Composition of the volatile oil from the leaves of *Ximenia americana* L.

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Abstract

The volatile oil of leaves of *Ximenia americana* was analysed by GC–MS, which resulted in the identification of 33 components representing 98% of the total oil. The major constituents identified were benzaldehyde (63.5%), hydroxybenzyl cyanide (13%) and isophorone (3.5%). Hydroxybenzyl cyanide is known as a primary breakdown product of glucosinolates that occur mainly in members of Brassicaceae family. The HPLC analysis carried out failed to confirm the possible synthesis or accumulation of glucosinolates in *X. americana*. Hence, the occurrence of hydroxybenzyl cyanide as well as that of benzaldehyde is discussed in this paper.

Keywords: Volatile oil; GC–MS; *Ximenia americana*; Benzaldehyde; Hydroxybenzyl cyanide; Isophorone; Glucosinolates

1. Introduction

Ximenia americana L. is a shrub or a small tree of the family Olacaceae which is found in the tropical regions of America and Africa (Hutchinson and Dalziel, 1954). In folk medicine, a number of therapeutic uses of all parts of *X. americana* were reported; roots are employed as antiseptic, for mental diseases, fever, jaundice and headache, whereas the leaves are used for measles, toothache and also as laxative (Omer and Elimina, 2003; Sofowora, 1982). The pulverized bark is used as remedy for ulcers and the infusion of fruit is applied for bloody diarrhoea (Hutchinson and Dalziel, 1954). This plant acts also against rheumatism, cancer and mouth infections (Kokwaro, 1976; Chhabra and Uiso, 1990).

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Because of its medicinal properties, the phytochemistry of *X. americana* has been investigated by several authors. In root extracts, an oleanolic acid saponin was identified (D'Agostino et al., 1994) as well as C_{18} acetylenic fatty acids (Fatope et al., 2000) and phenolic compounds (Mwangi et al., 1994). As far as investigation of our literature could ascertain, the chemical composition of leaves has been rarely investigated except for the early work that reported the presence of the cyanogenic glucoside, sambunigrin (Hutchinson and Dalziel, 1954). This prompted us to study the volatile fraction of the leaves of *X. americana*.

2. Material and methods

2.1. Plant materials

The aerial parts of *X. americana* were collected randomly in September (2000) at ~30 km from Ouagadougou (Burkina-Faso) and authenticated by Dr J. Millogo. A voucher specimen is kept in the herbarium of the University of Aix-Marseille-I (ref. Mar-2004-41).

2.2. Hydrodistillation

The leaves were air-dried at room temperature, crushed and then subjected to hydrodistillation through a conventional Clevenger-type apparatus for 2 h. The volatile oils were isolated three times from 3 × 100 g of dry leaves.

2.3. GC–MS analyses of the volatile constituents

GC–MS analyses of the volatile oil were performed with a Hewlett–Packard 5972 capillary GC–quadrupole MS system, fitted with a fused silica column (25 m × 0.2 mm; 0.15 μm film thickness) coated with DB5. The ionization energy was 70 eV. Helium was used as carrier gas with a flow rate of 1 ml min^{-1}. The oven temperature was set at 60 °C for 3 min then programmed from 60 °C to 220 °C at 3 °C min^{-1}. Different components were identified by published mass spectra database and retention indexes (Adams, 1995; Jennings and Shibamoto, 1980). Computer searches in HP mass spectral library were also applied. The relative amount (RA) of each oil component is expressed as percent peak area relatively to the total peak area.

2.4. HPLC analysis of glucosinolates

Lyophylisated leaves and seeds of *X. americana* (200 mg) were soaked in 2 ml of boiling MeOH–H_2O (7:3) for 5 min. The glucosinolates were extracted, purified on a DEAE Sephadex A-25, desulphated by a reaction with an arylsuphatase. The desulphated glucosinolates were then separated on an RP column of Spherisorb ODS 2 (250 × 4.6 mm, 5 μm particle size) with an elution gradient (from 0 to 25% of MeCN in H_2O). The quantification was carried out by UV spectrophotometry at 229 nm and the desulphoglucosinolates were identified according to their UV spectra and their capacity factors (Quinsac, 1993). The analyses were repeated in duplicate independent experiments.

3. Results and discussion

The volatile oils of the leaves of *X. americana* were obtained by hydrodistillation in a yield of 0.027% (relatively to dry weight material). The GC–MS analyses indicated that this oil is a complex mixture of 33 identified constituents which represent about 98% of the total oil (Table 1). This oil is made up of 69% of aromatic compounds, 12.5% of lipidic compounds, and 13% of terpenoid constituents. Benzaldehyde is by far the dominant molecule (63.5%). The occurrence of different cyanogenic glycosides in leaves of *X. americana* has been reported earlier, among these, sambunigrin was shown as the most abundant component (Hutchinson and Dalziel, 1954). Accordingly, other workers have characterized a mandelonitrile lyase which catalyses the dissociation of the aglucone (*S*)-mandelonitrile to benzaldehyde and hydrogen cyanide (Kuroki and Conn, 1989) but these breakdown products may also be produced non-enzymatically in acid and elevated temperature conditions (Conn, 1980). As we checked, the hydrodistillations were

Table 1
Composition of the volatile oil from the leaves of *Ximenia americana*

Compound	RI[a]	%RA[b]
(*Z*)-Hexen-3-ol	867	0.6
(*E*)-2-Hexenol	874	0.1
4-Methylpentanol	875	0.6
Benzaldehyde	936	63.5
6-Methylhept-6-en-2-one	966	1.0
(*E*,*E*)-2,4-Heptadienal	971	0.6
Isophorone	1042	3.5
3,5-Octadien-2-one[d]	1052	0.4
trans-Linalool oxide	1065	0.8
Methyl benzoate	1076	0.5
cis-Linalool oxide	1081	1.6
6-Methylhepta-3,5-dien-2-one	1088	0.3
3-Oxa-2,2,4-trimethyl-4-vinylcyclohexanone	1093	1.0
Linalool	1098	2.3
Terpinen-4-ol	1184	0.1
α-Terpineol	1197	1.7
β-Cyclocitral	1224	0.2
Allyl hexanoate	1247	0.2
Geraniol	1277	0.2
Hydroxybenzyl cyanide	1322	13.0
(2*E*,4*E*)-Nonadienal	1337	0.2
Geranyl acetone	1452	0.6
Epoxy-β-ionone	1473	0.3
β-Ionone	1477	0.3
Dihydroactinidiolide	1493	0.1
Actinidiolide	1496	0.3
NI[c]	1506	1.3
(*Z*)-3-Hexenyl benzoate	1508	0.2
Caryophyllene oxide	1581	0.4
Humulene oxide II	1606	0.2
γ-Eudesmol	1633	0.2
Campherenone	1642	0.5
β-Eudesmol	1647	0.6
α-Eudesmol	1653	0.5
Total identified		97.9

[a] Retention index.
[b] Relative area.
[c] Not identified.
[d] Stereochemistry not determined.

carried out in acid conditions (pH = 5.53), suggesting that the high content of benzaldehyde found in the leaves of *X. americana* may be correlated with the presence of sambunigrin.

The second main constituent found is hydroxybenzyl cyanide (13%). 2-, 3- and 4-hydroxybenzyl cyanides are listed among the breakdown products of glucosinolates (Fahey et al., 2001). The latter compounds are mainly found in members of Brassicaceae family where they co-exist with myrosinase. Because the non-enzymatic thermal degradation of glucosinolates may yield components that are identical to those of the conventional enzymatic hydrolysis (MacLeod and Rossiter, 1986), our data raise the question of the occurrence of intact glucosinolates in the whole plant of *X. americana*. Lyophylisated leaves were analysed by HPLC but no glucosinolates were identified. Further studies were carried out from seed materials since these constitute a particularly rich source of glucosinolates (Fenwick et al., 1983). The HPLC analysis results in an absence of glucosinolates above the detection threshold of the method used; 0.1 μg/g of seed. The traces obtained may be interpreted either as minor components which chromatographic properties are similar to those of glucosinolates or as the result of the contamination of our samples with extracts of the routinely analysed glucosinolate-containing plant materials. Accordingly, the presence of hydroxybenzyl cyanide in the leaves of *X. americana* is rather the fact of the addition of cyanure ions to benzaldehyde. This classical reaction may have occurred during the hydrodistillation of leaf materials in aqueous acid medium.

Our results raise the question of the original (in situ) flavour components of *X. americana* because under hydrodistillation conditions, the oil is boiled out after placing plant material into water. This often leads to pH changes and chemical rearrangements of oil components as clearly shown in a comparative study of cedar wood oil extraction through hydrodistillation and stream distillation (Adams, 1991). In order to check whether benzaldehyde and hydrobenzyl cyanide were actual volatile oil components, solvent extracts in diethyl ether and pentane were analysed. None of these components were detected in the extracts which confirm their occurrence as artifact due to hydrodistillation.

The norisoprenoid, isophorone was identified in leaves of *X. americana* in a rate of 3.5%. This compound was shown as a carcinogenic agent (Their et al., 1990; Bucher et al., 1986) which seems to be in contradiction with the traditional use of the plant in cancer treatment. As reported, many compounds may exhibit both carcinogenic and anticarcinogenic effects (Kohlmeier et al., 1995) but it is not excluded that the occurrence of compounds other than volatile constituents may act in the anticarcinogenic process.

Among the minor constituents found in this oil, it should be noted that several of them such as β-cyclocitral, β-ionone and actinidiolide are carotenoid derivatives as well as isophorone.

In conclusion, this study reveals that the volatile oil constituents of leaves of *X. americana* may vary significantly depending on the conditions of extraction. The Olacaceae family comprises 28 genera among which 13 are monospecific. Olacaceae are paraphyletic, a cladistic approach led to the identification of four lineages and the genus *Ximenia* (tribe Ximenieae) belongs to clade 2, together with genera *Curipira*, *Douradoa* and *Malania* (Malecot et al., 2004). Few chemical investigations regarding the volatile oil composition of members of the family Olacaceae have been reported so far: Bucek et al. (1987) showed that the oil obtained from stream distillation of *Ptychopetalum olacoides* (clade 3, tribe Olaceae) was mainly composed of α-pinene, α-humulene and β-pinene. New sesquiterpenes were isolated from Olacaceae, namely, scodopin (Wiart et al., 2001) from solvent extract of *Scorodocarpus borneensis* (clade 1, tribe Anacoloseae) and manicol (Banwell and Cameron, 1996) from *Dulacia guianensis* (clade 3, tribe Olaceae). None of the above-mentioned terpenic constituents was found in the volatile oil of *X. americana*. This suggests that isophorone may be of chemotaxonomic significance to the monotypic genus *Ximenia* which belongs to the tribe Ximenieae. Another question pointed out in this study is that surrounding the occurrence of chemicals identified as rodent carcinogens in plants used in folk medicine.

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