AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 30, NUMBER 3, SEPTEMBER, 2001

EDITOR:
B. O. OSOTIMEHIN

ASSISTANT EDITOR:
A. O. UWAIFO

ISSN 1116 — 4077

Afr. J. Med. med. Sci. (2001) **30**, 213-215

Antioxidant and anti-inflammatory activities of *Mallotus oppositifolium* in model systems

EO Farombi*¶, O Ogundipe and JO Moody****

** Drug Metabolism and Toxicology Unit, Department of Biochemistry and **Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Nigeria*

Summary

Chemical analysis of the powdered leaves and root of *Mallotus oppositifolium* revealed the presence of alkaloids, cardiac glycosides and phenolic compounds with a higher concentration residing in the leaves than in the root. Antioxidant and anti-inflammatory activities of the crude extracts in hexane and methanol were evaluated by the β-carotene linoleate model system and the carrageenan induced rat paw oedema animal model. Both systems identified significant biological activity in the methanolic crude extract of the leaf. Thin layer chromatographic (TLC) analysis of the crude extract in methanol identified four phenolic spots two of which were flavonoid in nature ($UV_{254nm, 356nm}$, $FeCl_3$ and $AlCl_3$ visualization)

Keywords: *Antioxidants, anti-inflammatory, phenolics, B-carotene, Mallotus oppositifolium*

Résumé

L'analyse chimique des poudres de fenilles et de racines de mallotus oppositifolium avait revele la presence des alkaloides des glycosidas cardiac et des composes phenolique avec les fortes concentrations au miveau des fenilles par rapport aux racines. Les activities antioxidantes et anti-inflammatoires des extraits cruds dans de l'hexane et du methanol avaient ele evalue par le model de la β-caritene linorteate et l'induction des oedemes dans un modele animal (rat). Les 2 systems avaient identifie une activites biologique significative dans l'evtrait methanolique des fenilles. La chromatographic sur couche mince (TLC) de l'extrait dans du mehtanol avait identifie 4 spots phenoli que parmis lesquels 2 etaient de nature flavonoide ($UV_{254nm, 356nm}$ visualization au $AICL_3$ et $Fecl_3$)

Introduction

The use of plants as a source of relief for illness is as old as mankind with recorded practices dating back at least 4000 years [1].

Mallotus oppositifolium (Family Euphorbiaceae), commonly referred to as 'oju-eja' (Yoruba, Southern Nigeria) is a shrub or small tree which grows in old farms of secondary forest and thickets. It also thrives in the savannah vegetation and is native to Africa, India and Arabia [2].

The leaves are ingredients of common antimalarial, antiinfection and anti-inflammatory remedies used locally [3].

Production of reactive oxygen species (ROS) beyond the antioxidant capacity of a biological system gives rise to oxidative stress. Oxidative stress has been implicated in over 100 diseased conditions ranging from heart diseases to malaria, neurodegenerative disease, AIDS, cancer and in the aging process. At the same time it has been recognized that free radical scavengers and plant derived antioxidants such as ∝-tocopherols, ascorbic acid, biflavonoids and carotenoids can protect against tissue damage and tumorigenesis induced by a large number of chemical carcinogens [4,5]. In addition, there is a growing interest in the use of food browning reaction products as natural antioxidants and scavengers of ROS [6-8].

Inflammatory reactions in humans are usually characterized by pains, swelling and fever and a major inflammatory disease (rheumatoid arthritis) is one of the most distressing and disabling syndromes encountered in medical practice and although it is one of the oldest diseases, there is no drug leading to a permanent cure. The side effects of existing steroidal and nonsteroidal anti-inflammatory drugs have led to increasing efforts in search of novel compounds especially from plants which would possess long acting anti-inflammatory activity with minimum side effects. We have reported previously the anti-inflammatory activity of extractives from some plants [9].

In the present study, we have examined the antioxidant and anti-inflammatory potential of *M. oppositifolium* using β-carotene linoleate and the carrageenan-induced rat paw oedema animal model systems.

Materials and methods

Chemicals

Butylated hydroxylanisole (BHA), linoleic acid and tween 40 emulsifier were purchased from Sigma Chem Co., St Louis, MO. All other reagents were of analytical grade and were purchased from the British Drug Houses. Poole, Dorset.

Plant material

The leaves and roots of *M. oppositifolium* were collected in April 1996 at the Botanical garden of the University of Ibadan, Nigeria. Authentication of the plant was carried out by Mr. J. Akindele of the Botany Department, University of Ibadan and voucher specimen PG UI 110 was deposited at the herbarium of the Department of Pharmacognosy, University of Ibadan. The samples were air dried and communited in the Chemistry Department, University of Ibadan.

Phytochemical screening

Standard phytochemical screening procedures were utilized [10].

Preparation of extracts.

40g of the powdered leaves and 20g of the powdered roots were exhaustively and successively extracted with n-hexane and methanol with a soxhlet apparatus. All extracts were concentrated 'in vacuo' to low volumes and utilized at a concentration of 2mg/ml for antioxidant evaluation and at a dose of 100mg/kg suspended in 40% v/v tween 80 for anti-inflammatory evaluation.

Correspondence: Dr. E.O. Farombi, Drug Metabolism and Toxicology Unit, Department of Biochemistry, University of Ibadan, Ibadan, Nigeria. Email: ofarombi@skannet.com.

Thin layer chromatography (TLC)
Analytical: Silica gel GF_{254} (Merck) 0.25mm thick, activated at 100°C before use.
Solvent system (SSI): Ethyl acetate: methanol: water (10:1.3:0.7).
Visualizaion: $FeCl_3$, $AlCl_3$
$UV_{254, 356}$.

Determination of antioxidant activity using ß-carotene-linoleate model system
The antioxidant activity of each fraction was determined according to Miller [11] and as modified by Farombi *et al.* [8] using ß-carotene-linoleate model system. 2mg of ß-carotene was dissolved in 10ml of chloroform. Chloroform was removed using rotary evaporator at 40°C. 1 ml of this solution was transferred into a round-bottom flask 20 mg of purified linoleic acid, 200 mg of tween 40 emulsifier and 50 ml distilled water were added to the flask and shaken vigorously. 5 ml of the emulsion were transferred to tubes containing 5mg of each isolated fraction. Absorbance readings were recorded every 15 min at 50°C until the colour of ß-carotene in the control sample had been bleached by exposure to the high temperature. The results were compared with BHA.

Experimental animals
The animals used in this study were adult male albino Sprague-Dawley (120-150g) rats obtained from the animal house, Department of Biochemistry, University of Ibadan. The rats were maintained under standard laboratory conditions but fasted 12 hours just before each experiment.

Anti-inflammatory evaluation
Assay procedure
Inhibition of the carrageenan-induced hind paw oedema was used as a measure of anti-inflammatory activity. Groups of animals (n = 6) received 100mg/kg p.o. of test extracts or aspirin (ASA), suspended in 40% w/v tween 80 in normal saline. The control group of animals received only the vehicle (40% w/v tween 80 in normal saline).

One hour after drug administration, oedema of the rat right hind paw was produced by injecting into the subplantar surface 0.5% w/v carrageenan (Sigma) suspension. Increase in the linear paw diameter which was taken as an index of the paw volume was measured using a micrometer screw gauge immediately before injecting the carrageenan and at hourly intervals there after up till 5 hours. The volume of oedema was expressed for each animal as the difference in the diameter of the rat paw before and after the injection of the carrageenan. The results are documented as percentage anti-inflammatory activity for the difference in oedema diameter obtained using a standard formula [12,13].

Thin layer chromatographic analysis of the crude methanol extract on silica gel GF_{254} (0.25mm thick) revealed the presence of four phenolic components (green coloration with $FeCl_3$ spray) with Rf values of 0.12, 0.28, 0.45 and 0.62, two of these components (Rf 0.12 and 0.28) were flavonoid in nature (fluorescent yellow coloration, 1% $AlCl_3$, UV_{356nm})

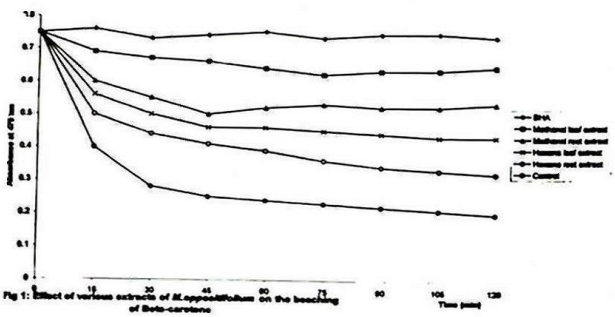
Results and discussion
The phytochemical screening of *M. oppositifolium* revealed the presence of secondary metabolites such as alkaloids, phenols, flavonoids, anthraquinones and cardenolides. A higher concentration of these reside in the leaves than in the stem and the root (Table 1).

Table 1: Phytochemical screening of the morphological parts of *Mallotus oppositifolium*.

	Leave	Stem	Root
Alkaloids			
Dragendorff	+	-	+
Mayer	+	-	+
Cardenolides			
Kedde	±	-	±
Keller killiani	±	-	±
Anthraquinones			
Bontrager's test	++	+	+
Tannins			
Ferric chloride	+++	+	++
Saponins			
Frothing test	±	±	±
Emulsifying test	±	±	±
Flavonoids			
Shibata's reaction	+++	+	++

Key
- = *Absent* ± = *Traces or possibility*
+ = *Present* ++ = *Moderately present*
+++ = *Abundant.*

Figure 1 shows the effect of isolated fractions on the bleaching of β-carotene. The results indicate the decrease in absorbance of β-carotene in the presence of various fractions ex



tracted from *M. oppositifolium* with the coupled oxidation of β-carotene and linoleic acid. The control sample without the addition of the isolated fractions oxidised most rapidly while the fractions elicited inhibition of bleaching of β-carotene to varying extents. The methanolic leaf extract with the highest percentage yield (21.4%) showed the strongest antioxidant effect by attenuating the bleaching of β-carotene. The hexane leaf and the methanolic root extracts showed weak antioxidant activity and their effects were short lived as these reduced after 60 min. The antioxidant activity of BHA in the β -carotene linoleate model system is superior to all the fractions isolated from *M. oppositifolium*.

The carrageenan-induced hind paw oedema was used as a measure of anti-inflammatroy activity due to its ease of administration. The crude methanolic leaf extract possessed the highest anti-inflammatory activity (65% inhibition) comparable with acetyl salicylic acid (aspirin^R), which was used as a standard and had (91% inhibition) at the same dose of 100 mg/kg (Table 2).

The development of oedema induced by carrageenan corresponds to the events in early exudation phase of inflammation, which is an important feature of the inflammatory pathway.

The significant anti-inflammatory activity exhibited by *M. oppositifolium* can be attributed to the presence

Table 2: Anti-inflammatory activity of crude extracts from *Mallotus oppositifolium*

Extract	%yield	Dose (mg/kg)	Oedema Diameter + S.e.m	% anti-inflam matory activity
HL	3.38	100	0.64±0.12	35.4
HR	0.05	100	0.88±0.25	11.1
ML	21.40	100	0.35±0.14	64.6
MR	1.90	100	0.58±0.12	41.4
ASA	-	100	0.09±0.14	90.9
TW	-	-	0.99±0.17	-

Key
HL - *Hexane leaf extract*
HR - *Hexane root extract*
ML - *Methanol leaf extract*
MR - *Methanol root extract*
ASA - *Acetyl salicylic acid (aspirin®)*
TW - *40% v/v Tween 80.*

of flavonoid compounds, which have been identified as potent anti-inflammatory agents [14-16]. Flavonoids and phenolic compounds have been employed in the treatment of a number of diseased conditions as antiinfective agents (15) Moreover, many flavonoids and phenolic compounds have been found to be strong free radical scavengers and antioxidants [17,18]. It is known that excessive free radical production and lipid peroxidation in vivo may cause many kinds of diseases. Thus, the antioxidation of flavonoids may be related to their pharmacological actions and so they may be used as protective agents in a number of diseases [19].

Our present findings therefore justify the use of *M. oppositifolium* in traditional medicine for the treatment of inflammatory diseases and for possible use as food antioxidant.

Acknowledgements

β-carotene was a generous donation of Dr. George Britton of the School of Biological Sciences, University of Liverpool, UK.

References

1. Dalziel JM and Hutchinson J. Flora of West tropical Africa, Crown Agents for overseas Government London 1963, 1-2.
2. Iwu MM. Handbook of African Medicinal Plants. CRC Press, Florida, 1993, pp 219-221.
3. Burkhill HM. The useful plants of West tropical Africa, Ed. 2 vol2 Families E-I. Royal botanic gardens, Kew 1994; 144-150.
4. Farombi EO and Britton G. Antioxidant activity of Palm oil carotenes in organic solution: effects of structure and chemical reactivity. Food chemistry1999; 64 (3): 315-3217.
5. Farombi EO and Britton G Antioxidant activity of Palm oil carotenes in peroxyl radical-mediated peroxidation of phosphatidyl choline liposomes. Redox Report 1999; 4 (1/2): 61-68.
6. Farombi EO, Nwankwo JO and Emerole GO. Possible modulatory effect of browned yam flour diet on chemically-induced toxicity in the rat. Food & Chemical Toxicology1997; 35: No 11, 975-979.
7. Farombi EO, Nwankwo JO and Emerole GO. Dose-dependent modulation of hepatic toxicities and microsomal peroxidation from carbon tetrachloride and bromobenzene in rats fed browned yam flour diet. Proceedings of the Federation of African Society on Biochemistry and Molecular Biology,1998; 1: 7-10
8. Farombi EO, Britton G and Emerole GO. Evaluation of the antioxidant and partial characterisation of extracts from browned yam flour. Food Research International 2000; 33 (6) 493-499.
9. Ajayiobe OO, Moody JO and Aziba PI. Anti-inflammatory activity of Newbouldia laevis extractives. Journal Pharmaceutical Science and Pharmacy Practice1997; 3: 1-3.
10. Trease GE and Evans WC. Pharmacognosy 14th edition, London W.B Sainders, 1996; pp 367.
11. Miller HE .A simplified method for the evaluation of antioxidants, J. Am. Oil. Chem. Soc 1971; 48: 91.
12. Bamgbose SOA and Naomesi BK. Studies on Cryptolepine. Inhibition of Carrageenan-induced oedema by cryptolepine. Planta Medica1981; 42: 392-396.
13. Mascolo N, Guusepua A and Francesco C. Biological screening of Italian medicinal plants for anti-inflammatory activity. Phytotherapy Research1987; 1: 28-31.
14. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochemical Pharmacology 1983; 32: 1141-1148.
15. Alcaraz MJ and Jimene MJ. Flavonoids as antinflammatory agents Fitoterapia1988; LIX (1): 25-38,
16. Bohlin L. Structure-activity studies of natural products with anti-inflammatory/immunomodulatory effects Proceedings of the Phytochemical Society of Europe, Hostettman K, Martson A, Mallard M and Huburger M (ed) 1993; Clarendon Press, Oxford, 137-163.
17. Scott R and Slater TF. Free radical scavenging activity of (+)-catechin and other flavonoids. In Slater TF; GarnerA (eds). Recent advances in lipid peroxidation and tissue injury. London: Brunel University 1981: 233-244.
18. Robak J and Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochemical Pharmacology1988; 37: 837-841.
19. Yuting C, Rongliang Z, Zhongjian J and Yong J. Flavonoids as superoxide scavengers and antioxidants. Free Radical Biology Medicine 1990; 9: 19-21