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A Comparative Study of Chemical Composition, Physicochemical and Antimicrobial Properties of Essential oils of *Schinus terebinthifolius* Fruits from Analamanga and Ihorombe

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ABSTRACT: The present study aims to compare the chemical composition, the physicochemical and antimicrobial properties of essential oils (EOs) from the fruits of *S. terebinthifolius* collected at Ihorombe and Analamanga. EOs were obtained by distillation using a Clevenger type apparatus and the chemical composition was determined by gas chromatography coupled to mass spectrometry. Physicochemical properties were appraised by using the protocol described in AFNOR standards and antibacterial activities were determined by the disc diffusion and liquid microdilution methods. According to the results, all the studied essential oils have similar physicochemical characteristics and the α -pinene, α -phellandrene, and [limonene + β -phellandrene] were found to be the major constituents. Each essential oil had broad-spectrum antimicrobial activities with a different degree and *B. cereus* ATCC 13061 are the most sensitive among the tested microorganisms. EO from Analamanga showed greater antibacterial potential against this bacterium with a minimum inhibitory concentration (MIC) of 1.087 $\mu\text{g/mL}$.

KEYWORDS: essential oils, comparison, *Schinus terebinthifolius*, fruits.

I. INTRODUCTION

With the emergence of chemoresistant pathogens, resulting from the excessive and inappropriate use of antimicrobial agents, plant extracts are widely used today in both developed and developing countries. Among plant extracts, essential oils have a prominent place [1, 2, 3]. While the chemotypes or chemical races are very common in essential oil plants, it is necessary to know their qualities and international standards before embarking on their exploitation.

Schinus terebinthifolius Raddi belongs to the family Anacardiaceae. This plant which is popularly known as "Pink pepper" was used and introduced to Madagascar, first for ornamental purposes, then for its multiple virtues. The fruits, leaves and other parts of this tree are capable of providing essential oils known especially in the Indian pharmacopoeia for their therapeutic properties. Previous study on pink pepper essential oil shows its antibacterial and antioxidant activities and suggests their potential as a biopreservative in foods [4].

The present study aims to compare the chemical compositions, the physicochemical characteristics and the antimicrobial properties of fruits essential oils of *S. terebinthifolius*. In the present paper, we report the extraction, the

physicochemical characterisation and antimicrobial potent of samples collected from Ihosy (Ihorombe region, south of Madagascar) and Mahabo Andoharanofotsy (region of Analamanga, centre of Madagascar).

II. RELATED WORK

Widespread use of synthetic chemicals has promoted the emergence of antibiotic-resistant pathogens, including multidrug resistant strains [5]. As alternatives, essential oils were mainly investigated by using different methods [1, 4, 5, 6, 7, 8]. Among all, physicochemical property and chemical composition are important because they allow the characterization and identification of an essential oil [1]. In addition, it was reported that essential oils consist mainly of two groups of compounds including terpenes and aromatic compounds [7, 8]. Among these compounds, monoterpenes and sesquiterpenes are the most predominant [8, 9].

Previous chemical analysis of essential oil of *S. terebinthifolius* from Brazil lead to the identification of 22, 13 and 37 compounds in the essential oil from the leaves, the fruits and the twigs, respectively. Among all, germacrene D, caryophyllene, α -pinene, β -pinene and γ -gurjunene are the most predominant compounds. The essential oils of this spice showed antibacterial potential against *E. coli* and *S. aureus* with a MIC value lower than the MIC of sodium nitrite (antimicrobial standard synthetic) [4].

III.METHODOLOGY

III.1. Plant Material: Fruits of *S. terebinthifolius* (Anacardiaceae) were collected at Ihosy (Ihorombe region, south of Madagascar) and Mahabo Andoharanofotsy (region of Analamanga, centre of Madagascar) in May and November 2021, respectively. Vouchers of specimens were presented to the botanists at the Flore Department of the Botanical and Zoological Park of Tsimbazaza (PBZT, Antananarivo) for identification.

III.2. Essential oil extraction: Fresh fruits were washed and air-dried. Air-dried fruits were ground. The essential oils were obtained from the air-dried and powdered fruits (250 g each) by steam distillation during 3 hours using a Clevenger type apparatus. All the essential oils obtained were separately stored in amber bottles away from light until using.

III.3. Physicochemical characterisation: Physicochemical parameters of EO1 and EO2 were determined according to the protocol described by AFNOR (Association Française des Normes) in AFNOR standards NF .T75-111, NF .T75-112, NF T 75 – 113, NF .T 75-103, NF.T75-104 and NF .T75-101 respectively for the relative density, refraction index, rotatory power, acid index, ester index and miscibility with ethanol [10].

III.4. Chromatographic Analysis: The chemical profile of EO1 and EO2 was established using a gas chromatography (GC-14A, Shimadzu, Japan), equipped with a flame ionization detector (FID). N₂ was used as carrier gas with pressure control (3 ml/min). The GC was connected to a CR8. All the essential oil samples were prepared with hexane (GC grade) and the following conditions were applied: Stationary phase (SE30) - Air (auxiliary gas) - Capillary column: BP5 in fused and non-polar silica (30m x 0.32mm x 0.5 μ m) - Oven temperature: 60°C (4.5 mins) to 230°C (3 °C / min) - Detector temperature: 280°C - Injector temperature: 260°C - Volume injected: 1 μ L.

III.5. Antimicrobial activity: Preliminary antimicrobial assays were carried out using the disc diffusion method in the M100 S24 protocol [11]. The following strains were tested: Fungi (*Candida albicans*), Gram-positive bacteria (*Bacillus cereus* ATCC 13061, *Staphylococcus aureus* ATCC 11632 and *Streptococcus pneumonia* ATCC 6301) and Gram-negative bacteria including, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella oxytoca* ATCC8724 and clinical isolates of *Escherichia coli* and *Salmonella enteritidis*. Determination of CMI and CMB were carried out on 96-well microplates according to the M7-A9 protocol described in CLSI (2012) [12]. For this, each test was run in duplicate and decreased concentrations of essential oils were tested. The concentration of the solvents used was maintained at less than 2% so that no inhibition of organisms or interference occurred.

IV.EXPERIMENTAL RESULTS

IV.1. Extraction yields: The yield was 4.88% for the EO1 from Analamanga and 4.48% for the EO2 from Ihorombe. The observed variation of yields which is relatively minor could be related to several factors such as the climate, the geographical area, the degree of freshness, and the drying period and/or due to slightly different experimental conditions of extraction. Indeed, these factors can have a direct impact on yields of essential oil [1, 13].

IV.2. Physicochemical properties: Table-1 shows the physicochemical properties of each analysed essential oil. The relative density of EO1 and EO2 obtained was 0.8698 \pm 0.0002 and 0.9003 \pm 0.0001, respectively. These values are



lower than 1, the density of water. Moreover, since the density is an important criterion for evaluating the quality of an essential oil, it can easily give insight into its naturalness [14].

The refractive indexes of the studied essential oils are also reported in Table-1. Its values which are slightly higher than that of distilled water (1.333) indicate their ability to reflect light [1, 14, 15]. Therefore, EO1 and EO2 could be considered to be of good quality in terms of purity.

Furthermore, EO1 from Analamanga and EO2 from Ihorombe are characterized by very low and similar acid indexes, and very high ester indexes. EO1 had the highest ester index and the acid values obtained in this study which give an idea about the free acid content of these essential oils were low. It may confirm that the essential oil was newly extracted and/or preserved in good conditions. Indeed, this parameter is an important indication of the age, quality, edibility and suitability of essential oils, and a high acid value indicated that the essential oil is becoming rancid [1, 16].

Table 1: Physicochemical and organoleptic properties of the essential oils of *S. terebinthifolius*

Physicochemical property	Essential oils	
	EO1	EO2
Refraction index n_D^{20}	1,4759 ± 0,0002	1,3787 ± 0,0002
relative density d_{20}^{20}	0,8698±0,0002	0,9003 ± 0,0001
Rotatory power α_D^{20}	+1°37±0,017	+1°64 ± 0,018
Acid index IA	0,280 ± 0,019	0,280 ± 0,036
Ester index IE	6,4515 ± 0,6090	5,329 ± 0,608

IV.3. Chemical composition: The chromatographic profiles of EO1 from Analamanga (refer to Figure 1) is largely comparable to EO2 from Ihorombe (refer to Figure 2). It may prove the similarity of the chemotype and confirm the chemotaxonomic link between the plants from which these essential oils were obtained. The corresponding chemical compositions are resumed in Table-2. About 31 chemical compounds are detected in both the EO1 and EO2. Among all, monoterpenes including α -pinene, α -phellandrene, and [limonene + β -phellandrene] are the major chemical constituents. Also, the compound Germacrene-D which is frequently found in trace is present in both EO1 and EO2 with a proportion more than 4%. The compound δ -3-carene which exists in trace amounts in EO2 is present within a high proportion both in the indicative value and EO1. These results are relatively different with those reported previously by Oliveira and collaborators in January 2024 [4]. Variations could be related to several factors such as plant age, maturation period, effect of drying, collection period, temperature, humidity, the sunshine, altitude, nature of the soil, nutrient availability and/or extraction method [17, 18].

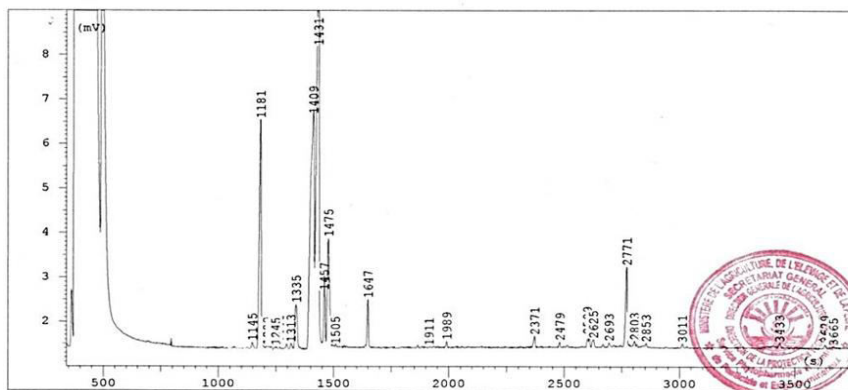


Fig. 1. Chromatogram of the essential oil (EO1) from the fruits of *S. terebinthifolius* collected at Mahabo Andoharanofotsy (Analamanga region) and analyzed by GC / MS.

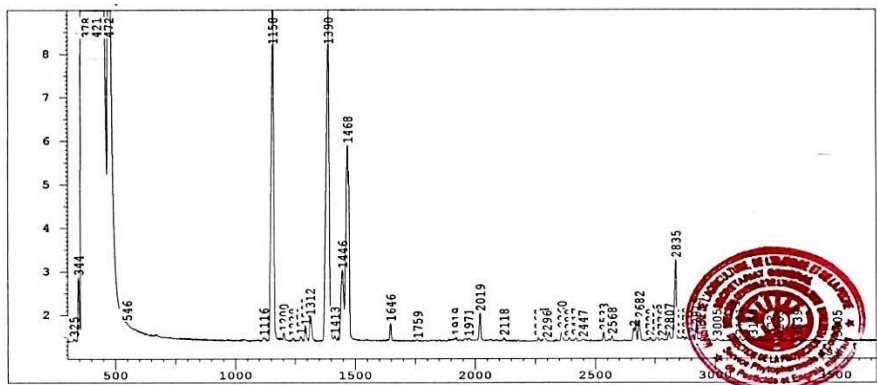


Fig. 2. Chromatogram of the essential oil (EO2) from the fruits of *S. terebinthifolius* collected at Ihosy (Ihorombe region) and analyzed by GC / MS.

Table 2: Comparison of the chemical compositions of *S. terebinthifolius* EO

chemical compounds	Standard	EO1 (in %)	EO2 (in %)
α -thuyene	0,24 - 1,10	0,35	0,33
α -pinene	14,17 - 21,27	12,68	24, 30
Camphene	0,20 - 0,91	0,26	0,39
Sabinene	-	0,35	0,29
β -pinene	4,48 - 8,70	0,31	0,84
Myrcene	1,30 - 3,0	3,40	1,64
α -phellandrene	4,45 - 18,94	27,56	29,91
δ -3-carene	9,32 - 36,10	33,73	-
p-cymene	6,51 - 19,95	3,05	5,83
Limonene + β -phellandrene	9, 31 - 17,32	7,07	18,44
r -terpinene	0,79 - 3,74	-	-
Terpinolene	0,94 - 1,60	1,87	0,78
δ -terpineol	0,31 - 0,67	-	0,15
Terpinene-4-ol	0,25 - 2,32	0,21	0,18
α -terpineol	0,10 - 0,40	-	0,14
Methylchavicol	0,64 - 1,60	0,30	1,33
δ -elemene	-	-	-
α -cubebene	0,21 - 0,41	-	0, 34
α -copaene	-	-	-
β -elemene	-	0,21	0,22
β -caryophyllene	0,11 - 0,78	0,88	0,85
r -muurolene	0,40 - 1,0	0,55	1,12
Germacrene-D	Traces à 1,0	4,11	4,74
β -bisabolene	0,2 à 5,4	-	-
α -muurolene	-	-	-
δ -cadinene	0,30 - 1,02	-	-
α -cadinene	0,43 - 1,13	0,15	0,85
Elemol	-	-	-
Elemicine	1,0 - 1,9	-	0,55
Nérolidol	-	-	-
Spathulenol	0,2 - 1,10	-	-

Standard: Direction de la Protection des Végétaux, Minister de l’Agriculture, de l’Elevage et de la pêche, Madagascar ; EO1: essential oil from Analamanga region; EO2: essential oil from Ihorombe region

IV.4. Microbiological studies:

The results of the disk diffusion assay are reported in Table-3. It show that the essential oils from *S. terebinthifolius* fruits exhibit broad-spectrum antimicrobial activities. The essential oil from Antananarivo EO1 displays the highest inhibition and the *B. cereus* strain is the most susceptible microorganisms, among all. Indeed, the diameter of the



inhibition zone is indicative of activity to the tested agent. Also, the results obtained with *E. coli* and *S. aureus* are in agreement with those previously reported [4].

Moreover, MICs and MBCs which were found during the liquid microdilution tests are presented in Table-4 and -5. These results indicate that activities of all the EO are better than that of referred antibiotics except for Érythromycine and Doxycycline. Also, it indicated that the tested *B. cereus* strain are trimethoprim resistant (MIC > 24 µg/mL). Among all the tested essential oils, EO1 and EO2 had the lowest MIC (1.087 µg/mL) and MBC (2.250 µg/mL), respectively. A lower MBC and MIC means that a minimum amount of the essential oil was needed to kill and to inhibit the growth of the tested microorganism [19]. Ranked in order of antibacterial activity, EO1 from Analamanga had the most potent antibacterial activity against the trimethoprim resistant *B. cereus*. We can then say that the essential oil of *S. terebinthifolius* has very interesting antimicrobial activity. Since terpenes are the major chemical constituents of these two essential oils, inhibition of the tested microbial strains may be due to the presence of these compounds. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption.

Table 3: Antimicrobial activity of essential oils (EO) from *S. terebinthifolius*

Tested strains		Inhibition zones diameters (in mm)	
		EO2 from Ihorombe	EO1 from Analamanga
Gram - positive bacteria	<i>B. cereus</i>	18,0 ^{***}	32 ^{***}
	<i>S. aureus</i>	11,0 ^{**}	17 ^{**}
	<i>S. pneumoniae</i>	11,0 ^{**}	15 ^{**}
Gram - negative bacteria	<i>K. oxytoca</i>	12,0 ^{**}	13 ^{**}
	<i>S. enteritidis</i>	11 ^{**}	15 ^{**}
	<i>E. coli</i>	10,5 ^{**}	16 ^{**}
	<i>P. aeruginosa</i>	07,5 [*]	11 ^{**}
Fungi	<i>C. albicans</i>	13 ^{**}	21 ^{***}

Extremely sensible^{***}, very sensible^{**}, sensible^{*}

Table 4 : Minimum Inhibitory Concentrations (MIC, µg/mL) for the *B. cereus* strain

Antibiotics	MIC endpoints (µg/mL)	<i>B. cereus</i> ATCC 14579	Interpretations
Amoxicilline	N. D.	> 24	N. D.
Aztreonam	N. D.	> 24	N. D.
Cephotaxime	S ≤ 8; I 16-32; R ≥ 64	> 12	I
Doxycycline	N. D.	< 0,37	N. D.
Érythromycine	S ≤ 0,5; I 1-4; R ≥ 64	< 0,37	S
Gentamicine	S ≤ 4; I 8; R ≥ 16	1,5	S
Acide nalidixique	N. D.	6	N. D.
Trimethoprime	R ≥ 4	> 24	R
Vancomycine	S ≤ 4	1,5	S

S = sensible; I = moderately sensible; R = resistant; N.A. = data not available.

Table 5 : MIC and CMB of EOs of *S. terebinthifolius* against *B. cereus*

Samples	MIC (in µg/mL)	MBC (in µg/mL)
EO2	1,125	2, 250
EO1	1,087	4,349

V. CONCLUSION

The present study has permitted the comparison of physicochemical profiles, chemical compositions and antimicrobial effects of essential oils of *S. terebinthifolius* fruits which were collected from the central EO1 and southern regions EO2 of Madagascar. Very similar physicochemical profiles are obtained for all these studied essential oils. Also, the chromatographic analysis GC-MS permits to identify 31 chemical compounds in both EO1 and EO2, mainly α-pinene, α-phellandrene, and [limonene + β- phellandrene]. Moreover, all these essential oils had broad-spectrum antimicrobial activities with a different degree, related to its origin. EO1 from Analamanga had the most

potent antibacterial activity against the tested *B. cereus* which is the most sensitive bacterium. The presence of terpenes could explain the antimicrobial activities of these essential oils.

In the future, more advanced analytical methods will be considered to analyze the essential oil and these results could contribute to the establishment of essential oil standards in Madagascar.

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