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Chemical Composition and Cytotoxic Activity of *Chenopodium ambrosioides* L. Essential Oil from Togo

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Abstract

The leaf essential oil of *Chromolaena odorata* L. (Chenopodiaceae) from Togo were steam-distilled, analyzed by GC and GC-MS for chemical composition and investigated *in vitro* for its potential cytotoxic activity on human epidermic cell line HaCat. The chemical composition showed that the main constituents of essential oil sample were respectively ascaridole (51.12 %), p-cymene (19.88 %), neral (8.70%) and geraniol (7.55%). The *in vitro* cytotoxicity bioassays on human cell line HaCaT revealed moderate toxicity level of *C. ambrosioides* essential oil $IC_{50}$with 700 µL.mL^{-1}. Pure commercial neral standard showed high toxicity with IC_{50} value of 100 µL.mL^{-1}). Conversely, pure ascaridole p-cymene and geraniol standards appeared almost non-toxic (IC_{50} >1000 µL.mL^{-1}), proving the major role played by neral in the overall toxicity showed by the *C. ambrosiodes* oil sample tested in this work.

Keywords: *Chenopodium ambrosioides*, Essential oil, Ascaridole, p-cymene, HaCaT cell line, Cytotoxicity.

Introduction

The genus *Chenopodium* of the family *Chenopodiaceae* is composed by 120 species, 45 of which are known to be distributed all over the world, which are reported to posses many medicinal properties (Guyot, 1992). The species *Chenopodium ambrosioides* L. commonly called Mexican tea is an herb that has been used for centuries to expel parasitic worms from the body (Chevallier, 1996). Especially *C. ambrosioides* is widely used in popular medicine as a vermifuge, emmenagogue and abortifacient (Comway & Slocumb, 1979). C. ambrosioides herb is also used both as anthelmintic, antiparasitic (Kliks, 1985; Giove Nakazawa, 1996; Quinlan *et al.,* 2002), antiplasmodial (Pollack *et al.,* 1990) and as condiment in small amounts that impart an acceptable flavour (Anonymous, 1950). An infusion can be used as a digestive remedy, being taken to settle a wide range of problems such colic and stomach pains (Chevallier, 1996). Externally, it has been used as a wash for haemorrhoids, as a poultice to detoxify snake bites and other poisons and is thought to have wound-healing properties (Chevallier, 1996). The plant is also used as a fumigant against mosquitoes (Gillij *et al.,* 2008) and is also added to fertilizers to inhibit insect larvae (Bown, 1995; Clovd and Chiasson, 2007). The plant can also cause dermatitis or other allergic reactions (Foster and Duke, 1995). *C. ambrosioides* essential oils chemotypes had insecticidal and acaricidal properties (Chiasson *et al.,* 2004).

Recently, particular attention has been given by researchers to the use of essential oils from tropical origin as active ingredients in various pharmaceutical formulations against some skin troubles (Orafidiya *et al.,* 2001; Orafidiya *et al.,* 2002) like human mycosis. Indeed, naturally occurring molecules of essential oils are more and more considered as valid additives to conventional antibiotherapies (Chaumont *et al.,* 2001; Koba, 2003).

Intensive work is done to record the possible cytotoxic potential of plants essential oils on human cell lines (Dubey *et al.,* 1997; Foray *et al.,* 1999; Hayes *et al.,* 1999; Koba *et al.,* 2007) and their other biological applications all over the world but according to our knowledge no any works has been reported in the relevant work regarding *C. ambrosioides* oil chemotypes in Togo.

The aim of the present research was to investigate about the chemical composition and the *in vitro* cytotoxic potential of

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C. ambrosioides L. essential oil from Togo. According to the literature report the assumption is made that *C. ambrosioides* can caused dermatitis or other allergic reactions (Foster and Duke, 1990) when in contact with skin and may be toxic to the human epidermic cell line HaCaT.

Materials and Methods

Plant material sampling and volatile oils isolation

Leaves and inflorescences of *C. ambrosioides* L. used in this work were harvested from plants at full flowering stage in the experimental field of the "Unité de Recherche sur les Agroressources *et la* Santé Environnementale" at the "Université de Lomé" on October 2005.

Plant specimen was identified by Pr. Akpagana, "Departement de Botanique, Faculté des Sciences" at the Université de Lomé (Togo), where Voucher specimen was deposited in the Herbarium.

A sample (50 g) of air-dried plant material was extracted by the hydro-distillation technique during 2 hours in a modified Clevenger-type glass apparatus (Craveiro *et al.,* 1976). The extracted crude essential oil was stored in hermetically sealed dark glass flasks with rubber lids, covered with aluminum foil to protect the contents from light and kept under refrigeration at $4^{O}C$ until use without any prior purification.

Essential oil analyses

Gas chromatography analysis

Gas chromatographic analysis was carried out on a Varian 3300 type gas chromatograph equipped with FID detector. An apolar capillary column DB-5 (30 m x 0.25 mm i.d.; film thickness 0.25 µm) and on a polar column Supelcowax 10 with the same characteristics as above mentioned were used. DB-5 column operating conditions were as follows: from 50 ^{O}C (5 min), $50^{O}C$ to $250^{O}C$ at the rate of 2 ^{O}C/min and Supelcowax 10 from $50^{O}C$ (5 min), 50 ^{O}C to $200^{O}C$ at $2^{O}C$/min. The injector and detector temperatures were respectively $250^{O}C$ and $300^{O}C$. The carrier gas was helium at a flow rate of 1.50 ml/min. Samples (0.2 µl) of non diluted essential oil were injected manually.

Gas chromatography-Mass spectrometry analysis

The GC/MS analysis was carried out on a Hewlett Packard 5890 SERIES II chromatograph, coupled with a mass spectrometer of the Hewlett Packard 5971 SERIES type operating in the EI mode at 70 eV. The capillary column type was DB_5-MS (30 m x 0.25 mm i.d.; film thickness 0.25 µm). The amount of sample injected and GC/MS parameters were the same as above.

Identification of components

The components of oil sample were identified by their retention time, retention indices relative to C_5-C_{18} n-alkanes, computer matching with Willet 275.L library and as well as by comparison of their mass spectra with the authentic samples or with data already available in the literature (Kondjoyan & Berdagué, 1996; Adams, 2001).

The percentage of composition of the identified compounds was computed from the GC peak area without any correction factor and was calculated relatively.

Biological assay

Materials

Dubelcco's Modified Eagle's Minimum Essential Medium (DMEM), foetal calf serum (FCS), trypsin (0.25%) were from D. Dutscher (Brumath, France). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO), polyoxyethylene 20 sorbitan monoleate (Tween 80®), N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) (HEPES), were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Phosphate Buffered Saline (PBS) without calcium and magnesium was purchased from VWR International (Cergy-Pontoise, France).

Pure ascaridol, p-cymene, geraniol and neral from commercial origin were purchased from Sigma Chemical Co. St. Louis USA.

HaCaT Cell culture

HaCaT, an immortalized human keratinocyte line was a generous gift from Nathalie Gault (Commissariat à l'Énergie Atomique, Bruyère Le Châtel, France) (Boukamp *et al.,* 1988 ; Gault *et al.,* 2002). Cells were routinely grown in Costar plastic flasks in monolayer cultures in DMEM medium supplemented with 10 % (V/V) FCS, and 5 M of HEPES + 80 $mg.L^{-1}$ of gentamicine. They were grown in a humidified atmosphere of 5 % CO_2 in air. The medium was routinely renewed 2, 4 and 6 days after passage and when confluence was reached; cells were trypsinized and split for subcultures (seeding density 3500 cells/cm^2 in a 75 cm^2 flask) or used for cytotoxicity assays. Cells were used for experiments within 10 passages to ensure cell line stability. All the experiments were carried out at $37^{O}C$.

Cytotoxicity assay

Treatment of cell cultures with tested essential oils and their commercial components

HaCaT cells were seeded at a density of 6 x 104 cells per well in 100 µL culture medium containing 10 % FCS on 96 multiwell culture plates and incubated overnight for adherence. The next day, the medium was removed and cells were incubated in FCS-free medium containing increasing concentrations (from 50 to 1000 $\mu L.mL^{-1}$) of *C. ambrosioides* essential oils or their major components from commercial origin (Pure ascaridole, p-cymene, geraniol and neral) solubilised in 1/10 Tween 80® in culture medium (with < 0.1% Tween 80® an ethanol vehicle). Each experiment was carried out in triplicate.

Determination of cell viability

After the exposure period, the reaction medium was removed and the adhering cells washed with PBS. One hundred microliter of MTT solution (0.5 $g.L^{-1}$ in medium) was added to each culture well. After incubating for 4 hours at $37^{O}C$, the MTT reaction medium was removed and formazan-blue was solubilised in 100 µL DMSO. This assay is based on the reduction of yellow tetrazolium salt MTT by the mitochondrial succinate deshydrogenase to form an insoluble formazan-blue product. Only viable cells with active mitochondria reduce significant amounts of MTT (Mosmann, 1983) and formazan-blue formation absorbance was recorded in an $EL_{X800}UV$, Universal Microplate Reader spectrophotometer at 570 nm. Values of absorbance were converted into percentage of residual viability. Usually, inhibition concentration 50 % (IC_{50}) is chosen as the best biological marker of cytotoxicity.

The inhibition (I) of the essential oils dilutions in percent was calculated as follows:

$$I = \left(\frac{A_o - A_1}{A_o}\right) \times 100$$

A_o is the absorbance of the control reaction (containing all reagents except the tested compounds), and A_1 the absorbance with the tested substances (crude essential oils or pure commercial component).

The IC_{50} (inhibition concentration 50 %) value represented the concentration of the tested compounds or essential oils that caused 50 % cells inhibition.

Results and Discussion

Chemical composition of tested essential oils

The colourless essential oil of *C. ambrosioides* was obtained in yields of 0.8 % based on dried extracted material. The chemical composition of the studied oil samples are listed in Table I. Eleven compounds were identified in the *C. ambrosioides* oil sample representing 99.03 % of the detected compounds that included ascaridole (51.12 %), p-cymene (19.88 %), neral (8.70 %) and geraniol (7.55%) as major components. Other notable components identified in this oil were α-terpinene (6.35 %), carvacrol (2.10 %). This oil consisted of five monoterpene hydrocarbons (28.94 %) and six oxygenated monoterpenes (70.09 %).

Table I. Chemical composition of *Chenopodium ambroisioides* L. essential oil from Togo

Compounds	Retention indices		Peak area
	RI[ii]	RI[iii]	[%][i]
Monterpene hydrocarbons			28.94
α-terpinene	1023	1189	6.35
p-cymene	1033	1273	19.88
trans- β-ocimene	1069	1250	0.51
γ-terpinene	1041	1252	1.52
terpinolene	1095	1283	0.68
Oxygenated monterpenes			70.41
p-mentha, 1, 2, 8 triene	1109		0.32
ascaridol	1237		51.12
neral	1238		8.70
geraniol	1253		7.55
carvacrol	1299	2229	2.10
isoascaridol	1302		0.62
Total identified			99.35

[i]Peak area percentage is based on apolar DB-5 column, and values represent average of three determinations
[ii]Retention indexon apolar DB-5 column
[iii]Retention index on polar Supelcowax 10 column

This composition was near to previous reports on *C. ambrosioides* oil from Madagascar (Cavalli *et al.*, 2004), but differed from those described in Brazil by Jardim *et al.* in 2008 with (Z) ascaridole (61.4 %), (E) ascaridole (18.6 %), p-cymene (2.0 %) as major components and in India by Gupta *et al*. in 2002 with α-terpinene (63.6 %) p-cymene (19.5 %) and ascaridole (6.2 %) as major components of the oil.

Cytotoxicity of tested essential oil samples

The percentage of HaCaT cell viability and the IC_{50} values recorded for tested essential oil sample and its four major constituents from commercial origin are shown in Table II.

Tested essential oil induced significantly increased cell cytotoxicity, from the lower to higher concentrations ranging from 50 to 1000 µL.mL^{-1}. Two types of profiles were observed: (i) tested *C. ambrosioides* oil and pure neral, standards showed cytotoxicity towards HaCaT with following

Table II. *In vitro* viability of human skin cell line HaCat exposed to *C. ambroisioides* essential oils and their major constituents

Concentrations µL/mL	*Cell viability* (%)				
	Essential oil	Major constituents of essential oils			
	C. ambroisioides	Ascaridole	p-cymene	Geraniol	Neral
Control (0)	100 ± 00	100 ± 00	100± 00	100 ± 00	100 ± 00
50	96 ± 1.73	135.66 ± 1.15	126 ± 1.15	98.66± 0.57	85.66± 1.15
100	93 ± 1.00	131 ± 1	120 ± 1	110.66± 1.15	50.66± 0.57
150	90.66± 0.57	128 ± 00	115± 00	114.33± 1.15	47.33± 0.57
200	89± 1	123.66± 1.15	110 ± 1.15	116± 1.73	44.66± 0.57
250	85.33± 0.57	120.33± 0.57	108± 0.57	119.33± 1.15	40.33± 0.57
300	83.66± 0.57	118.33± 2.08	105± 1.52	125.33± 0.57	38.66± 0.57
350	80.33± 0.57	115.33± 0.57	103± 0.57	129.33± 1.15	30.33± 0.57
400	75.66± 0.57	110 ± 1.73	102± 1.52	134.33± 0.57	25.66± 0.57
450	71.00± 1.00	108 ± 1	98.66± 0.57	137.66± 0.57	21.00± 1
500	69.33± 1.15	104.66± 0.57	95 ± 0.57	140.33± 0.57	19.33± 1.15
600	56.66± 1.52	93.66± 1.52	93.33 ± 1	142.66± 0.57	16.66± 1.52
700	50.66± 1.52	93.66± 1.15	92.66± 0.57	144.66± 0.57	11.66± 1.52
800	45.66± 1.15	92.33± 0.57	93.33± 0.57	147.33± 1.15	10.66± 1.15
900	40.66± 0.57	90.66± 1.15	92.66± 1.52	150.33± 0.57	5.66± 0.57
1000	30.66± 0.57	90.33± 1.15	93.66± 1.15	154.33± 1.15	4.66± 0.57

Viability expressed as mean ± S.D, n = 3.

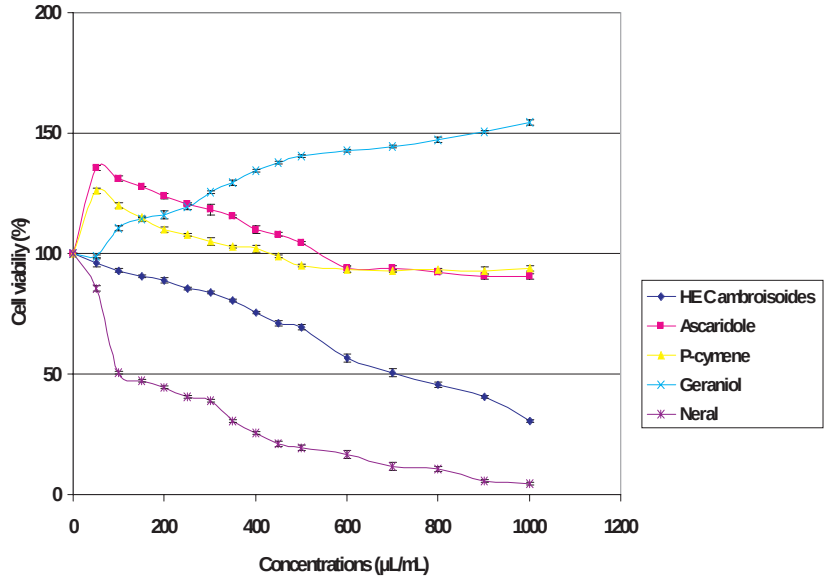

Fig. 1. *In vitro* viability of human cell line HaCat exposed to *C. ambroisiodes* essential oil and their major coumpounds

respective IC_{50} values (100, 700 µL.mL^{-1}) shown in Table II; (ii) pure ascaridole, p-cymene et geraniol standard did not show any cytotoxicity up to 1000 µL.mL^{-1} with respectively cell viability 90.33, 93.66 and 154 %.

These experimental results showed that, of the four major constituents of tested essential oil, neral, was the most toxic for HaCaT cell line. Consequently the cytotoxic effect of the essential oils of *C. ambrosioides* found in this investigation was undoubtedly due to neral. Our previous works on *in vitro* cytotoxicity of *C. citratus* and *C. nardus* essential oils from Togo on cell line HaCaT assumed what we experimentally established to a large extent in this investigation, and provide numerical data on the toxicity of the essential oils rich in citral on human skin cell line (Koba *et al.*, 2009). Apart from its inherent toxicity, some authors have reported that citral, a mixture of both geranial and neral stereoisomers, had a significant ability to suppress oxidative stress possibly through induction of the endogenous antioxidant glutathione system, providing a new insight into skin cancer (Nakamura *et al.*, 2003). Besides, ascaridole, p-cymene and geraniol, as some of the main constituents of the *C. ambrosioides* sample tested in this work, did not show any cytotoxicity. But they could probably expressed their potential as cytoprotectors or as antioxidants in subsequent oxidative stress assays.

Conclusion

In this paper, the investigation of chemical composition of the essential oil of *C. ambrosioides* along with the evaluation of its cytotoxic activity on human cell line HaCaT is quite a typical applied research. The present study has shown a potential cytotoxic activity of *C. ambrosioides* essential oil on human cell line HaCaT. But this essential oil of *C. ambrosioides*, with a percentage composition identical to our tested oil chemotype in this investigation, when used in appropriate doses, could be quite suitable as active components in pharmaceutical formulations for skin treatment and its damages repairing. Further required clinical investigations will include other important studies like contact allergy tests, which might be linked to the use of essential oils as active ingredients.

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