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ORIGINAL RESEARCH PAPER

VOLATILE CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL FROM *Securidaca longepedunculata* Fers. GROWING IN BENIN

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Abstract: The essential oil obtained by hydrodistillation of roots bark of *Securidacaca longepedunculata* Fers. (Polygalaceae) growing in Savalou, Biguinan and Gbegrou (Benin) were analyzed using capillary GC and GC/MS. Seven compounds representing (99.5%; 99.1%; 99.3%) respectively of the oils were identified. The major compound was found to be methyl salicylate respectively (98.0%; 98.6%; 98.7%). The antimicrobial activity of these oils was found to be high, and medium antiradical activity was observed.

Keywords: *antimicrobial activity, antiradical activity, essential oils, methyl salicylate, Securida longepedunculata Fers.*

INTRODUCTION

Securidaca longepedunculata Fers. (syn. *Securidaca spinosa* Sim.; *Lophostylis pallida* Klotzsch.) is a shrub or sub-shrub, always green, spread in savannas. Sometimes at least part of its sheets loses. The flowers appear especially during the rainy season and the fruits ripening in dry season. It is a species soudano-guineo-saheliennes spread in all intertropical Africa [1]. An infusion of the sheets, consumed in morning, fights the painful rules [2]. The dry barks of root, in aqueous maceration, are used in convulsives crises of the child. The plant is indicated for the treatment of the envenimations by bite of snake [3-5]. The powdered root bark is used against headaches sniffing. It is strongly sternutatory (causes sneezing). The plant is also used against snake bites. The aerial part, powdered, is used in case of bleeding [5]. It is commonly used as a medicine in many parts of Africa. In Nigeria, the plant is commonly used for the treatment of inflammatory conditions and as laxative. An oral administration of a decoction of the root has been shown to produce a sedative effect, mainly attributed to its content of oleanolic acid glycoside [6]. Extracts of the plants from Nigeria has been reported to possess both gastrointestinal and trypanocidal effects [7, 8] and as antimicrobial [9]. Elsewhere considerable antimalarial [10], insecticidal [11, 12] and insect repellent activities [13] have been confirmed for extracts from various parts of the plant. *S. longepedunculata* is widely used for several diseases, in America (Venezuela) traditional medicine uses the plants' dried barks for the treatment of epilepsy [14]. In Africa (Ghana, Nigeria) the plant decoction is prescribed by Ghanaian healers to treat asthma and other diseases associated with smooth muscle contraction [15] and as antinociceptive and antidepressant [16]. Moreover, the traditional use of *S. longepedunculata* root bark against the hepatic infection has been reported and their anti-allergenic effects were verified *in vivo* [17, 18]. The root barks of *S. longepedunculata* have very high antioxidant and anti-inflammatory properties [19].

On the chemical point of view, the seed oils have an abundant of hydroxydienoic fatty acids and acetotriacylglycerols [20]. Ergonine alkaloids [21] and tannins of preseneganine skeleton [22] have been characterized from the plant. Previous research works on the volatile oils have been focused on the root. Costa *et al.* identified methyl 4-hydroxybenzoate as the dominant volatile compound of the ether extract of the root oil of the plant [23]. Lognay *et al.* reported the occurrence of methyl salicylate and 2-hydroxy-6-methoxybenzoic acid methyl ester in *S. longepedunculata* root bark from Senegal [24]. Later, Jayasekara *et al.* also identified methyl salicylate (2-hydroxy benzoic acid methyl ester) (93%) as the main volatile component in the methanol extract of the root bark of the plant from Ghana [25]. Methyl salicylate (100%) was also reported by Nebie and co-workers [26]. In addition, nine others compounds of the benzoic acid derivatives were also characterized as the dominant compounds in die root bark oil of the plant from Burkina Faso. Methyl salicylate was identified as the compound responsible of the fumigant property of the root of the plant [23]. The root barks of this plant have anti-inflammatory and antioxidant properties with regard to their phenolic contents. More lately, Adebayo *et al.* showed that the essential oils of leaves collected in Nigeria are dominated by methyl salicylate (89.6%), major compound of the root bark [27].

In this paper, we report the chemical composition of three essential oils obtained by hydrodistillation of the roots bark of *S. longepedunculata* Fres. collected in three areas

of Benin, as well as the results of their antiradical and antimicrobial (antifungal and antibacterial) properties evaluation.

MATERIALS AND METHODS

Plants material and extraction of essential oils

The plant material was collected in areas of Benin at Savalou (Sample 1), Biguinan (Sample 2) and Gbegrou (Sample 3), in August 2010. A voucher specimen [AA 6380/HNB] was deposited in the Herbarium of the University of Abomey-Calavi. Batches of 200 g of fresh roots bark were submitted to hydrodistillation for 2 h using a Clevenger-type apparatus; after decantation, the oils were dried over anhydrous Na_2SO_4 and stored in sealed vials below 10°C until using.

Chemical analysis

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 × 0.25 mm i.d. film thickness 0.25 µm) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m × 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}).

GC-MS analysis

GC-MS analyses were performed using a Hewlett Packard apparatus equipped with a HP5 fused silica column (30 m × 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 [28, 29].

Antiradical activity

Antiradical scavenging activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) following the Mellors and Tappel method [30], adapted to essential oil

screening [31]. DPPH [1898-66-4] was purchased from Sigma-Aldrich 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 were added 100 µL of a methanolic solution of the antioxidant reference BHT at different concentrations. The essential oils were tested in the same manner. 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 the following equation:

$$SC\,\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \qquad (1)$$

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} ($mg.L^{-1}$).

Antifungal activity

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 5 mL oxytetracycline (0.1%), this medium was cast in limp of Petri dish 9 cm in diameter at a rate of 17 mL.

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 speed out on the sterilized mould medium (Yeast Extract Glucose Agar: YEGA) and uniformly. The present limp was incubated at 25 ± 1°C five days awared from day light.

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 ± 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 [32].

The antifungal activity was evaluated by the following equation [33]:

$$I = \left(1 - \frac{d}{d_c}\right) \times 100 \qquad (2)$$

with I - antifungal index, 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).

Test of determination of the fongiostatic or fungicidal activity

With the experimental concentrations where neither growth, nor germination was observed, we tested the fungiostatic or fungicidal activity. 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, it's the fungicidal activity of the natural extract and in the contrary case it's spoken about fongiostatic activity.

Antimicrobial activity

Preparation of the essential oil emulsion

2 mL of Mueller Hinton broth added with 0.02 g/L of phenol red were added 40 µL of essential oil and 1 and 2 drops of Tween 80. We introduced it inside a hemolyse test tube. The mixture is homogenized.

Preparation of suspensions

This preparation was made in the same manner for the three stocks used. A pure colony of each stock was suspended in 10 mL of Mueller Hinton broth. After incubation at 37°C during one night, we obtained 10^9 germs/mL a microbial suspension with a turbidly equals to 2 on McFarland scale (10^6 CFU/mL).

Determination of Minimal Inhibitory Concentration (MIC)

The method used is reported by [34]. 100 µL of bubble Mueller Hinton broth containing 0.02 g/L of phenol red were distributed in all the 96 wells of microplaque. We added 100 µL of tested extract (initial solution) to each well of the first column except that of the second line and we carried out successive dilutions of reason 2 well per well, column by column to the last well of the last column where 100 µL were rejected. We put 100 µL thereafter bubble Mueller Hinton broth not containing phenol red in the first well second-rate and carried out successive dilution of reason 2. We sowed all the wells except those of the first line by introducing 100 µL there bacterial suspension with 10^6 CFU/mL. On the place of the bacterial suspension, we put bubble Mueller Hinton broth without phenol red in the wells of the first line. The microplaque one was finally covered with paper parafilm and was incubated at 37°C during approximately 18 hours.
It should be noted that the first line constitutes the negative witness and the second, positive witness. With the reading, obtaining a yellow color indicates a bacterial multiplication. The persistence of initial red color means the absence of growth of the germs. The MICs are the weakest concentration for which there is no visible growth. It is thus the low concentration of the well where there is no turn with the yellow.

Statistical analysis

All data were expressed as the mean ± standard error of triplicate measurements; standard deviations did not exceed 5%.

RESULTS AND DISCUSSION

Chemical composition

The yields of the essential oils obtained by hydrodistillation of fresh roots bark of *Securidaca longepedunculata* collected in three locations of Benin are given in Table 1. They range from 0.30 to 0.52%; these results are lower than those obtained in previous studies [26], but similar to that observed with the same species from the Nigeria [27]. The chemical compositions of these essential oils are shown in Table 2.

Table 1. *Yields (% w/w) of essential oils obtained from fresh roots bark of* ***Securidaca longepedunculata Fers.*** *from Benin*

Sample	Date and place of harvest	Yield (% w/w)
1	August 2010 (Savalou, Benin)	0.36
2	August 2010 (Biguinan, Benin)	0.52
3	August 2010 (Gbegrou, Benin)	0.30

Table 2. *Chemical composition (% w/w) of essential oils of roots bark of* ***Securidaca longepedunculata Fers.*** *from Benin*

RI*	Component	Sample 1	Sample 2	Sample 3	Mode of identification
		(% w/w)			
1029	*p*-cymene	0.1	-	0.1	GC, MS, RI
1063	γ-terpinene	0.1	0.1	0.1	GC, MS, RI
1113	*p*-α-dimethyl styrene	0.1	0.1	0.1	MS, RI
1222	**methyl salicylate**	**98.0**	**98.6**	**98.7**	MS, RI
1369	eugenol	0.1	0.1	0.1	GC, MS, RI
1449	NI	0.6	0.5	0.6	MS, RI
1771	germacrene D	0.1	0.1	0.1	MS, RI
1866	NI	0.1	0.1	0.1	MS, RI
1871	benzyl salicylate	0.1	0.1	0.1	MS, RI
Grouped components (%)					
Monoterpene hydrocarbons		**0.3**	**0.2**	**0.3**	
Oxygenated monoterpenes		**98.1**	**98.7**	**98.8**	
Sesquiterpene hydrocarbons		**0.1**	**0.1**	**0.1**	
Oxygenated sesquiterpenes		**0.1**	**0.1**	**0.1**	
Total identified		**98.6**	**99.1**	**99.3**	

Sample **1** = Savalou; Sample **2** = Biguinan; Sample **3** = Gbegrou;
RI* - Retention index relative to *n*-alkanes (C_9-C_{20}) on a HP5 column;
GC - identification was based on retention times of authentic compounds on a Varian CP-3380GC with a fused silica capillary column;
MS - identification was 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 [28, 29];
RI - tentatively identified based on comparison of retention index of the compounds compared with published data [28, 29];
NI - not identified.

Seven compounds were identified amounting to (98.6%; 99.1% and 99.3%) respectively of the oils constituents. Methyl salicylate was the abundant constituent (98.0%; 98.6% and 98.7%). This major compound was reported as a characteristic constituent of the

roots bark oil from trees growing Senegal, Ghana and Burkina Faso [24-26] and of leaf oil from trees growing in Nigeria [27]. The chemical composition of essential oil of the roots bark of *S. longepedunculata* was described for the first time in Benin.

Antiradical activity

The antiradical activity of the essential oil of the sample 1 was also evaluated and compared to that of the commercial antioxidant BHT (butylated hydroxytoluene) which was widely used as a preservative. The following results were obtained (Figures 1 and 2). The percentage of inhibition was less than 50% at a concentration of 5 g/L; more concentrated solutions (5-20 g/L) have been evaluated in an attempt to determine the SC_{50}:

- SC_{50} (BHT) = 7.50 ± 0.45 mg/L;
- SC_{50} (*S. longepedunculata*) = 500 ± 30 mg/L.

On another hand, an average antiradical activity of this essential oil could be observed according to the DPPH method.

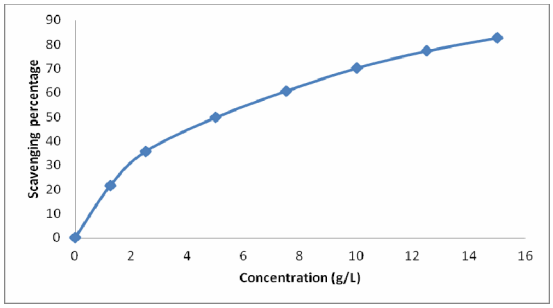

Figure 1. *Reactivity of essential oils of* ***Securidaca longepedunculata*** *with respect to the 1,1-diphenyl-2-picrylhydrazyl (DPPH)*

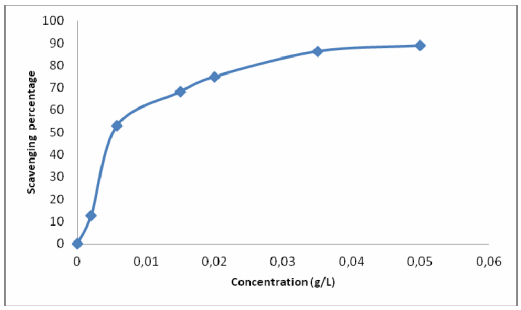

Figure 2. *Reactivity of essential oils of butylated hydroxytoluene (BHT) with respect to the 1,1-diphenyl-2-picrylhydrazyl DPPH*

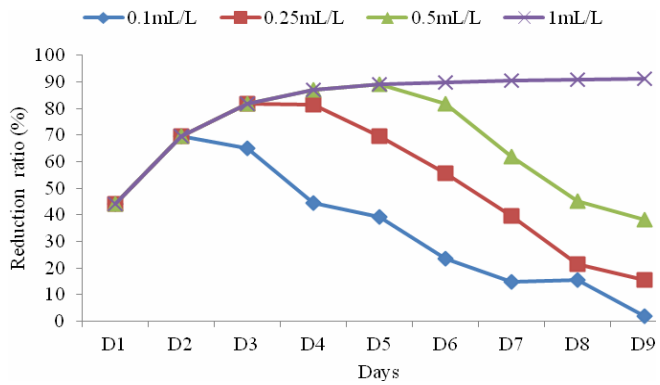

Figure 3. *Action of the oil essential of* ***Securidaca longepedunculata*** *with various concentrationson the mycelial growth of* ***Aspergillus ochraceus***

Antifungal activity

The antifungal activity of the essential oil of the sample 1 was evaluated. The following results were obtained (Figure 3).
We noted a progressive increase in ratio reduction going from 44 to 91.36% during the 9 days (216 hours) with an essential oil concentration 1 mL/L (1000 ppm).
For a concentration of 0.5 mL/L (500 ppm) of essential oil, a progressive increase in ratio reduction (capacity antifungal) was noted of 1 day (after 24 hours) at the 5 day (120 hours) which passed from 44 to 89.06% and a reduction in this rate as from day 6 today 9,which falls from 81.75 to 38.27%; whereas for a concentration of 0.25 mL/L (250 ppm) the increasing of the capacity antifungal stops after 72 h followed by its reduction. On the other hand the growth of the reduction ratio stops after 48 h for a concentration of 0.1 mL/L (100 ppm) then we observed a significant antifungal capacity in reduction from 64.94 to 1.85%.
The essential oil of roots bark of *Securidaca longepedunculata* is most active against *Aspergillus ochraceus*; it showed a total inhibition of the mycelial growth (fungiostatic/fungicidal) to a higher concentration (≥ 1000 ppm).
After having reintroduced the mycelial disc of the Petri dish having for concentration 1000 ppm essential oil in a culture medium nine without natural extract, we noted that this essential oil carried on a fungicidal activity against *Aspergillus ochraceus*.
This activity is probably due to the presence of the majority compound (methyl salycilate) or has a synergy between the majority compound and the minority compounds.

Antimicrobial activity

The essential oil of the roots bark of *Securidaca longepedunculata* almost does not have an antimicrobial activity against *Staphylococcus aureus*. It is slightly active against *Escherchia coli* ATCC 25922. We noted a very interesting activity of this essential oil on *Candida albicans*, Table 3.

Table 3. *Antimicrobial activity (Minimal Inhibitory Concentration: MIC value, mg/mL) of essential oil of roots bark of* ***Securidaca longepedunculata***

Microbial stock	Minimal Inhibitory Concentration (MIC) (mg/mL)
Escherichia coli ATCC 25922	12.79 ± 0.77
Staphylococcus aureus ATCC 25923	-
Candida albicans ATCC 14133	0 .40 ± 0.02

CONCLUSION

The essential oil extracted from the barks of root of *S. longepedunculata* is rich in methyl salicylate with a strong content (> 98%). It detains a very interesting antimicrobial activity against *Candida albicans* and a relative antifungal one against *Aspergillus ochraceus*. Somewhere it presents an average antiradical activity.

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