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Research Article

Chemical composition and anti-inflammatory activity of essential oils of *Melaleuca alternifolia* from Senegal

Alioune Diallo[1], Yoro Tine[1*], Madieye Sene[2], Cheikhouna Gaye[1], Benjamin Ndiaye[1], Idrissa Ndoye[1], Alassane Wele[1], Julien Paolini[3] and **Jean Costa[3]**

1. Laboratory of Organic and Therapeutic Chemistry, Faculty of Medicine, Pharmacy and Dentistry, Cheikh Anta Diop University, BP: 5005 Dakar-Fann, Senegal.
2. Laboratory of Pharmacology and Pharmacodynamics, Faculty of Medicine, Pharmacy and Dentistry, Cheikh Anta Diop University, BP: 5005 Dakar-Fann, Senegal.
3. University of Corsica, UMR CNRS 6134 SPE, Natural Resources Team, Campus Grimaldi, BP 52, F-20250 Corte, France.

Abstract

This study aimed to determine the chemical composition of three essential oil samples obtained from the leaves of *M. alternifolia* (Myrtaceae) collected in Kaolack (Senegal) and to assess their *in vivo* anti-inflammatory activity. The corresponding oils were obtained by hydrodistillation and analyzed by GC/FID and GC/MS. The anti-inflammatory activity of the essential oil was evaluated by carrageenan-induced rat paw oedema method. Oil yields from dried leaves were ranged from 1.5 to 1.8%. The essential oils mainly consisted of terpinen-4-ol (28.8-33.0%), followed by geranial (18.1-19.6%), neral (12.0-12.4%), *p*-cymene (9.8-11.3%) and γ-terpinene (8.7-9.6%). The essential oil administered per os very significantly prevented the development of inflammatory edema of the rat paw induced by carrageenan at doses of 25, 50 and 100 mg/kg. The effect observed was most pronounced between 3 and 5 hours. It was dose-dependent between 25 and 50 mg/kg. The activity at 50 mg/kg was greater and identical to that of aspirin, used as a reference molecule and administered at a dose of 100 mg/kg. We can conclude that the essential oil of *M. alternifolia* possesses potential anti-inflammatory activity, supporting the traditional application of this plant in treating various diseases associated with inflammation.

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Corresponding Author
Prof. Dr. Yoro Tine
E-mail: yoro.tine@ucad.edu.sn
Tel: +221774591945

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Melaleuca alternifolia, essential oils, anti-inflammatory activity, GC-MS.

1. Introduction

Inflammation is a defense reaction of the body to various stimuli, which can be of physical, chemical, infectious or biological origin. Unfortunately, inflammation is accompanied by various undesirable symptoms, e.g. edema, erythema and pain [1–3]. Their therapeutic management using anti-inflammatories is subject to numerous adverse effects, drug interactions and contraindications. Thus, there has recently been an increasing interest in highly effective anti-inflammatory agents from natural sources with as little adverse reaction as possible [1–3].

Melaleuca alternifolia is a plant native to Australia and its essential oil (tea tree oil; TTO) is widely used for medicinal purposes due to its excellent antiseptic activity and antioxidant properties. TTO has a complex chemical composition [4–8]. Six natural chemotypes in *M. alternifolia* have been described, each producing an oil of distinct chemical composition and identified by the presence of terpinen-4-ol (terpinen-4-ol/γ-terpinene/α-terpinene [9,10] and terpinen-4-ol/1,8-cineole/terpinolene [11]), 1,8-cineole (1,8-cineole [11]; 1,8-cineole/terpinolene

[11] and 1,8-cineole/terpinen-4-ol [11]) or terpinolene (terpinolene/1,8-cieneole [11,12]). The relative concentrations of the main compounds terpinen-4-ol, 1,8-cineole and terpinolene determine the commercial quality of the oil [13]. Thus, the aim of this study was to characterize the chemical profile and anti-inflammatory activity of the essential oils from leaves of *M. alternifolia* collected in Kaolack (Senegal).

2. Materials and methods

2.1. Plant material

Three samples of *M. alternifolia* fresh leaves were collected on February 19, 2023 in Kaolack (Senegal). The plant material was identified by the technicians from the Department of Botanical of the Fundamental Institute of Black Africa (IFAN) of the University Cheikh Anta Diop of Dakar.

2.2. Extraction of essential oils

Plant materials were air-dried for 14 days at room temperature. Samples were hydrodistilled (5h) using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [14]. The yields of essential oils (w/w, calculated on the dry weight basis) were given in Table 1.

2.3. GC and GC/MS Analysis

The chromatographic analyses were carried out using a *Perkin-Elmer Autosystem XL GC* apparatus (Walthon, MA, USA) equipped with a dual flame ionisation detection (FID) system and fused-silica capillary columns, namely, Rtx-1 (polydimethylsiloxane) and Rtx-wax (poly-ethyleneglycol) (60 m × 0.22 mm i.d; film thickness 0.25 µm). The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C for 35 min: hydrogen was employed as carrier gas (1 mL/min). The injector and detector temperatures were maintained at 280°C, and samples were injected (0.2 µL of pure oil) in the split mode (1:50). Retention indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C5–C30) by linear interpolation using the Van den Dool and Kratz (1963) equation with the aid of software from *Perkin-Elmer* (Total Chrom navigator). The relative percentages of the oil constituents were calculated from the GC peak areas, without the application of correction factors.

Samples were also analysed with a *Perkin-Elmer Turbo mass* detector (quadrupole) coupled to a *Perkin-ElmerAutosystem XL*, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C (35 min): hydrogen was employed as carrier gas (1 mL/min). The following chromatographic conditions were employed: injection volume, 0.2 µL of pure oil; injector temperature, 280°C; split, 1:80; ion source temperature, 150°C; ionisation energy, 70 eV; MS (EI) acquired over the mass range, 35–350 Da; scan rate, 1 s.

Identification of the components was based on: (a) comparison of their GC retention indices (RI) on non-polar and polar columns, determined from the retention times of a series of n-alkanes with linear interpolation, with those of authentic compounds or literature data; (b) on computer matching with commercial mass spectral libraries [15–17] and comparison of spectra with those of our personal library; and (c) comparison of RI and MS spectral data of authentic compounds or literature data.

2.4. Animals

Wistar rats (130–180 g) of both sexes were raised in the laboratory of pharmacology and pharmacodynamics of the Faculty of Medicine and Pharmacy of the University Cheikh Anta Diop of Dakar. Animals were randomly assigned to groups and maintained in plastic boxes at controlled room temperature (25–28 °C) with free access to food and water, under a 12:12 h light/dark cycle. All the experimental procedures were carried out during the day (08:00 a.m. to 05:00 p.m.) and were in accordance with the guidelines for animal care set out by the research ethics committee of the Cheikh Anta Diop University of Dakar for Animal Use in Research which was conducted in accordance with the internationally accepted principles for laboratory animal use and care. The animals submitted to oral administration of the EOMA or drugs were fasted for 12h before the experiments and acclimatized for at least 2h before the experiments.

2.5. Antiinflammatory activity

Carrageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drugs. The anti-inflammatory activity of the essential oil of *M. alternifolia* from sample 1 was evaluated by carrageenan-induced rat

Table 1. Chemical composition of the essential oils of *Melaleuca alternifolia*

N[a]	Compounds	lRI[b]	RIa[c]	RIp[d]	1	2	3
1	α-Thujene	929	922	1023	2.1	1.9	2
2	α-Pinene	963	931	1015	1.7	1.7	1.5
3	β-Pinene	978	974	1108	1.8	1.5	1.4
4	Myrcene	1007	982	1154	2.3	2.3	1.9
5	***p*-Cymene**	1010	1013	1264	**11.3**	**9.8**	**10.5**
6	Limonene	1022	1021	1200	2.9	2.9	2.6
7	**γ-Terpinene**	1046	1048	1239	**8.7**	**9.6**	**8.8**
8	Terpinolene	1077	1080	1278	2.7	3.4	2.8
9	**Terpinen-4-ol**	1161	1163	1590	**33.0**	**28.8**	**30.8**
10	Neral	1215	1214	1679	**12.0**	**12.4**	**11.6**
11	Geraniol	1235	1233	1843	1.8	2.8	2.8
12	**Geranial**	1244	1247	1731	**18.1**	**18.8**	**19.6**
	Compound Classes						
	Hydrocarbon monoterpenes				33.5	33.1	31.5
	Oxygenated monoterpenes				64.9	62.8	64.8
	Total identified (%)				98.4	95.9	96.3
	Yields (w/w vs dry material)				1.8	1.5	1.6

[a] Order of elution is given on apolar column (Rtx-1).
[b] Retention indices of literature on the apolar column (lRIa) [25].
[c] Retention indices on the apolar Rtx-1 column (RIa).
[d] Retention indices on the polar Rtx-Wax column (RIp).

paw oedema method [18].

The rats were divided into 5 groups of 5 animals each. Essential oil of *M. alternifolia* (EOMA) was dissolved in 0.9% NaCl solution and administered *per os* at different dose levels. Rats of Group I were given normal saline (10 mL/kg, bw) and treated as negative control. Rats in Group II were administered acetyl salicylic acid (100 mg/kg, bw) and considered as standard. Rats from Group III to Group V were given increasing doses of essential oil solution of *M. alternifolia* (25, 50, 100 mg/kg, bw). Acute paw edema was induced by injecting 0.1 mL of 1% (w/v) carrageenan solution, prepared in normal saline. After 1 h, 0.1 mL, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the left hind paw. The linear paw circumference will be measured at hourly intervals for 5 h. The increased edema was measured using digital calliper 60, 180 and 300 min (T1h, T3h and T5h) after carrageenan injection.

The importance of edema was assessed by determining the mean percentage increase (%INC) of linear paw diameter according to the following formula:

$$\%\,INC = \frac{Dt - D0}{D0} \times 100$$

Dt = Paw diameter at t time
D0 = Initial paw diameter

2.6. Statistical analysis

The means of contortions in treated groups were compared to the control with the Student t-test. A value of $p < 0.05$ had been considered as significant and n = 5 represent the number of rats in each group. The means of rat hind paw volumes were compared by an analysis of variance (ANOVA), in order to prove homogeneity between groups.

The means of percentages of rat hind paw oedema variations at 1 and 5 h were also compared to control group with t-test. A value of $p < 0.05$ had been considered significant and n = 5 represents the number of rats in each group. Statistical analysis was done using a GraphPad Prism 5 software.

3. Results and discussion

3.1 Chemical composition of essential oils

The essential oil yields, calculated with relative to the mass of dry plant material, were between 1.5 and 1.8%. The analysis of the leaf essential oils by GC/FID and GC/MS allowed the identification of 12 compounds accounting for 95.9 to 98.4% of the total compositions (Table 1). Essential oils were dominated

by oxygenated monoterpenes (62.8-64.9%) and hydrocarbon monoterpenes (31.5-33.5%). The essential oils mainly consisted of terpinen-4-ol (28.8–33.0%), followed by geranial (18.1-19.6%), neral (12.0-12.4%), *p*-cymene (9.8-11.3%) and γ-terpinene (8.7-9.6%). To our knowledge, we report for the first time this chemotype in *M. alternifolia*. Apart from terpinen-4-ol, a characteristic compound of this species, geranial and neral were present at significant levels. In addition, α–terpinene, one of the constituents of the most common chemotype (terpinene-4-ol/γ-terpinene/α-terpinene) of this species [19–22], was among the dominant components. However, we note the absence of α-terpinene. *p*-Cymene, present at a significant level in our study, was reported by two studies as being part of the majority components of *M. alternifolia*: in Slovakia (15%) [23] and in Brazil (20%) [24].

3.2. Antiinflammatory activity

The results of in-vivo anti-inflammatory activity of the essential oil of *M. alternifolia* (EOMA) from sample 1 on carrageenan induced edema on rat's paw are given in Figure 1.

The EOMA administered per os very significantly prevented the development of inflammatory edema of the rat paw induced by carrageenan at doses of 25, 50 and 100 mg/kg. The effect observed was most pronounced between 3 and 5 hours. It was dose-dependent between 25 and 50 mg/kg. The activity at 50 mg/kg was greater and identical to that of aspirin, used as a reference molecule and administered at a dose of 100 mg/kg.

In this connection, it should be noted that the 5 major components of the oil, of terpinen-4-ol (33.0%), geranial (18.1%), neral (12.0%), *p*-cymene (11.3%) and γ-terpinene (8.7%) are reported to have anti-inflammatory properties. Terpinen-4-ol, the main component of the essential oil of *M. alternifolia*, suppresses inflammatory mediator production by activating human monocytes [26]. Terpinen-4-ol can suppress the production of inflammatory mediators in LPS-stimulated human macrophages [27]. Recently, terpinen-4-ol has been shown to have an anti-arthritic effect which may be attributed to the downregulation of pro-inflammatory cytokines [28]. Citral, chiral enantiomers of neral and geranial, reduce the nociceptive and inflammatory response in rodents [29]. Citral also inhibits oxidative activity, nuclear factor kappa B (NF-κB) activation, and cyclooxygenase-2 (COX-2) expression [30]. Neral has been shown to have more potent anti-inflammatory activity than geranial, including significant inhibition of cytokine secretion (TNF-α, IL-6 and IL-1β) and expression of inflammatory molecules (pro-IL-1β, iNOS, COX-2 and NLRP-3) of LPS-stimulated macrophages [3]. *p*-cymene has been reported to possess antinociceptive and anti-inflammatory activities [31–33]. Studies have shown that, in different models of inflammation, γ-terpinene treatment attenuated inflammatory parameters such as edema and pro-inflammatory cytokine production, as well as cell migration into the inflamed site [34].

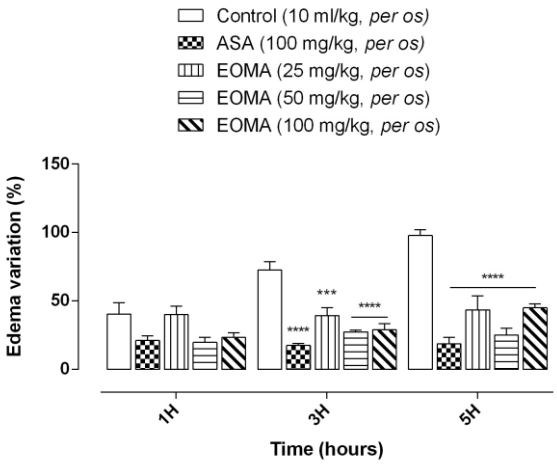

Figure 1. Effect of Essential Oil of *M. alternifolia* on Rat Paw Oedema Thickness in Carrageenan Model. **p < 0.01, ***p < 0.001, ****p < 0.0001 Vs Control Group. EOMA: Essential oil of *M. alternifolia*; ASA: Acetylsalicylic Acid

4. Conclusions

This study reported for the first time the chemical composition and anti-inflammatory activities of essential oils of *M. alternifolia* from Senegal. The essential oils mainly consisted of terpinen-4-ol, geranial, neral, *p*-cymene and γ-terpinene, exhibit anti-inflammatory activity at low doses in a model of carrageenan-induced inflammatory edema in rats. The EOMA possesses potential anti-inflammatory activity, supporting the traditional application of this plant in treating various diseases associated with inflammation. In perspective, we will carry out a study of the chemical variability of the essential oils of *M. alternifolia* in Senegal, as well as the evaluation of other biological activities.

Abbreviations

EOMA: Essential oil of *M. alternifolia;* Per os: Route of administration of medication by mouth.

Authors' contributions

Designed and coordinated the study, Y.T.; M.S.; A.W.; J.P.; J.C.; carried out the extraction and chemical characterization of essential oils, Y.T.; A.D.; C.G.; I.N.; B.N.; A.W.; J.C.; J.P.; evaluated the anti-inflammatory activity of this oil, M.S.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

Authors have no conflict of interest.

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