Anti-oxidant and Anti-inflammatory Activity of *Manniophyton fulvum*

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**Abstract**  
*Manniophyton fulvum* Muell. Arg. (Euphorbiaceae), is used for wound healing, inflammatory conditions and against dysentery, and diarrhea in central Africa. In addition, the plant is an important delicacy for the pygmy chimpanzee (*Pan paniscus*). Up to now, no scientific proof exists to justify such practices. To unravel the rationale behind the folkloric uses, the leaves of the plant were extracted in methanol and chemically analysed for its secondary metabolites. The crude methanolic extract was assayed for its anti-oxidant activity using 2,2-diphenyl-1-picrylhydrazyl hydrate:DPPH assay and for its anti-inflammatory potential using hens’ eggs test-chorioallantoic membrane assay: HET-CAM. The crude methanolic extract showed a significant anti-oxidant activity (IC$_{50}$: 1.06 µg/ml) as well as a strong dose dependent anti-inflammatory activity (92% inhibition at 250 µg/pellet). Via bio-assay fractionation, the main activity can be enriched in the medium polar fraction. Interestingly, no embryotoxic effects and or membrane irritation was observed in the purified fraction. These results clearly demonstrate the rationale behind the uses of this plant against the ascribed ailments, both by the traditional medical practitioners (TMP) in Africa and by *Pan paniscus* as a food component.

**INTRODUCTION**  
The onset of inflammation is partly the result of oxidative stress, a consequence of the imbalance between pro-oxidants and anti-oxidants. Oxidation and superoxidation stress hypothesis have put this fact forward as an explanation for the aetiology and pathology of some human diseases and animal fitness (Yokosawa et al., 1997). It is therefore plausible that anti-inflammatory drugs may play a vital role in the immune response (Sur et al., 2001). Hence, the search for potent anti-oxidants may play a vital role in alleviating problems caused by inflammations especially in chronic cases. *Manniophyton fulvum* is a member of the Euphorbiaceae family, the decoction of the leaves is used in Democratic Republic of Congo (DRC), Cameroon and in other African countries against a variety of human afflictions related to inflammatory conditions (Iwu, 1993; Nguimatsia et al., 1998) in addition of being an important food component for *Pan paniscus* (the pigmy chimpanzee: the “Bonobos”). To the best of our knowledge the plant has never been studied before. Because of the correlation between oxidation and inflammation (Sur et al., 2001) and informed by the folkloric uses of the plant, we deemed it necessary to study systematically these two factors.

**MATERIALS AND METHODS**

**Materials:** Collection of Plant Material and Extraction

*Manniophyton fulvum* Muell. Arg. was collected in Kinshasa (May, 2000),
Democratic Republic of Congo (DRC) and identified by the taxonomist of the Institute of Pharmacy, University of Kinshasa XI, where a voucher specimen is deposited. 5 kg of the fresh leaves were sun-dried and powdered to afford 1750 g, which were extracted cold in 100% methanol by percolation for 72 h. The brown organic phase was filtered through Whatman paper No 1, concentrated in vacuo and freeze-dried to give 450 g. The freeze-dried material was submitted to chromatographic and subsequent standard analytical methods for the presence or otherwise of bioactive ingredients (Harborne, 1984). It was assayed and fractionated with organic solvents to give n-Hexane (He), Chloroform (Ch), ethylacetate (Ea), n-butanol (Bu) and aqueous (Aq) fractions for further assays. The yield was calculated in percentage.

Methods: Anti-oxidant Activities
1. Rapid-TLC Screening for Anti-oxidant Activity. The freeze-dried methanol powder and its fractions were dissolved in 100% methanol, spotted on silica gel sheets, developed in methanol:ethylacetate (2:1; v/v), air-dried and sprayed with 0.2% solution of 2,2-diphenyl-1-picrylhydrazyl hydrate: DPPH (Cuendet et al., 1997). The plates were visualised for the presence of whitish spots, indicating antioxidant positive response.
2. DPPH Assay. The DPPH assay was carried out as described by Cuendet et al. (1997). 50 µg of various dilutions from the different fractions were mixed with 5 ml of a 0.004% methanol solution of DPPH. After 30 min incubation, the absorbancy of the sample was read at 512 nm using a spectrophotometer. Ascorbic acid was used as a positive control.

Anti-inflammatory Assay: HET-CAM
1. Phase 1: Pellet Preparation. 7.25 mg sodium dodecyl sulfate (SDS) was dissolved with or without a specified concentration of the test drug in 1 ml of a hot (about 60°C) 2.5% agarose solution. 10 µl of the gelling solution was used for the pellet preparation.
2. Phase 2: Incubation. The modified method of D’Arcy and Howard (1967) was used. The fertile hen eggs were incubated for 75 h at 37°C and relative humidity of 80%. The eggs were placed in a horizontal position and rotated several times. They were opened in the snub end after aspiration of 10 ml of albumin from a hole at two thirds of the height from the pointed end. The eggs were traced with a scalpel and thereafter the shell was removed with forceps. The aperture was covered with keep fresh paper and the eggs were incubated at 37°C for 75 h at a relative humidity of 80%. One pellet per egg was put on the newly formed chorio-allantoic membrane (CAM) which was about 2 cm in diameter. The eggs were incubated for 24 h and then evaluated. For every test, 10 eggs were utilised. To evaluate the effect as positive irritation control, CAMs were treated with SDS only, as positive control, hydrocortisone (HC) was tested at a concentration of 50 µg/pellet in the presence of SDS at a concentration of 72.5 µg/pellet. As a blank, CAMs were treated with agarose solution only.
3. Phase 3: Interpretation of Experiments. The inhibition or otherwise of the membrane irritation was observed. A positive effect, corresponding to anti-inflammatory activity, exists if the irritation of the membrane induced by SDS decreases (i.e. the star like picture around the granuloma) and the blood vessel net appears normal. The number of eggs with a positive effect was given in per cent and indicated the anti-inflammatory activity of the drug tested. (Marchesan et al., 1998). However, the death of the embryo was recorded when observed.

Statistical Analysis
The experiment (HECTAM) was carried out in triplicate (Table 2) and the data are expressed as mean ± SD and the statistical significance between groups was analysed by means of an analysis of variance (ANOVA), followed by student – New man – Keul’s test. P values less than 0.05 was considered as indicative of significance.
RESULTS AND DISCUSSION

Extraction, Preparation and Phytochemical Screening

During the extraction, the yield of 35% and 9% over fresh weight were recorded for the dried powder and the crude methanolic extract respectively. The chemical analysis, using the standard procedures described by Harborne (1984), revealed the presence of flavonoids, tannins, phlobatannins, saponins and traces of alkaloids in the methanol extract. After being tested, the methanol extract was fractionated to yield (w/w), n-hexane (10.5%), the lowest, chloroform (15.2%), ethylacetate (25.53%), n-butanol (19.85%) and aqueous fractions (27.39%) the highest (Table 1, Fig. 1), for further bio-assay studies. The most active fraction (ethyl acetate) was found to contain flavonoids.

Anti-oxidant Activity (DPPH) Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) reagent was used to visualise the active compounds and to measure the level of radical scavenging potential of the methanolic extract and its fractions. As a spray reagent, the presence of whitish reactions were identified on different chromatograms spotted with prepared methanol and fractions. The reagent was also used spectrophotometrically to measure their IC₅₀. In both cases the ethyl acetate fraction displayed the best resolution in term of number of positive spots on TLC and 50% inhibition, IC₅₀ 0.60 µg/ml as against IC₅₀ 1.06 µg/ml in the “mother” methanolic extract (Fig. 2). The hexane fraction showed no antioxidant spot on TLC, however its 50% inhibition was high, IC₅₀ 4.90 µg/ml as compared to the positive control (IC₅₀ 0.64 µg/ml). The result is an indication that most of the active components are concentrated in the ethyl acetate fraction. In addition of being implicated in the pathogenesis of several diseases (Peterhans, 1997), oxidation reactions are also a great concern for the food industry since they cause considerable deterioration to fresh and processed foodstuffs thereby limiting their shelf life. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) and natural anti-oxidants such as tocopherol and ascorbate derivatives are widely used in food preservation because they provide good protection for unsaturated fats and oils. Recently however, BHT and BHA have been suspected to be dangerous for human health (Safer et al., 1999). From these findings, this plant represents a potential target for the isolation of potential anti-oxidant natural compounds which could serve in food industry.

Anti-inflammatory Activity (HET-CAM) Assay

Serial dilutions of methanol and EtOAc fractions displayed, a dose response trend between 500 and 72.5 µg/pellet (Fig. 3) in restoring to normal an inflammatory condition (membrane irritation ) induced by sodium dodecyl sulphate (SDS). Therefore the typical picture of membrane irritation: inflammation of blood vessel around the granuloma to give a star-like structure returned to normal 24 h after drug application. Within the dilution zone, the inhibition effect of membrane irritation was graded and found in the average to be in the “Good effect zone” according to Marchesan et al. (1998). Total inhibition (100%) was found at 500 µg/pellet in the methanol extract and the lowest inhibition (81%) was found at 72.5 µg/pellet in the EtOAc fraction. It is noteworthy to mention that purification of the methanol extract enhanced the anti-inflammatory activity and kept the 10% toxic effect observed in the methanolic extract at 500 µg/ml out of the most active EtOAc fraction (Fig. 3). The activities of the EtOAc fraction and the methanolic extract were comparable to that of hydrocortisone at concentration of 72.5 µg/pellet instead of 50 µg/pellet, the fraction was still having a “Good effect” (81%). These results point to the fact that the plant may contain bioactive ingredients in particular flavonoids, since they were found in high concentration in the plant extract and in the most active fraction: ethyle acetate, and number of them with the right substitution pattern, have been implicated in a variety of inflammatory relieving processes (Yokosawa et al., 1997). These findings have justified the uses of this plant in traditional medicine in Africa in curing chronic inflammatory conditions. In addition, its implication as a food
component for *Pan paniscus* has been substantiated because its anti-oxidant response coupled with the recorded anti-inflammatory potential could provide fitness and some level of immunity to diseases. Further studies are however necessary to isolate and characterise the anti-oxidant and anti-inflammatory components for use in food industry and medicine.

**ACKNOWLEDGEMENTS**

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**Literature Cited**


**Tables**

**Table 1. Comparative yield (%) of the various fractions from EtoAc.**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>He</th>
<th>CH</th>
<th>Ea</th>
<th>Bu</th>
<th>Aq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (%)</td>
<td>10.5</td>
<td>15.2</td>
<td>25.53</td>
<td>19.85</td>
<td>27.39</td>
</tr>
</tbody>
</table>

**Table 2. Anti-inflammatory (HET-CAM) response of the tested drugs.**

<table>
<thead>
<tr>
<th>Extract/Fraction</th>
<th>Dose (µg/pellet)</th>
<th>Inhibition (%) ± SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeoH (me)</td>
<td>500</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td>MeoH (Ne)</td>
<td>250</td>
<td>92 ± 1.00</td>
</tr>
<tr>
<td>Ethyl acetate (Ea)</td>
<td>250</td>
<td>94 ± 1.50</td>
</tr>
<tr>
<td>Ethyl acetate (Ea)</td>
<td>125</td>
<td>90 ± 2.00</td>
</tr>
<tr>
<td>Ethyl acetate (Ea)</td>
<td>72.5</td>
<td>81 ± 1.30</td>
</tr>
<tr>
<td>Sodium Dodecyl Sulfate (SDS)</td>
<td>72.5</td>
<td>10 ± 1.00</td>
</tr>
<tr>
<td>Hydrocortisone (HC)</td>
<td>50</td>
<td>84 ± 1.50</td>
</tr>
</tbody>
</table>

* Values represent mean ± SD from triplicate experiments

P < 0.001
Figures

Fig. 1. Comparative yield (%) of the various fractions from EtOAc.

Fig. 2. Anti-oxidant response (DPPH) of fractions from EtOAc.

Fig. 3. Anti-inflammatory (HET-CAM) activity of methanol and serial EtOAc fractions.