

# Chemical Composition and Antioxidant Activity of the Essential Oil of *Chromolaena Odorata* Harvested in the Region of the Mountain District of Côte D'ivoire

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## ABSTRACT

The aromatic plants have occupied an important place in the daily life of man. The excessive and repetitive use of drugs has led the body to develop resistance to their curative effects. Many authors have focused on the study of the therapeutic properties of essential oils in order to provide new remedies. They are used as a source of bioactive molecules of natural origin.

The objective of this work is to contribute to the valorization of medicinal and aromatic plants of the Ivorian flora. We propose to determine the chemical composition and to evaluate the antioxidant activity by spectrophotometry of the essential oil. The plant material consists of the leafy twigs of *Chromolaena odorata*. The technical of steam distillation using a four-compartment stainless steel device was used to extract the essential oil from the plant matrix.

The analysis of the essential oils was carried out on a GC chromatograph (7890A, Agilent Technologies) coupled to a mass spectrometer (5975C, Agilent Technologies). The antioxidant potential of the extracts was evaluated using the Blois method. The essential oil obtained by steaming, with an aromatic odor and pale green color has a yield of (0.082 ± 0.004)%.

Analysis of the chromatogram and mass spectra identified 24 phytocompounds (99.92%). The phytochemical composition is dominated by hydrocarbon sesquiterpenes (44.21%) followed by hydrocarbon monoterpenes (29.48%) and other compounds (26.23%). The major compound is  $\alpha$ -pinene (17.79%), 6-propen-1-enylbicyclo [3.1.0] and hexan-2-one (14.95%).

The essential oil extract of *C. odorata* exhibits low antioxidant activity compared to vitamin C.

**Keywords:** Antioxidant activity, *Chromolaena odorata*, Essential oil, Ivory Coast.

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## INTRODUCTION

Different aromatic plants are characterized by the biosynthesis of odorous and volatile molecules which constitute what are called essential oils.<sup>1</sup> They have, all the times occupied an important place in the daily life of man since he uses them to flavor dishes, to perfume and to heal himself.<sup>2</sup> The excessive and repetitive use of drugs has led the body to develop resistance to their curative effects. These remedies like antibiotics and antioxidants have shown their limits. Many authors have focused on the study of the therapeutic properties of essential oils in order to provide new remedies. They are used as a source of bioactive molecules of natural origin enjoying biological activities, including antimicrobial and antioxidant activities.<sup>3</sup> Thus the natural antioxidants of plants are interesting and attractive. Much effort and research is directed towards natural antioxidant agents for their effects on oxidative stress linked to certain diseases.<sup>4</sup> Antioxidant protection against damage due to oxides in the immune system mechanism plays a crucial role in the prevention or/and progression of certain chronic pathologies.<sup>5</sup> For this interest, essential oils considered to be bioactive natural substances occupy a good choice in the discovery of new therapeutic molecules, and attract the interest of several studies in the view of the number of their countable biological properties. They

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are being studied for their use as an alternative to synthetic products in the treatment of infectious diseases and in various pathologies associated with oxidative stress.<sup>3</sup>

Therefore, as part of a contribution to the enhancement of aromatic plants and essential oils from the Ivorian soil, we are interested in determining the chemical composition and evaluating the antioxidant activity of EO from *Chromolaena*

*odorata* collected in the Mountain District region. The species are native to Central America. It is a semi-lignified perennial herbaceous plant, very fragrant, diffuse and fast-growing perennial that can reach 3 to 3.5 m in height.<sup>6</sup> *C. odorata* is widely used in traditional medicine. The fresh leaves and extracts of *C. odorata* are used in some developing countries for traditional treatment of mild burns, tissue wounds and skin infections.<sup>7</sup> In the Central African Republic, a hot infusion of the leaves promotes rapid healing of wounds.<sup>8</sup> In Côte d'Ivoire in the southern Comoé region more precisely in Adiaké, it is used to stop bleeding during an injury.<sup>9</sup> The results, from studies carried out in India, show that the aqueous and ethanolic extracts of the leaves are endowed with antioxidant power.<sup>10</sup> The results of studies carried out in Thailand show that the extracts of the leaves of the species could be indicated to treat bacterial infections of the skin.<sup>11</sup> Oils of *C. odorata* can inhibit the lipoxygenase L1 of soybean, model of the human lipoxygenase 5LO.<sup>12</sup> The antioxidant and antibacterial properties of essential oils of *C. odorata* have been demonstrated by several studies.<sup>13,14</sup> Work on the chemical composition has been carried out. Daouda T. work on EOs shows that the chemical composition is dominated by hydrocarbon sesquiterpenes (74.06%), the major constituents are E-caryophyllene (31.75%) and germacrene D (22.57%).<sup>15</sup> The predominant compounds in the studies conducted by Pamo and al in Cameroon are bicyclogermacrene (12.55%), Geigerene (11.85%) and  $\alpha$ -pinene (9.36%).<sup>16</sup> In Togo,  $\alpha$ -pinene (15.96%),  $\beta$ -pinene (8.06%), geijerene (19.44%), and germacrene-D (14.03%) are the major compounds in the work of Koumaglo KH.<sup>6</sup> In Nigeria, work by Moses indicates that the composition of essential oil (EO) extracted by hydrodistillation from the dry leaves of the species is dominated by hydrocarbonated monoterpenes (55.2%). The majority compounds are  $\alpha$ -pinene (42.2%),  $\beta$ -pinene (10.6%) germacrene D (10.6%) and  $\beta$ -copaen-4-ol 9.4%.<sup>14</sup>

This is why, in this work in order to contribute to the valorization of medicinal and aromatic plants of the Ivorian flora, we propose to determine the chemical composition and to evaluate the antioxidant activity by spectrophotometry of the essential oil of *C. odorata*.

## MATERIALS AND METHODS

### Plant Material

The plant material consists of the leafy twigs of *C. odorata*. The harvest was carried out on 12/10/2020 on the side of a hill in the Domoraud neighborhood in Man in the Mountain District region. The plant was identified and authenticated by a technician at the National Floristic Center (CNF) of Abidjan (Ivory Coast) using the existing herbarium (N<sup>o</sup>H UCJ 003622).

### Methods

#### Extraction of Essential Oil

The technique of steam distillation using a four-compartment stainless steel device was used to extract the essential oil

from the plant matrix (MV). The boiler (60 L capacity) is connected to a large tank by a stainless-steel pipe. The large tank (height: 100 cm, internal diameter: 51 cm or a volume of 0.2 m<sup>3</sup>) contains four grids attached to a removable rod. On the grids, the aerial part (7.5 kg) of withered plant material was placed (extraction 24 hours after harvest). From this tank, the water vapor drives from the volatile compounds into a third tank (height: 100 cm, internal diameter: 41 cm, i.e., a volume of 0.13 m<sup>3</sup>) which serves as a refrigerant. The essential oils (EOs) are obtained in a fourth compartment serving as a recovery system. The essential oil is put in pill boxes wrapped in aluminum foil and then stored in a freezer at around 4°C.<sup>17</sup>

#### Determination of the Phytochemical Composition

The analysis of the EO diluted in dichloromethane (1:100) was carried out on a GC chromatograph (7890A, Agilent Technologies) coupled to a mass spectrometer (5975C, Agilent Technologies). A sample of HE (1- $\mu$ L) was injected into an HP-5MS capillary column at 250°C. The oven temperature was programmed at 40°C for 5 min, then at 2°C/min for 15 min up to 250°C, with a flow rate of 10°C/min up to 300°C. Helium was used as carrier gas with a flow rate of 1-mL/min. The MS detector had a temperature of 280°C and a voltage of 1.4 kV. The ions with a mass / charge ratio of between 40 and 500 were detectable. The identification of the compounds was carried out by comparison of the retention indices, calculated from retention times and mass spectra obtained with those from the National Institute of Standards and Technology (NIST) database and from the literature.<sup>18</sup>

$$RI = 100 \left[ n + \frac{T_R(C_i) - T_R(C_n)}{T_R(C_{n+1}) - T_R(C_n)} \right]$$

RI: retention index or KI: Kovats index

Ci: Compound unknown to EO; Cn: linear alkane whose retention time is just before that of the unknown compound of the EO; n: carbon number of the linear alkane; C<sub>n+1</sub>: linear alkane whose retention time is just after that of the unknown compound; T<sub>R</sub> (C<sub>n</sub>): Retention time of linear alkane with n carbon atoms eluted before the unknown compound; T<sub>R</sub> (C<sub>n+1</sub>): Retention time of the linear alkane with n + 1 carbon atoms eluted after the unknown compound.

#### Evaluation of antioxidant activity

The antioxidant potential of the extracts was evaluated using the Blois method.

The DPPH is dissolved in absolute ethanol to obtain a solution of 0.3 mM molar concentration. The solutions to be tested: are diluted in absolute ethanol in order to have the following concentrations in  $\mu$ g/mL: 2.5; 5; 10; 20; 50; 125; 250 and 500.

2.5 mL of test solution are introduced into dry and sterile hemolysis tubes and 1-mL of ethanolic solution of DPPH is added. After shaking, the tubes are placed in the dark for 30 min, protected from light. For each solution to be tested, a blank is prepared consisting of 2.5 mL of pure absolute ethanol supplemented with 1-mL of ethanolic solution of DPPH. For each solution to be tested, a blank is prepared

consisting of 2.5 mL of pure absolute ethanol supplemented with 1-mL of ethanolic solution of DPPH.

For the negative control, a solution of DPPH is prepared by diluting 1-mL of the ethanolic solution of DPPH in 2.5 mL of ethanol. For the positive control, a solution of vitamin C (ascorbic acid) is used, the absorbance of which is measured under the same conditions. The measurement of the residual absorbance is carried out at 517 nm. It is translated into percentage inhibition by the following formula.<sup>19</sup>

$$\%I = \left( 1 - \frac{\text{Abs test}}{\text{Abs DPPH}} \right) \times 100$$

%I: Percentage inhibition. Abs test: Absorbance of ethanolic solution of essential oil and DPPH., AbsDPPH: absorbance of blank (ethanolic solution of DPPH).

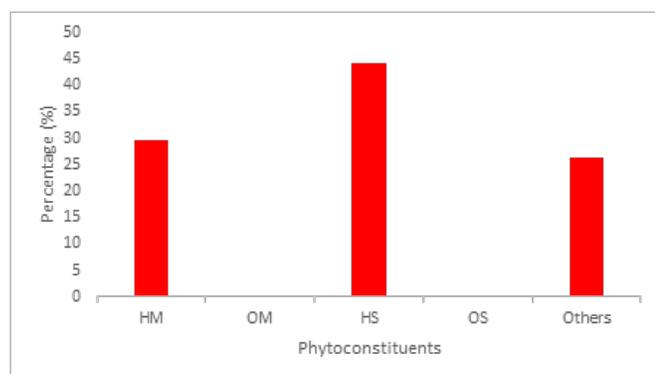
## RESULTS AND DISCUSSION

### Extraction Results

The essential oil obtained by steaming, with an aromatic odor and pale green color has a yield of  $(0.082 \pm 0.004)$  %. Its density is approximately  $0.82 \pm 0.04$ . This yield is appreciably equal to that of the work of the authors Koumaglo and collaborators which is 0.08%.<sup>6</sup> This similarity could be because the extracts are collected in a mountainous region, in Man as in Togo. But according to other authors the yield could depend on environmental conditions, the harvest period, and the technique of extraction.<sup>20,21</sup>

### Phytochemical Composition of Essential Oil

Analysis of the chromatogram and mass spectra identified 24 phytochemicals (99.92%) (Table I). The phytochemical composition (Figure 1) is dominated by hydrocarbon sesquiterpenes (44.21%) followed by hydrocarbon monoterpenes (29.48%) and other compounds (26.23%). We note that oxygenated monoterpenes and sesquiterpenes are absent in our EO sample. The major constituents, six in number, are: germacrene-D (21.82%) (A) thereafter, there is  $\alpha$ -pinene (17.79%) (B), 6-propen-1-enylbicyclo [3.1.0] hexan-2-one (14.95%) (C), 3,4-diethenyl-3-methylcyclohexene (10.15%)



**Figure 1:** Proportions of EO phytoconstituents of leafy twigs of *Chromolaena odorata*

HM :Hydrocarbon monoterpenes ; OM : oxygenated monoterpenes; HS: Hydrocarbon sesquiterpenes; OS Oxygenated sesquiterpenes

(D),  $\beta$ -caryophyllene (7.94%) (E) and  $\beta$ -pinene (7.22%) (F). This composition is similar to that of the various authors from Cote d'Ivoire<sup>15,22</sup> and partly from Cameroon<sup>16</sup> and Togo.<sup>6</sup> But with different proportions which may be due to certain ecological factors, the age of the plant, the period of the vegetative cycle, the origin of the plant and the distillation technique. The Figure 2 shows the chemical structures of the major phytochemicals of essential oil (EO).

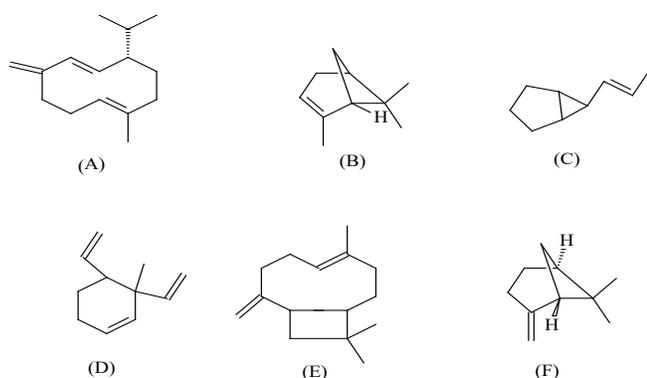
### Antioxidant Activity

The antioxidant activity was assessed spectrophotometrically. The absorbance of DPPH was measured at 517 nm. The results (Figure 3) show that the EO extract of *C. odorata* exhibits low antioxidant activity compared to vitamin C. This result is proved by the determined IC50 values. The IC 50 value of vitamin C is 0.31  $\mu$ g/mL while that EO extract of *C. odorata*

**Table I:** Phytochemical composition of EO of leafy twigs of *C. odorata*.

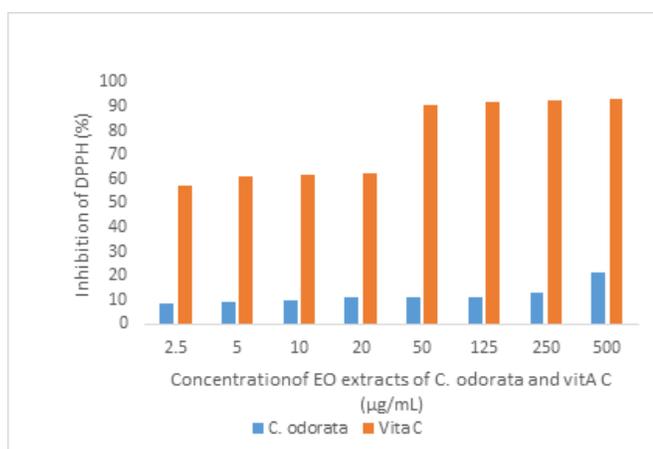
N°	RT	RI	Compound	PM	%
1	12.61	924.44	$\alpha$ -pinene	136	17.79
2	15.33	966.68	$\beta$ -pinene	136	7.22
3	16.74	988.58	$\beta$ -myrcene	136	1.14
4	19.09	1022.63	D-limonene	136	0.80
5	20.99	1036.70	E- $\beta$ -ocimène	136	0.26
6	20.78	1046.20	$\alpha$ -Ocimene	136	2.07
7	25.69	1115.10	Pépillene	150	0.14
8	26.52	1126.80	1,3-cycloheptadiene	94	1.01
9	27.01	1133.80	3,4-diéthényl-3-méthylcyclohexène	148	10.14
10	36.97	1278.10	6-propèn-1-enylbicyclo[3.1.0]hexan-2-one	136	14.95
11	40.44	1330.98	Terpinolene	136	0.20
12	42.77	1367.35	$\alpha$ -copaène	204	2.01
13	43.89	1384.72	(-)-cis $\beta$ -elemene	204	0.65
14	45.41	1408.75	$\beta$ -caryophyllene	204	7.94
15	46.09	1420	$\beta$ -cubebene	204	0.36
16	47.48	1442.87	$\alpha$ -caryophyllene	204	2.24
17	49.22	1471.40	GermacrèneD	204	21.82
18	49.83	1481.34	1-épibicyclasesquiphellandrène	204	0.69
19	50.15	1486.58	$\gamma$ -elemene	204	2.06
20	50.59	1493.94	$\beta$ -gurjunene	204	1.03
21	51.29	1505.66	$\gamma$ -cadinene	204	0.37
22	51.88	1515.83	$\beta$ -cadinene	204	4.48
23	53.61	1546.03	Patchoulene	204	0.32
24	55.09	1571.71	Trans-caryophyllene	204	0.24
			Hydrocarbonmonoterpenes		29.48
			Oxygenatedmonoterpenes		0
			Hydrocarbonsesquiterpenes		44.21
			Oxygenatedsesquiterpenes		0
			Others		26.23
			Total		99.92 %

RT: Retention time; RI: retention index; PM: Molecular weight and %: percentage



**Figure 2:** Chemical structures of the majors phytochemicals of EO of *C. odorata*.

A :germacrene-D; B :  $\alpha$ -pinene ; C : 6-propen-1-enylbicyclo [3.1.0]hexan-2-one; D : 3, 4-diethenyl-3-methylcyclohexene ; E :  $\beta$ -caryophyllene et F:  $\beta$ -pinene



**Figure 3:** Concentration dependent of inhibition of DPPH *C. odorata* : *Chromolaena odorata* ; Vita C : vitamin C

is 17.4  $\mu\text{g/mL}$ . The  $\text{IC}_{50}$  of EO extract is 56 times higher than that of vitamin C, the standard antioxidant. This proves that vitamin C is 56 times more effective than EO extract. The relative antioxidant activity of EO extract could justify the use of this plant in traditional medicine. This antioxidant activity could be linked to the presence of terpenes.<sup>23</sup>

## CONCLUSION

In the present study, we are interested in the valorization of *C. odorata* an aromatic plant used in traditional Ivorian medicine. The essential oil of the aerial part of the species, obtained by steam distillation, has a low yield ( $0.082 \pm 0.004\%$ ).

24 phytochemicals have been identified there. The phytochemical composition is dominated by hydrocarbon sesquiterpenes (44.21%) followed by hydrocarbon mono terpenes 29.48% and other compounds (26.23%). Hydrocarbon and oxygenated monoterpenes are absent. The majority compounds are: germacrene-D (21.82%),  $\alpha$ -pinene (17.79%), 6-propen-1-enylbicyclo [3.1.0] hexan-2-one (14.95%), 3,4-diethenyl-3-methylcyclohexene (10.15%),  $\beta$ -pinene (7.22%) and  $\beta$ -caryophyllene (7.94%).

The study of antioxidant activity by the DPPH test showed that the EO analyzed has less antioxidant activity than that of vitamin C, taken as the reference antioxidant. The  $\text{IC}_{50}$ s are 0.31  $\mu\text{g/mL}$  for vitamin C, 17.4  $\mu\text{g/mL}$  for *C. odorata*.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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