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Influence of Chemical Composition on the Antioxidant Activity and Toxicity of Essential Oils of *Cymbopogon nardus* (l.) Rendle and *Eucalyptus camaldulensis* dehnh Acclimatized in Benin

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Abstract Essential oils (EO) of aromatic plants have always been used by humans for healing, perfume or food. But an oil using does not always give desired results. Faced with this difference in results, it would therefore be advisable to control the chemical composition in order to optimize the biological activity of each EO. This work is interested in the study of influence of the chemical composition on the antioxidant activity and the toxicity of EO of two plants cultivated in Benin. EO of fresh leaves of *Cymbopogon nardus (L.) Rendle* (C.n) and of *Eucalyptus camaldulensis Dehnh* (E.c), collected around 7 AM, 1 PM and 7 PM, are obtained by hydrodiffusion. The chemical composition evaluated by GC/FID and GC/MS varies qualitatively and quantitatively depending on the time of harvest. The main compounds identified are monoterpenes and sesquiterpenes which the rate varies. The antioxidant activity of EO tested by DPPH method also varies. The antioxidant activity of C.n EO is interesting at 1 PM (EC_{50} = 0.97 mg/mL) and weak at 7 AM (EC_{50} = 1.62 mg/mL). In contrast, that of E.c is better at 7 PM (EC_{50} = 6.23 mg/mL) and weak at 1 PM (EC_{50} = 42.64 mg/mL). This variation in oil activity could be explained not only by the variation in chemical composition but also by the presence of certain compounds recognized as antioxidants. Our oil samples tested on *Artemia salina Leach* larvae are less toxic (LC_{50} > 26 µg/mL) than camptothecin (LC_{50} = 13.27 µg/mL), a reference compound.

Keywords: *Cymbopogon nardus (L.) Rendle*; *Eucalyptus camaldulensis Dehnh*; essential oil; antioxidant activity; toxicity

1. Introduction

For thousands of years, humans have drawn from their environment the knowledge necessary for their survival and well-being. He passes on the experience of medicine and all types of natural remedies to develop traditional medicine. Diseases are sometimes created by the overproduction of free radicals [1] in the body [2]. Antioxidant compounds, in addition to their use as preservatives in food [3], they are also involved in the treatment of many diseases [2, 4, 5]. Among other things, aromatic plants, being natural sources of active ingredients, are essential in traditional medicine. Several studies have demonstrated the different biological activities of aromatic plants [6-8]. Thus by these properties, the essential oils (EO) of these plants could therefore be used for various purposes. EO are gaining ground and are used in many fields: cosmetics, food, well-being and of course health. Although used in many areas, essential oils are far from safe. To do this, their use involves mastering their properties and their dangers, all of which is linked to the variation in their chemical composition.

Cymbopogon nardus (L.) Rendle (C.n) and *Eucalyptus camaldulensis* Dehnh (E.c) two aromatic plants adopted in Africa and belong respectively to the Poaceae family and to the Myrtaceae family. The EO extracted from these plants have many pharmacological properties. The essential oil of E.c exhibits antimicrobial, acaricide, insecticide and herbicide properties [9]. It also has antifungal [10] and antioxidant [11] properties. As for C.n, its essential oil has ovicidal and larvicidal activities [12]. Its antimicrobial, analgesic [13], antitrypanosomal and antiplasmodial [14] properties are also known.

In addition to the various properties, the chemical composition of the oils of these plants are studied [10, 11]. But the study of the variation of antioxidant activity and toxicity in relation to the composition of essential oils remains to be elucidated, hence the interest of this work.

2. Materials and methods

2.1-Materials

2.1.1- Plant material

Fresh leaves of *Cymbopogon nardus* (L.) Rendle (C.n) and *Eucalyptus camaldulensis* Dehnh (E.c) constitute the plant material. These leaves were harvested in March 2014 at around 7 AM, 1 PM and 7 PM in the botanical garden of the University of Abomey-Calavi (Benin).

2.1.2- Chemicals and drugs

In the context of this work, the reagents used are such as: 2,2-Diphenyl-1-picryl-hydrazyl (DPPH), (S) - (+) - camptothecin, ascorbic acid, methanol and those previously described by Allanto et *al*. [15]

2.1.3- Biological material

The toxicity tests are carried out on brine shrimp larvae (Artemia salina Leach) obtained after hatching from the eggs.

2.2-Methods

2.2.1- Extraction of essential oils

The extraction was carried out by hydrodiffusion with an improved Clevenger type apparatus [16], generally lasting three hours, for each harvest batch and repeated three times. The essential oil, after decanting and drying over sodium sulfate, is collected in a flask and then stored at 4°C in a cold room for analyzes.

2.2.2-Chemical analysis of essential oils

The analysis of essential oils was carried out with the GC/FID and the GC/MS coupling. The analysis is carried out on a FOCUS GC equipped with an HP 5MS capillary column of dimensions 30 mx 0.25 mm with 0, 25 µm film thickness. In order to confirm the specificity and selectivity of the GC method, GC-EIMS analyzes were performed on a TRACE GC 2000 series, as described by Kpoviessi et *al*. [14].

2.2.3-Identification of constituents

By comparison of the spectral data and the retention indices with those of the bibliographical references, the constituents of the various essential oils are identified as described by Kpoviessi et *al*. [14].

2.2.4-Antioxidant test

The DPPH method was adopted as described by Brand Williams et *al*. [17] and Kpadonou et *al*.[18].

2.2.5-Toxicity test

The larval toxicity test was carried out according to the method of Michael et *al*.[19] and described by Kpadonou et *al*.[18]

3. Results and Discussion

3.1-Chemical composition of essential oils of Eucalyptus camadulensus and Cymbopogon nardus

The chemical composition of the essential oils of *Eucalyptus camadulensus* and *Cymbopogon nardus* harvested at different times of the day is determined by GC / FID and GC/MS. The compounds having a percentage greater than or equal to 1%, ten and thirteen in number respectively for C.n and E.c are given in Table 1.

Table 1: Majority compounds (% ≥ 1) of essential oils of *Eucalyptus camadulensus* and *Cymbopogon nardus*

[a]Compound	KI	*Eucalyptus camadulensus*			*Cymbopogon nardus*		
		7 h	**13 h**	**19 h**	**7 h**	**13 h**	**19 h**
α-pinene*h	939	1.4±0.04	1.1±0.03	0.9±0.01	-	-	-
α-phellandrene*h	1017	2.4±0.04	0.4±0.04		-	-	-
o-cymene*h	1023	22.7±0.01	21.2±0.04	23.4±0.03	-	-	-
limonene*h	1028	-	-	-	2.2±0.04	2.4±0.04	2.4±0.03
1,8-cineole*o	1033	12.5±0.04	14.3±0.03	14.8±0.01	-	-	-
terpinolene*h	1055	44.5±0.03	35.6±0.05	41.9±0.03	-	-	-
(+)-4-carene*h	1128	2±0.02	2.3±0.02	1.2±0.00	-	-	-
terpinen-4-ol*o	1177	9.6±0.03	14.9±0.01	12.3±0.04	-	-	-
(+)-α-terpineol *o	1189	0.6±0.04	1.4±0.03	0.8±0.01	-	-	-
β-citranollal*o	1192	-	-	-	35.6±0.04	36.6±0.02	33.3±0.03
β-citronellol*o	1244	-	-	-	11.7±0.01	11.6±0.02	11.4±0.04
nerol*o	1294	-	-	-	25.1±0.02	25.1±0.00	24.5±0.01
carvacrol*o	1298	0.3±0.03	1.4±0.01	0.6±0.00			
geranyl acetate *o	1344	-	-	-	1.1±0.02	1.1±0.02	1.6±0.00
β-elemeneh**	1353	-	-	-	1.9±0.05	2.4±0.03	2.8±0.03
germacrene-Dh**	1477	-	-	-	1.3±0.05	1.6±0.01	2±0.02
δ-cadineneh**	1523	-	-	-	1.1±0.02	1.4±0.00	1.8±0.03
elemolh**	1556	-	-	-	9±0.04	7.5±0.04	8.2±0.02
cubenolo**	1579	-	-	-	2.0±0.02	1.3±0.04	1.3±0.03
globulolo**	1583	0.2±0.04	0.9±0.02	0.7±0.03			
τ-cadinolo**	1639	-	-	-	1.0±0.02	0.9±0.04	1.2±0.03
α-cadinolo**	1650	-	-	-	2.0±0.00	2.0±0.01	2.5±0.02
(Z,E)-farnesolo**	1699	-	-	-	0.4±0.04	0.2±0.04	0.1±0.05
TOTAL		96.2±0.32	93.5±0,28	96.6±0,16	94.4±0.37	94.1±0.31	93.1±0.34

[a]Compounds listed in order of elution from HP-5 MS column; b: Kovats indices (KI) on HP-5 MS column;
*monoterpenes;**sesquiterpenes; ***non terpenes; h hydrocarbons; o oxygenated; (-): absence or not detected**.**

The major compounds identified in oils are only monoterpenes and sesquiterpenes (Figure 1). Their rate varies depending on when the plant is harvested during the day. The monoterpenes constitute the majority chemical group and the sesquiterpenes the minority.

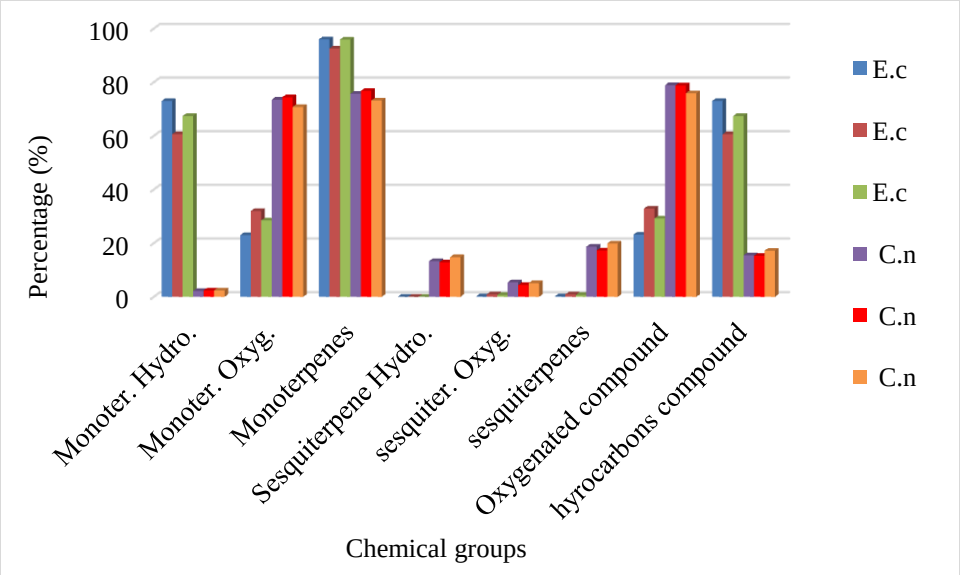

Figure 1: Variation in the rate of chemical groups of essential oils of Eucalyptus camadulensus and Cymbopogon nardus

E.c oils contain more hydrocarbon compound (60.6% -73%) than oxygenates (23.2% - 32.9%). This is not the case for those of C.n. They contain more oxygenates (75.9% - 78.9%) than hydrocarbons (15.3% -17.2%). In E.c oil, the oxygenated monoterpenes at a higher rate in the morning drop around 1 PM before increasing around 7 PM For hydrocarbons, their rate increases from 7 AM to 1 PM before taking the lowest value of the day at 7 PM. According to the statistical analysis of Student's t-test, all of its values are significantly different from each other ($p < 0.05$). These two subgroups, oxygenated and hydrocarbon-based monoterpenes, compensate for each other, thus maintaining the level of monoterpenes during the day. Sesquiterpenes showing only their oxygen subgroup have their maximum level at 1 PM and the minimum at 7 AM. These results show a significant difference according to the Student *t-test* ($p < 0.05$) (Figure 1).

The hydrocarbon monoterpenes in C.n oils have their rate which varies very little during the day. According to statistical analysis, the values of 1 PM and 7 PM are not statistically different from each other but are with the value of 7 AM ($p < 0.05$). So the rate of hydrocarbon monoterpenes increases from 7 AM to 1 PM and becomes constant until 7 PM. This is not the case for oxygenates. From 7 AM to 1 PM, their rate increases and decreases, taking the lowest rate of the day around 7 PM (Figure 1). All of these values show significant differences between them according to the Student *t-test*. The level of monoterpenes is preserved during the day by compensation of their two hydrocarbon and oxygen subgroups.

The major compounds in oils can be grouped into two groups depending on how their levels change during the day. For E.c oils, the group of compounds reaching their maximum level at 1 PM such as globulol, carvacrol, (+) - α-terpineol, (+) - 4-carene and terpinen-4-ol and that of compounds which reach their minimum at 1 PM as 1,8-cineole and terpinolene classified in order of increasing rate. In C.n oils, classified in order of their rate, geranyl acetate, cadinene, germacrene-D, α-cadinol, elemene and limonene constitute the group of compound which grow during the day, and (Z, E) -faresol, cubenol, citronellol and nerol the group of those which decreasing (Table 1). The variation of the rate of these compounds could be explained by the interconversion catalyzed by light during the day [21].

Several scientific works have studied the chemical composition of these essential oils. Citronellal, composed of more than 35% of C.n oil, is also obtained as the major compound by Kpoviessi et *al*. [14], Abéna et *al*. [13] and Nyamador et *al*. [11] but at different percentages. On the other hand, for the oil of E.c, the terpinolene obtained as the majority compound (44%) is not that obtained by Nait et *al*. [22] and Haouel et *al*. [23]. So the chemical composition of oils varies not only depending on the time of harvest but also depending on where the plant is harvested.

3.2-Antioxidant activity of essential oils

In order to justify the use of the leaves in traditional medicine, the antioxidant test of essential oils was carried out by the method of DPPH. The results of the antioxidant activity and the antiradicalire power, expressed respectively in terms of EC_{50} (the Concentration of Extract reducing 50% of DPPH) and AP (Anti-radical power), are given in table 2. The more EC_{50} is weak the more the activity is interesting. The analysis of Table 2 reveals a variation in the antioxidant activity and the anti-radical power of the oils depending on the time of harvest during the day.

Lower in the morning at 7 AM (EC_{50} = 1.62 mg/mL and PA = $61.73*10^{-2}$), the antioxidant activity of Cn increases at 1 PM (EC50 = 0.97 mg/mL and PA = 1.03) before dropping slightly around 7 PM (EC_{50} = 1.26 mg/mL and PA = $79.36 * 10^{-2}$) (Figure 2 Table 2). Statistical analysis shows a significant difference between these values obtained during the day. Antioxidant activity is therefore more interesting at 1 PM when the sun is at its zenith. For E.c, the essential oil has the lowest activity of the day at 1 PM (EC_{50} = 42.64 mg/mL and PA = $2.34 * 10^{-2}$). The 7 AM (EC_{50} = 9.51 mg/mL and PA = $10.52 * 10^{-2}$) and 7 PM (EC_{50} = 6.23 mg/mL and PA = $16.05 * 10^{-2}$) activities are most interesting (Figure 2 table 2). A significant difference is seen from statistical analysis. The best activity during the day is obtained at 7 PM. This activity is lower than that obtained by Bayala et al [24] (% INH = 43.4 ± 4.13 for a concentration of 8 mg/mL which corresponds to EC_{50} = 9.21 mg/mL and PA = 10.86 by deduction). The best activity of E.c oil obtained at 7 PM is lower than that of C.n. So the oil of C.n is more antioxidant than that of E.c. The presence of monoterpenes, the major constituents of oils, and known for their antioxidant power, could justify the activity of oils [25-27].

Terpinolene, the majority compound representing more than 35% of E.c oil, is recognized for its anti-stress properties [28]. Its rate decreases from 7 AM (45.5%) to 1 PM (35.6) before increasing around 7 PM (41.9%) which is close to the rate of the antioxidant activity of oil during the day. The rate of terpinolene could then explain the evolution of antioxidant activity. Likewise, in C.n oil, citronellal, the major compound, peaks at 1 PM as the oil's antioxidant activity. This compound, being an oxygenated monoterpene, is known for its antioxidant capacity [29]. The appearance of the percentage of this compound during the day could therefore explain the evolution of the antioxidant activity of the oil. The antioxidant activity of oils is also explained by the presence of compounds such as α-pinene, α-terpineol, terpinen-4-ol, limonene, germacrene-D (Table 1) having an antioxidant capacity and acting in synergy [30 -32]. Compared to ascorbic acid (EC_{50} = 0.02 mg/mL) price as a reference compound, our oil samples exhibit lower antioxidant activity. The essential oils of E.c and C.n would therefore constitute a non-negligible source of treatment due to their reducing power of free radicals.

Table 2: Antioxidant activity and antioxidant power

Samples		**Daytime**	**Inhibitory concentration (EC_{50}) mg/mL)**	**Antioxidant power (PA = 1 / EC_{50})**
Plants	C. n	7 AM	1.62 ± 0.01^{d}	$61,73* 10^{-2}$
		1 PM	0.97 ± 0.01^{b}	1,03
		7 PM	$1.26\pm0 .01^{c}$	$79,36* 10^{-2}$
	E. c	7 AM	9.51 ± 0.05^{e}	$10,52* 10^{-2}$
		1 PM	42.64 ± 0.05^{f}	$2.34* 10^{-2}$
		7 PMh	6.23 ± 0.05^{d}	$16.05* 10^{-2}$
Positive Control	ascorbic Acide		0.02 ± 0.00^{a}	50

The values of the same column with different letters ($^{a.b.c}$) are statistically different by Student's t-test ($p < 0.05$)

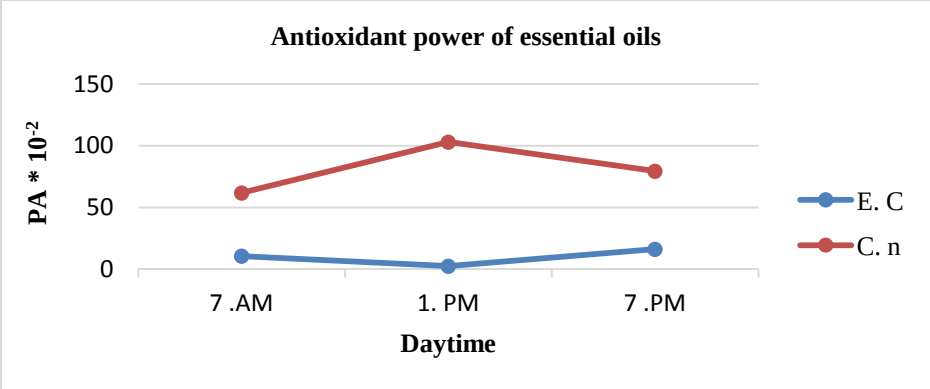

Figure 2: Evolution of the antioxidant power of essential oils during the day

3.3-Oil toxicity

The in vitro toxicity test carried out on shrimp larvae. The results expressed as LC_{50} (concentration from which at least 50% of the larvae contained in the reaction medium are killed) are given in Table 3. Analysis of the table. it appears that the larval toxicity of oils varies from plant to plant and depending on when the plant is harvested during the day.

The higher the LC_{50} value, the less toxic the compound. The toxicity of Ec oils decreases from 7 AM (LC_{50} = 8575.63 µg/mL) to 7 PM (LC_{50} = 6589.72 µg/mL through 1 PM (LC_{50} = 7273.25 µg/mL) (Figure 3). Statistical analysis shows a significant difference between its three values. On the other hand for C.n oils the toxicity decreases from 7 hours (LC_{50} = 27.495 µg/mL) to 1 PM (LC_{50} = 110.902 µg/mL) before increasing around 19 hours (LC_{50} = 26.39µg/mL). According to the Student t-test, the values of 7 AM and 7 PM are not significantly different from each other, but they are compared to that of 1 PM. Therefore the toxicity is practically the same at 7 AM and at 7 pm E.c oil (LC_{50} >6500 µg/mL) is less toxic than C.n oil (26.39 <LC_{50} <110.9 µg/mL). But they are less toxic than camptothecin, reference compound (LC50 = 13.27µg/mL). In order to confirm the toxicity or not of these essential oils, it would be important to carry out in vivo *t-tests*, of these oils. Only, it will be necessary to respect the dosage limits in its administration.

Table 3: Toxic power of oils

Samples		**Daytime**	**Toxicity (CL_{50}.µg/mL)**
Plants	C. n	7 AM	27.495
		1 PM	110.902
		7 PM	26.39
	E. c	7 AM	8575.63
		1 PM	7273.25
		7 PM	6589.72
Positive control	Camptothecin		13.27±0.02

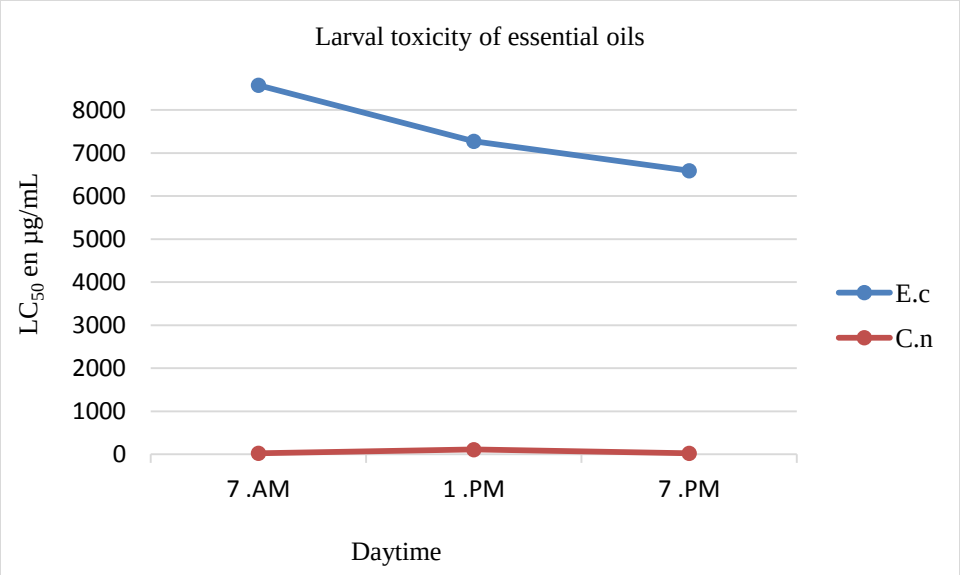

Figure 3: Evolution of the toxicity of EOs during the day

4. Conclusion

The essential oils of *Cymbopogon nardus* (L.) Rendle and *Eucalyptus camaldulensis* Dehnh, two plants cultivated in Benin, have a chemical composition that varies depending on when the plant is harvested during the day. These oils consist primarily in increasing order of levels, sesquiterpenes and monoterpenes. This variation could be justified by the various environmental factors influenced by sunlight. Likewise, the antioxidant activity of essential oils varies depending on when the plant is harvested during the day. Cymbopogon nardus oil is more antioxidant with the 1 PM sample and that of Eucalyptus camaldulensus with the 7 PM sample. Like antioxidant activity, the toxicity of oils also varies. It is weaker than that of camptothecin, a reference compound. This confirms the use of these plants in traditional medicine in Benin. In vivo studies could provide more appropriate answers to questions concerning the mechanisms of toxicity.

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