., 2002) at a concentration of 10 mg/kg given twice daily for 6 days, per os in microsphere capsules.

Giovannelli et al. (2000) works on complex polyphenol and tannins from wine, concluded that they might both have a protective and a therapeutic potential in oxidative damage-related pathologies. In 1996, after topical test for heterogeneous tannins’ ability to inhibit the biomarkers of tumour promotion in mouse skin in vivo, Perchellet et al. (1996) concluded that some foliage tannins have potent antioxidant and anti-inflammatory activities.

However, in our experiments, we observed that the most active crude extract (MeOH-S) had the lower tannins content (Table 4) and that the aqueous decoction containing about 10% tannins had no significant activity at a dose of 2000 μg/cm².

Furthermore, the yields of tannins in fractions F1 (12.33%) and F3 (10.57%) can not explain the difference between the observed anti-inflammatory activity since F1 contained more tannins and was proportionally less active than F3. On the other hand, concentrations of flavonoids in F1, F3 and F4 were related to the anti-inflammatory activity. Nevertheless, the most active fraction (F2) was highly lipophilic.

Purification and characterisation of the compounds from this fraction are in process.

5. Conclusion

The MeOH extracts of Alchornea cordifolia leaves showed strong dose-dependent anti-inflammatory activity after topical application. This activity may be explained at least partially by unidentified lipophilic compounds and by flavonoids (e.g. hyperoside and quercetin) but the degree of activity was not related to tannin contents. This work is the first report on the presence of quercitrin in Alchornea cordifolia. Nevertheless, other compounds probably also contribute to anti-inflammatory activity.

Further studies will be undertaken to elucidate the mechanism of action by which the extracts exert their anti-inflammatory activity and eventually isolate active compound(s).

However, our results give a rational support to the traditional use of Alchornea cordifolia in tropical Africa as an anti-inflammatory agent.

Acknowledgements

Part of this work received support from DMO Internation, Tramix S.A. D.E.P. Marie is under an AUF (Agence Universitaire de la Francophonie) post-doctoral fellowship. D. Brkic is under contract in the frame of a Research Training Network founded by the European Commission (HPRN-CT-1999-00054). The authors wish to thank M.-C. Crutzen-Fayi for her skilful technical assistance.

References


Ogunbamila, F.O., Samuelsson, G., 1990. Antibacterial and antifungal activities of nine medicinal plants from Za-


