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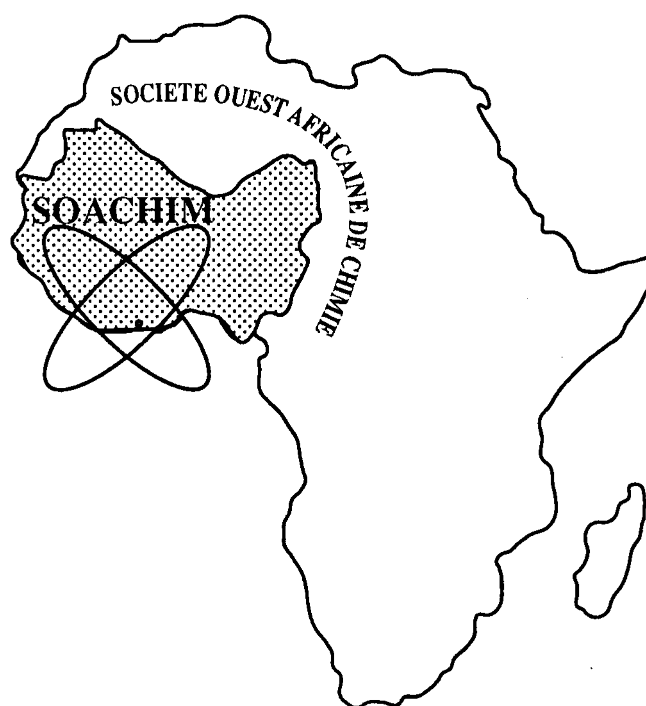

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Chemical Composition and biological Activities of Essential Oil from Benin *Diplolophium africanum* Turcz stem leaves

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Summary : The essential oil obtained by hydrodistillation of stem leaves of *Diplolophium africanum* (Apiaceae) collected in Samson (Borgou) in the Western North of Benin was analyzed using capillary GC and GC/MS. Thirty one compounds representing (99.8 %) of the oil were identified. The major compounds were found to be *p*-mentha-1,3,8-triene (66.3 %) **,** α-pinene (14.4 %), *p*-α-dimethyl styrene (4.9 %) and limonene (3.4 %). The antiradical activity of the oil was found to be low compared to that of butylated hydroxytoluene (BHT) and highly antimicrobial activity was showed.

Keywords: *Diplolophium africanum*; Apiaceae; essential oil; antiradical activity, antifungal activity antibacterial activity; stem leaves.

Composition chimique et activités biologiques de l'huile essentielle extraite des tiges des feuilles de *Diplolophium africanum* Turcz du Bénin

Résumé : L'huile essentielle extraite des tiges des feuilles de *Diplolophium africanum* (Apiaceae) récoltées à Samson (Borgou) au Nord Ouest du Bénin a été analysée par CG et CG/SM. Trente un composés représentant (99,8 %) ont été identifiés dans l'huile essentielle. Les composés majoritaires sont le *p*-mentha-1,3,8-triene (66,3%)**,** l'α-pinène (14,4 %), le *p*-α-diméthyl styrène (4,9 %) et le limonène (3,4 %). Cette huile essentielle présente une forte antimicrobienne et une activité antiradicalaire relativement faible par rapport à celle du composé de référence le butyl hydroxyl toluène (BHT).

Mots clés: *Diplolophium africanum*; Apiaceae; huile essentielle; activité antiradicalaire, activité antifongique ; activité antibactérienne ; tiges des feuilles.

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1. Introduction

Diplolophium africanum Turcz is an seasonal plant growing on hardy subterranean rhizomes in a savannah of *Cymbopogon giganteus* and *Loudetia* sp. during the growing period. In its natural habitat, the young shoots appear in May and the plant is entirely dried out in November [1]. The leaves are very fragrant when dissected and the inflorescences are compound umbels. The plant is used in native medicine in Rwanda where it is called Agaso and is known to be toxic to cattle. Root extracts have been shown to inhibit root growth in young wheat [2].

A preliminary study showed the oil to possess insecticidal properties towards the bruchids *Callosobruchus maculates* F. and *C. subinnotatus*, predators of niebe (African name for a variety of bean) and the *cowpea weevil* [3].

On the chemical point of view, Jirovetz and al identified β-phellandrene as the dominant volatile compound of both essential oil samples of stems and leaves from Cameroon [4]. Another chemotype of this plant was identified by Koumaglo et al. (1994) with main constituents as α-pinene (38 %) and β-pinene (20 %) [5]. Scoparone determined by spectrometric methods has been identified as the active principale of this plant and its inhibitory effect on the growth of wheat rootlets was isolated from *Diplolophium africanum* for the first time with a minimal inhibitory concentration value of 62.5 µg/mL [6]. The chemical characteristics of its essential oil has been identified by Naves in 1948 in Guatemala [7].

The present study emphasies both on the chemical composition of essential oil extracted from the stem leaves of *Diplolophium africanum* Turcz collected in some area of Benin and the evaluation of its biological properties like antiradical and antimicrobial activities.

2. Experimental

2.1. Plants material

The stem leaves of this plant were collected in Savalou area of Benin in August 2010. The sample was authentificated by Mr. Akoegninou of the Herbarium of Abomey-Calavi, Department of Vegetal Biology where voucher specimen [AA6378/HNB] was kept for future reference.

2.2. Isolation of essential oil

The essential oil was obtained by hydrodistillation of the pulverized fresh stem leaves (200 g) using a Clevenger-type apparatus for 2 h. The resulting essential oils were dried after decantation over anhydrous sodium sulphate and stored at 15 °C in the absence of light.

2.3. Analysis

2.3.1. Chemical analysis

2.3.1.1.GC-FID analysis

The oils were analyzed on a Varian CP-3380 GC equipped with a HP5 (100 % dimethylpolysiloxane) fitted with a fused silica capillary column (30 m x 0.25 mm i.d. film thickness 0.25 µm); temperature program 50 – 200 °C at 5 °C/min, injector temperature 220 °C, detector temperature 250°C, carrier gas N_2 at a flow rate of 0.5 $mL.min^{-1}$. Diluted samples (10/100, v/v, in methylene chloride) of 2.0 µL were injected manually in a split mode. The percentage compositions were obtained from electronic integration measurements without taking into account relative response factors. The linear retention indices of the components were determined relatively to the retention times of a series of n-alkanes (C_9-C_{20}).

2.3.1.2. GC-MS analysis

GC-MS analyses were performed using a Hewlett Packard apparatus equipped with a HP5 fused silica column (30 m x 0.25 mm; film thickness 0.25 µm) and interfaced with a quadruple detector (Model 5970). Column temperature was programmed from 70 to 200 °C at 10 °C/min; injector temperature was 220 °C. Helium was used as carrier gas at a flow rate of 0.6 $mL.min^{-1}$, the mass spectrometer was operated at 70 eV. 2.0 µL of diluted samples (10/100, v/v, in methylene chloride) were injected manually in the split mode.

The identification of individual compounds was based on the comparison of their relative retention times with those of authentic samples on the HP5 column and by matching the linear retention indices and mass spectra of peaks with those obtained from authentic samples and/on the NBS75K.L and NIST98.L libraries and published data [8, 9].

2.3.2. Antiradical activity

Antiradical scavenging activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) following the Mellors and Tappel method [10], adapted to essential oil screening [11].

1,1-diphenyl-picrylhydrazyl [1898-66-4] was purchased from Sigma-Aldrich chemistry and the solutions were prepared with analytical grade solvents purchased from standard commercial sources.

DPPH, was dissolved in ethanol to give a 100 µM solution. To 2.0 mL of the ethanolic solution of DPPH was added 100 µL of a methanolic solution of the antioxidant reference BHT at different concentrations. The essential oils were tested in the same method. The control, without antioxidant, is represented by the DPPH ethanolic solution containing 100 µL of methanol. The decrease in absorption was measured at 517 nm after 30 min, at 30 °C. All the spectrophotometric measures were performed in triplicate with a SAFAS UV mc2 spectrophotometer, equipped with a multicells/multikinetics measure system and with a thermostated cells-case.

The free radical-scavenging activity of each solution was calculated according to the following equation: $SC\ \% = [(A_{(blank)} - A_{(sample)}) / A_{(blank)}] \times 100$ [12]

Antiradical activity, defined as the concentration of test material required to cause a 50 % decrease of the initial DPPH absorbance, was determined graphically and expressed as SC_{50} ($mg.\ L^{-1}$).

2.3.3. Antifungal activity

2.3.3.1. Preparation of the culture medium

11.5 g agar of yeast extract (Yeast extract AGAR) and 10 g of anhydrous glucose are mixed with 500 ml of distilled water for the preparation of culture medium. After sterilization and addition of oxytetracycline (0.1 %) 5 ml, this medium was cast in limp of Petri dish 9 cm in diameter at a rate of 17 mL.

2.3.3.2. Detection of the moulds

A quantity of vegetable weighed from gardening culture, fresh tomato fruits and banana leaves was diluted in sterile peptone water in order to detected fungi responsible of their deterioration. 30 min after homogenizing each sample, 0.1 mL of the inocula was spread out on the sterilized mould medium (Yeast Extract Glucose Agar: YEGA) and uniformly. The present limp was incubated at 25 °C ± 1 °C five days awared from day light.

2.3.3.3. Transplantation and mycelial growth

The moulds detected after examination and identification then, are transplanted (subcultured) using a disc of 6 mm in diameter which carries spores from the anamorph mould on the surface of Petri dish containing the former medium YEGA containing tested essential oils at different concentrations or no (positive control). The moulds subcultured were incubated at 25 °C ± 1 °C. The mycelial growth was appreciated every day by measuring the average of two perpendicular diameters passing by the middle of the disc, from the first day till the seventh one at, least 7 days [13].

The antifungal activity was evaluated by the following equation:

$$I= [1-(d/d_c)]x100$$ [14].

I : index antifungal**;** **d :** diameter of growth of Petri dish treated out of essential oil**;** **d_c:** diameter of growth of the control (witness) [Petri dish without essential oil]

2.3.3.4.Test of determination of the fongiostatic or fungicidal activity

With the experimental concentrations where no growth, nor germination was observed, we tested the fungiostatic or fungicidal activity was tested. This test consists in taking the mycelial disc not germinated at the end of the incubation of the Petri dish and reintroducing it in a new culture medium (former one) nine without natural extract. If the mycelial growth is always inhibited, the fungicidal activity of the natural extract and in the contrary case, it's spoken about fongiostatic the activity.

2.4. Antibacterial activity

2.4.1. Essential oil emulsion.

2 ml of Mueller Hinton broth added with 0.02 g/L (w/v) of phenol red were added 40 µl of essential oil and 2 drops of Tween 80 and has been introduced in an hemolyse test tube and homogenized.

2.4.2.Preparation of bacteria suspensions

This preparation was carried out from the three stocks of tested bacteria. A pure colony of each stock was suspended in 5 ml of Mueller Hinton broth. After incubation at 37 °C for 2 hours, we obtained 10^6 cfu/mL corresponding to the scale of McFarland standard.

2.4.3. Determination of Minimal Inhibitory Concentration (MIC)

The method used was reported by Yehouenou et al [15].

100 µl of bubble Mueller Hinton broth containing of phenol red to 0.02 g/L were distributed in all the 96 wells of microplate. 100 µl of essential oil emulsion (initial solution) were added well of the first column except that of the second line and we carried out successive dilutions of reason 2 were carried out well by well till the 12th one and the remaining aliquot (100 µL) were rejected. 100 µl of Mueller Hinton which not containing phenol red were introduced on the first well of the first columns and successive dilutions of reason 2 were carried out as before. All the wells of the second column received 100 µL of bacteria suspension except the first line which represents the negative control and the second line, the positive control. The microplate one was finally covered with paper parafilm and was incubated at 37 °C during approximately 18 hours.

2.5. Statistical analysis

Data were subjected to analysis of variance (ANOVA). They were expressed as the mean ± standard error of triplicate measurements; standard deviations did not exceed 5 %.

3. Results and discussion

3.1. Chemical composition

The essential oil was obtained in 0.37 % yield from the stem leaves of *Diplolophium africanum*; this yield is similar to that already observed with the same species from Togo [5]. The chemical composition of the essential oils was given in **Table I**.

Table I: Chemical composition of stem leaves essential oil of *Diplolophium africanum* from Benin

$RI_{cal\ (5°C)}$	Component	%	Identification methods
931	α–Thujene	0.3	MS, RI
943	**α-Pinene**	**14.4**	GC, MS, RI
955	Camphene	0.1	GC, MS, RI
979	Sabinene	0.2	MS, RI
985	β-Pinene	3.4	GC, MS, RI
994	Myrcene	1.3	GC, MS, RI
1008	*trans*- 3-Hexenyl acetate	0.2	MS, RI
1010	α-Phellandrene	0.5	MS, RI
1020	α-Terpinene	0.1	MS, RI
1030	*p*-Cymene	0.5	GC, MS, IR
1036	**Limonene**	**3.4**	GC, MS, RI
1050	*trans*-β-Ocimene	0.3	MS, RI
1064	γ-Terpinene	0.7	GC, MS, RI
1102	***p*-α-dimethyl Styrene**	**4.9**	MS, RI
1114	**1,3,8-*p*-Menthatriene**	**66.3**	MS, RI
1158	*trans*-Verbenol	1.0	MS, RI
1186	Lavandulol	0.1	MS, RI
1192	α-Terpineol	0.1	GC, MS, RI
1198	*o*-Cumenol	0.1	GC, MS, RI
1206	γ-Terpineol	0.1	MS, RI
1212	2-methyl Anisol	0.2	MS, RI
1231	*trans*-Carveol	0.1	MS, RI
1241	(E)-Ocimenone	0.1	MS, RI
1245	*ortho* methyl Phenol	0.4	MS, RI
1276	*cis*-Carvone oxide	0.1	MS, RI
1422	*trans*-β-Caryophyllene	0.2	GC, MS, RI
1481	7-epi-1,2-dehydro-Sesquicineol	0.1	MS, RI
1497	Germacrene -D	0.2	MS, RI
1568	(E)-Nerolidol	0.3	MS, RI
1590	Caryophyllene oxyde	0.1	MS, RI
Grouped components (%)			
Monoterpene hydrocarbons		**96.4**	
Oxygenated monoterpenes		**2.5**	
Sesquiterpene hydrocarbons		**0.4**	
Oxygenated sesquiterpenes		**0.5**	
Total identified		**99.8**	

RI*****, Retention index relative to n-alkanes (C_9-C_{20}) on a column DB5;
GC, identification based on retention times of authentic compounds on a DB5 fused silica capillary column;
MS, identification based on comparison of retention index of the computer matching of the spectra of peaks with ESSENCES, NBS75K.L and NIST98.L libraries and published data[8,9],
RI, tentatively identified based on comparison of retention index of the compounds compared with published data[8,9].

The structures of the type *p*-menthane were identified to be the major compounds and they were characterized by a high percentage of *p*-mentha-1,3,8-triene (66.3 %) accompanied of α-pinene (14.4 %). The chemical composition of our sample was not comparable to those previously described for sample from other sources and is characterized by a majority of hydrogenated derivatives *p*-menthane skeleton, mainly *p*-mentha-1,3,8-triene, which account for two thirds of the essence.

3.2. Antiradical activity

The antiradical activity of the oils was also evaluated and compared to that of the commercial antioxidant BHT (Butylate Hydroxy Toluene), which was widely used as a preservative. The percentage of inhibition was less than 50 % at a concentration of 5 $g.L^{-1}$. More concentrated solutions (5-20 $g.L^{-1}$) had been evaluated in an attempt to determine the SC_{50}. The following results were obtained : SC_{50}(*D. africanum*) = (10.7 ± 0.53) $g.L^{-1}$; SC_{50}(BHT) = (7.50 ± 0.37) $mg.L^{-1}$.
Very low antiradical activity of this essential oil was observed according the DPPH method: Values (See above).

3.3. Antifungal activity

The antifungal activity of the essential oil of *Diplolophium africanum* was evaluated. The following results were obtained (**Fig. 1**).

For concentrations 50 ml/l (50000 ppm) and 75 ml/l (75000 ppm) out of essential oil of *Diplolophium africanum*, there was an increase in reduction rate which stops after 48 hours and it was noted a considerable rate reduction whereas for a concentration of 100 ml/l (100000 ppm) the increase of rate reduction is observed after 24 hours until day 4 (96 hours) before witnessing a progressive reduction rate (**Fig. 1**).

It should be noted that the increase in rate reduction observed on the level on **Figure 1** explained the inhibition of the mycelial growth by essential oil whereas the intensive reduction in reduction rate translates the mycelial growth of *Aspergillus ochraceus*. On the level of this essential oil noticed a light inhibition with a concentration equal to 100 ml/l over 96 hours duration after the transplantation of *Aspergillus ochraceus*. The essential oil rather was fongiostatic than fungicidal**.**

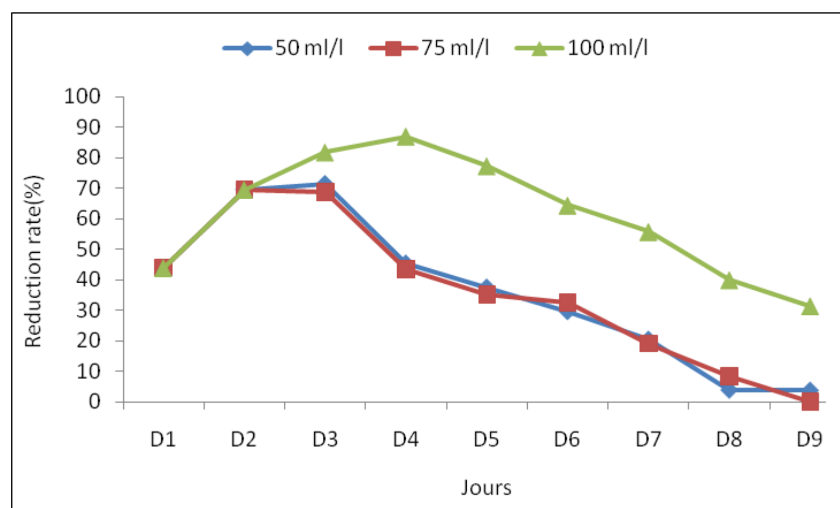

Figure 1 Action of the oil essential of *Diplolophium africanum* with various concentrations on the mycelial growth of *Aspergillus ochraceus*

3.4. Antimicrobial activity

The essential oil of *Diplolophium africanum* has same the CMI on *Escherchia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 (8.48 ± 0.42 mg/ml) which is higher than the CMI on the level of *Candida albicans* ATCC 14133 ((0.53 ± 0.03) mg/ml). This oil has same the MIC on *Escherchia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 ((8.48 ± 0.42) mg/ml) which is higher than the MIC on the level of *Candida albicans* ATCC 14133 ((0.53 ± 0.03) mg/mL). The essential oil extracted from the stem leaves of *Diplolophium africanum* carried on an antimicrobial activity because from their MIC ((8.48 ± 0.42) mg/mL). We noted a weak activity antimicrobial of essential oil on *Candida albicans*. The result obtained on the level of antimicrobial test would be due to the chemical profiles of this essential oil **(Table I& II).**

Table II: Antimicrobial activity (Minimum Inhibitor Concentration: MIC value, mg/ml) of essential oil of stem leaves of *Diplolophium africanum*

Microbial stock	Minimum Inhibitor Concentration (MIC) (mg/ml)
Escherichia coli ATCC 25922	8.48 ± 0.42
Staphylococcus aureus ATCC 25923	8.48 ± 0.42
Candida albicans ATCC 14133	0.53 ± 0.03

4. Conclusion

The essential oil extracted from the stem leaves of *Diplolophium africanum* showed a high level of hydrocarbons monoterpens (96.4 %). Its detained a very interesting antimicrobial activity on *Candida albicans* with a MIC of (0.53 ± 0.03) $mgmL^{-1}$ but presented a few antiradical activity. Fongiostatic property was observed from the stem leaves essential oil.

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