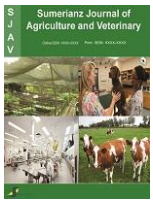
Sumerianz Journal of Agriculture and Veterinary, 2021, Vol. 4, No. 1, pp. 1-6
ISSN(e): 2617-3077, ISSN(p): 2617-3131
Website: https://www.sumerianz.com
DOI: https://doi.org/10.47752/sjav.41.1.6



Original Article Open Access

TLC/GC-FID Analysis and Antioxidant Activity of *Melicope madagascariensis* (Baker) T.G. Hartley (Rutaceae) Essential Oil from Madagascar East Coast Rainforest

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Article History
Received: December 5, 2020
Revised: January 11, 2021
Accepted: January 15, 2021
Published: January 18, 2021

Abstract

The aim of the present study was to evaluate the chemical composition and antioxidant activity of *Melicope madagascariensis* essential oil from Madagascar East Coast Rainforest. Essential oil from the leaves of this plant species was extracted by hydro-distillation and analyzed by TLC and GC-FID. The extraction revealed a yield is of 0.4% for a mass of 290 g of fresh leaves. Having a bland, pungent taste, the oil has a green-black color, a slightly unpleasant odor with a clear mobile consistency. Its density is 0.8765 and refractive index 1.4956 at 20 °C and is dextrorotatory (+0.1°) at 24 °C for its rotational power. The results of GC/FID analysis revealed that the essential oil contains (Z)-β-ocimene (12.7%) and (E)-β-ocimene (25.7%) as major compounds. The preparative TLC fractionation (using DPPH radical as revelator) and GC-FID analysis of the essential oil revealed the presence of compounds of the sesquiterpene family namely trans-nerolidol (4.32%), phenylpropanoid like methyl eugenol (5.9%), terpene oxide like caryophyllene oxide (3.66%), cinnamic esters like cinnamyl acetate (4.79%) and monoterpene alcohol like linalol (2.81%). The study on *M. madagascariensis* essential oil is herein reported for the first time. Further studies including cytotoxicity assay in order to evaluate the selectivity/therapeutic index of this essential oil are needed before developing it as novel antioxidant drug.

Keywords: Plant biodiversity; Secondary metabolites; Essential oil; TLC/GC-FID; Madagascar.

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1. Introduction

Madagascar possesses an inescapable richness in flora; this ecological diversity gives it resources an opportunity for therapeutic or food-processing research purposes [1]. The large island has around 84% of endemicity rate in vegetative flora [2, 3]. Its ecological diversity has led us to take a close look at the genus and species that deserve to be studied in depth and if it is still possible to contribute to the scientific data banks of the Malagasy flora. The plant species *Melicope madagascariensis* is a tree traditionally used as exhilarating agent, purgative, and in the treatment of liver, kidney and stomach disorders, bronchitis, mumps and malaria. Members of the genus *Melicope* are rich sources of furoquinoline alkaloids, methoxyflavones and acetophenones [4].

As part of our ongoing project on plant secondary metabolites of pharmaceutical relevance from Madagascar rainforest, we carried out phytochemical investigation on the essential oil of *M. madagascariensis*, which is one of the eleven *Melicope* species endemic to Madagascar. The present study constitutes thus a first scientific work on this plant species essential oil. In this perspective, the plant material was extracted by hydro-distillation in order to obtain its essential oil with the aim to evaluate its chemical composition and antioxidant activity [5-11].

2. Material and Methods

- Studied species: *Melicope madagascariensis*, an endemic plant species from the humid forest of the east coast of Madagascar [4].
- Part used: fresh leaves essentially crushed
- Extraction method used: Hydro-distillation to obtain an essential oil.
- Method for the determination of physico-chemical properties [5-11]:

o Determination of organoleptic characteristics

o Physical determination

- Relative density : $d_{20}^{20} = \frac{m_2 - m_0}{m_1 - m_0}$
- Refractive index : $n_D^t = n_D^{t'} + 0.0004\,(t' - t)$
- Pouvoir rotatoire : $[\propto] = \frac{\alpha_D^t}{c} \times 100$

Chemical determination

- Acide index : $IA = \frac{5.61\,V}{m}$
- Ester index : $IE = \frac{28.05}{m} \times (v_0 - v_1)$

o Analysis of the essential oil by GC

2.1. Operating Conditions

- GC : PE Clarus 580 with automatic injector
- Column : ELITE-WAX (30m*0.32mm*025mm) ; Furnace: 50°C to 245°C (5°C/min)
- Detector: FID; Carrier gas: Hydrogen, pressure: 0.33 bar (4.8psi)
- Injection: split mode (1/75); Integration: percentage of threshold area: 0.02%.
- Determination of antioxidant activity [1, 3, 5-9, 12]:

Evaluation by DPPH trapping on TLC, qualitative study: The method consists in depositing the essential oil to be tested on a TLC plate which has been developed with a mixture of solvents (n-hexane/diethilic ether; 9/1; v/v). The chromatographic plate was soaked in 0.004% DPPH methanolic solution for a few seconds. After drying, the migration stains for substances with antioxidant activity appear as pale yellow or yellow on a purple background.

Scavenger effect of the DPPH radical, comparison with ascorbic acid as a control: demonstrated according to the compilation of methods described by [1-3, 7, 9, 12]. A 0.004% DPPH solution ($0.2 < DO < 0.6$ at λ=517 nm) was prepared by solubilizing 2mg DPPH in 50 ml methanol. A volume of 20 µL of essential oil (at various concentrations) was added to 2 ml of the 0.004% DPPH solution. The reaction mixture was shaken vigorously and incubated for 30 min in the dark.

After incubation, the absorbances (OD) were measured at 517 nm against the blank (DPPH/methanol solution) **[1]**. Ascorbic acid was used as a synthetic reference antioxidant. Three tests were performed.

oGC identification of semi-purified compounds due to TLC isolation at radical DPPH trapping [1, 2, 8, 12].

2.2. Operating Conditions

- Use of n-hexane for GC analysis
- Same operating condition as for essential oil of *Melicope madagscariensis*

3. Results and Discussions

o Results of organoleptic tests :

Color: Green-black

Odor: Unpleasant
Taste: Bland and spicy
Consistency: Clear mobile

o Yield of essential oil extraction of *Melicope madagascariensis*

Material mass: 290 g
Mass of the essential oil: 1, 29 g
Y = 0, 4%

o Results of physical parameters :

Relative density: 0.8765 at 20°.
Refractive index: 1.4956 at 20°.
Rotational power: +0.1°(C°:1mg/ml) at 24°C

o Results of the chemical parameters :

Acid index: IA: 11.22
Ester index: IE: 201.96

o Results of the analysis on GC

The essential oil of *Melicope madagascariensis* specifically contains the following chemical compounds:

- family of monoterpenes: (Z)-β-ocimene (12.7%); (E)-β-ocimene (25.7%); α-pinene (8.56%) in majority percentage ,
- monoterpenic alcohols [13]: trans-piperitol (7.42%), linalool (0.73%); b-citronellol (0.33%); geraniol (0.08%); β-elemol (0.07%).
- phenol-methyl-ether: methyl chavicol or "estragol" (6.25%); methyl eugenol (1.12%).
- sesquiterpenes: β-selinene (2.06%); (E)-β-caryophyllene (3.21%); caryophyllene oxide (0.96%); α-copaene (0.75%); curcumene (0.41%) aromadendrene; (0.14%) allo-aromadendrene (0.15%); ar-curcumene (0.08%).
- sesquiterpenol: terpinen-4-ol (0.04%), α-terpineol (0.32%), β-elemol (0.07%)
- The table 1 gives the chemical composition of the essential oil of the leaves of *M. madagascariensis.*

Table-1. Chemical composition of the essential oil of the leaves of *M. madagascariensis*

N°	Constituents	RT [min]	RI p	Percentage [%]
1	α-pinene	2.801	527.6	8.56
2	Camphene	3.193	571.7	0.03
3	β-pinene	3.644	613.7	0.22
4	β-myrcene	4.428	667.5	1.00
5	Limonene	4.957	702.9	0.25
6	1,8-cineole	5.114	711.0	0.07
7	(*Z*)-β-ocimene	5.658	739.0	12.71
8	(E)-β-ocimene	5.988	756.0	25.74
9	Terpinolene	6.540	784.5	0.04
10	Citronellal	11.153	989.5	0.20
11	α-copaene	11.360	993.8	0.75
12	α-gurjinène	11.992	1013.1	0.97
13	Linalol	12.833	1046	0.73
14	Menthyl acetate	13.298	1064.2	0.33
15	(*E*)-β-caryophyllene	13.826	1084.9	3.21
16	Terpinen-4-ol	13.960	1090.1	0.04
17	Aromadendrene	14.064	1094.2	0.14
18	Allo-aromadendrene	14.496	1111.3	0.15
19	Menthol	14.957	1129.7	0.68
20	Methyl chavicol	15.536	1152.7	6.25
21	Neral	15.75	1161.3	0.23
22	α-terpineol	16.233	1180.5	0.32
23	Germacrene-D	16.468	1189.9	0.04
24	β-selinene	16.680	1198.3	2.06
25	*Trans*-piperitol	17.691	1240.5	7.42
26	ar-curcumene	17.997	1253.3	0.08
27	b-citronellol	18.073	1256.5	0.33
28	Curcumene	18.192	1261.4	0.41
29	Geraniol	19.790	1328.9	0.08
30	Caryophyllene oxide	22.409	1442.0	0.96
31	Methyl eugenol	23.253	1479.0	1.12

32	β-elemol	24.662	1544.2	0.07
33	Eugenol	26.25	1620.6	0.08
Total				**75.31**

*RI: Retention Index

3.1. Antioxidant Activity

The preparative TLC fractionation of the essential oil showed antioxidant activity. The eluent used was the mixture n-hexane/diethyl ether (9/1: v/v). The free radical inhibition by the essential oil was evaluated using ascorbic acid as positive control. The ascorbic acid used here as control is a compound resulting from chemical synthesis and 99% purified, giving it a real reference for its antioxidant activity [1, 7].

- Identification by GC of the compounds of the antioxidant fraction obtained by preparative TLC

The fraction from the preparative TLC was analyzed by GC (Table 2) for a comparative study of its antioxidant activity compared to the control which is ascorbic acid at 99% purity.

Figure-1. Spectrum of the essential oil of leaves of *Melicope madagascariensis* by GC

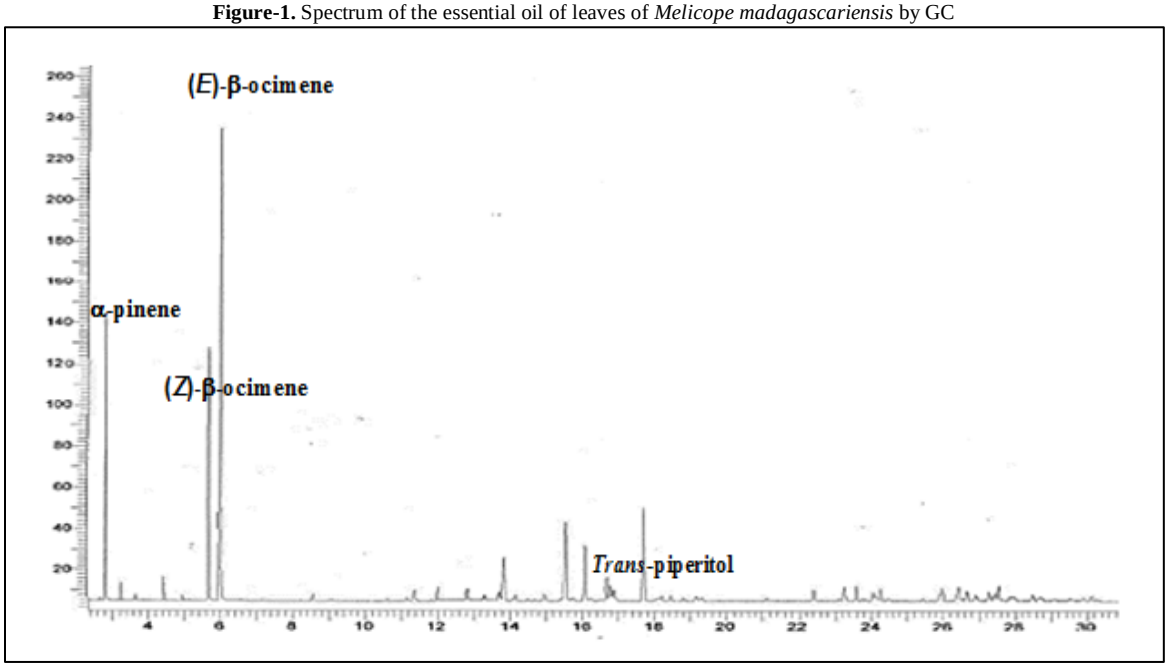


Figure-2. The noticeable presence of yellowish fluorescence (right) on the purple background with ascorbic acid (left)

The table 2 gives Antioxidant activity of *M. madagascariensis* leaves essential oil

Table-2. Antioxidant activity of *M. madagascariensis* leaves essential oil

Concentration (mg/ml)	**Essential oil of *Melicope madagascariensis***		**Ascorbic acid**	
	OD	**P (%)**	**OD**	**P (%)**
120.000	0.151	56.23	0.006	98.26

60.000	0.280	18.84	0.007	97.97
30.000	0.305	11.59	0.008	97.68
15.000	0.318	7.82	0.009	97.39
7.500	0.337	2.31	0.010	97.10
3.750	0.342	0.86	0.011	96.81
1.875			0.012	96.52
0.937			0.014	95.94
0.468			0.022	93.62

(Apparatus used: Bionate Spectrophotometer 3 at 0.324A -517 nm essential oil ThermoFischer)

- Identification by GC-FID of the compounds of the antioxidant fraction obtained by preparative TLC

The fraction from the preparative TLC was analyzed by GC-FID for a comparative study of its antioxidant activity compared to the control which is ascorbic acid at 99% purity. The fractionation by preparative TLC and analysis on GC showed that the crude oil contains anti-free radical-based oxygenated compounds. The percentage of identified molecules is 24.56%.

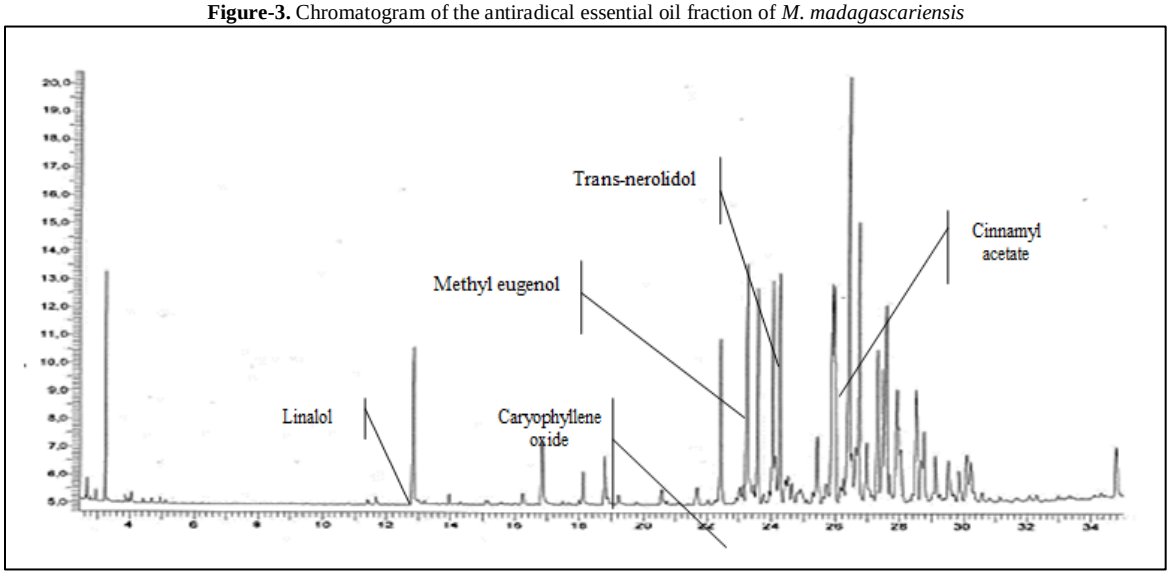
Table 3 gives the chemical composition of the fraction responsible for the antioxidant activity of the essential oil of *M. madagascariensis.*

Table-3. Chemical composition of the fraction responsible for the antioxidant activity of the essential oil of *M. madagascariensis*

N°	Component Name	Rt (min)	RI p	Percentage (%)
1	Camphor	11.662	1000.2	0.18
2	Linalol	12.830	1045.9	2.81
3	Terpinen-4-ol	13.954	1089.9	0.19
4	α-terpineol	16.230	1180.4	0.32
5	Citronellol	17.992	1253.1	0.10
6	Curcumene	18.116	1258.3	0.69
7	Caryophyllene oxide	22.401	1441.6	3.66
8	Methyl eugenol	23.245	1478.7	5.90
9	Cinnamaldehyde	23.459	1488.1	0.53
10	Trans-nerolidol	24.044	1514.8	4.32
11	β-elemol	24.643	1543.3	0.50
13	Cinnamyl acetate	25.900	1603.1	4.79
14	Eugenol	26.244	1620.3	0.57
Total				**24.56**

The figure 3 gives the chromatogram of the antiradical essential oil fraction of *M. madagascariensis.*

Figure-3. Chromatogram of the antiradical essential oil fraction of *M. madagascariensis*



This fraction contains monoterpenic ketones (camphor: 0.18%), monoterpenic alcohols (citronellol: 0.1%), phenylpropanoid (cinnamaldehyde: 0.53%), sesquiterpenol (trans-nerolidol : 4.32%), cinnamyl acetate (4.79%). It has

been noticed that their content have increased and are even more important and majority compared to the initial contents of the essential oil initially considered:

- monoterpenic alcohols: linalol (2.81%),
- sesquiterpenol: terpinen-4-ol (0.19%), α-terpineol (0.32%), β-elemol (0.5%)
- sesquiterpene: curcumene (0.69%), caryophyllene oxide (3.66%),
- phenol-méthyl-ether: methyl eugenol (5.9%).

4. Conclusion

This study focused on the evaluation of chemical composition and the antioxidant activity of *Melicope madagascariensis* essential oil harvested from Madagascar East Coast Rainforest. The results revealed that the essential oil from *M. madagacariensis* species possess antioxidant activity.

The fractionation of this essential oil by preparative TLC and GC-FID analysis revealed the presence of the following molecules: camphor (0.18%), citronellol (0.1%), cinnamaldehyde (0.53%), trans-nerolidol (4.32%), cinnamyl acetate (4.79%), linalool (2.81%), terpinen-4-ol (0.19%), α-terpineol (0.32%), β-elemol (0.5%), curcumene (0.69%), caryophyllene oxide (3.66%), methyl eugenol (5.9%).

The study on *M. madagascariensis* essential oil is herein reported for the first time. Further studies including cytotoxicity assay in order to evaluate the selectivity/therapeutic index of this essential oil are needed before developing it as novel antioxidant drug.

Acknowledgement

The authors are indebted to the Polytechnic High School, the National Center for Environmental Researches, the Botanical and Zoological Park of Tsimbazaza, the University of Gbado-Lite and the University of Kinshasa (Democratic Republic of the Congo) for their technical assistance.

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