

Analysis of the Essential Oil of the Aerial Parts of the Medicinal Plant *Aristolochia indica* Linn. (Aristolochiaceae) from South-India

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The essential oil of the aerial parts of *Aristolochia indica* Linn. (Aristolochiaceae) from South-India was analyzed by gas chromatographic-spectroscopic (GC-FID and GC-MS) and olfactoric methods to identify those compounds responsible for the characteristic odor as well as partly for the folk medicinal use of this plant. Especially sesqui- and monoterpenes were found to be dominating constituents of this essential oil, such as: β -caryophyllene, α -humulene, ishwarone, caryophyllene oxide I, ishwarol, ishwarane and aristolochene as well as linalool and α -terpinolene.

The odor impression of the sample is described and the possible biological activity of some single volatiles shortly discussed.

Keywords: *Aristolochia indica* Linn., Aristolochiaceae, essential oil, aerial parts, odor, Indian folk medicine

Introduction

In continuation of a scientific cooperation on the analysis of volatiles of medicinal plant used in Indian folk medicine the essential oil of the aerial parts of *Aristolochia indica* was investigated.

Aristolochia indica Linn., commonly known as „Indian birthwort“ [1], belongs to a family of plants (Aristolochiaceae) which comprises nearly eighteen genera and over six hundred species of mostly perennial climbing shrubs [5]. The genus, *Aristolochia*, known to contain about 500 species, is distributed mainly in sub-tropical and tropical regions of the world. Of the many species of this genus, only five or six have been subjected to careful chemical analysis. In some papers dealing with the chemical composition of this plant, used in Indian folk medicine as an emmenagogue and as an abortifacient [1], especially sesqui-, di- and triterpenes were found to be dominant and of high importance for the above mentioned biological effects [1,5-8, 16-18], but studies only of the volatiles from *Aristolochia indica* could not be found upto now.

Results and discussion

The essential oil of the aerial parts of *Aristolochia indica* was olfactorically evaluated as follows: Smoky, leather-like, woody-earthy with weak green-caryophyllene-terpinene and floral-fruity side-notes.

Using gas chromatographic - spectroscopic systems more than 50 components could be identified in the essential oil of the aerial parts of *A. indica* (see Table 1). As main compounds (concentrations higher than 1%, calculated as %-peak-area of GC-FID analyses) were found: β -caryophyllene (58.4%), α -humulene (17.5%), ishwarone (2.8%), caryophyllene oxide I (1.4%), ishwarol (1.2%), linaool (1.1%) and α -terpinolene (1.0%).

Table 1: Composition of the essential oil of the aerial parts of *Aristolochia indica* Linn. from South-India.

Compounds ¹	conc. (%) ²	Identification ³
β -caryophyllene	58.4	GC, GC-MS, O
α -humulene	17.5	GC, GC-MS, O
ishwarone	2.8	GC-MS, R [5]
caryophyllene oxide I	1.4	GC, GC-MS
ishwarol	1.2	GC-MS, R [7]
linalool	1.1	GC, GC-MS, O
α -terpinolene	1.0	GC, GC-MS, O
ishwarane	0.8	GC-MS, R [6,11]
aristolochene	0.7	GC-MS, R [6,11]
cis-3-hexenol	0.5	GC, GC-MS, O
germacrene D	0.5	GC, GC-MS
octen-3-ol	0.4	GC, GC-MS, O
3-hexenyl acetate	0.4	GC, GC-MS, O
camphor	0.4	GC, GC-MS, O
nonanol	0.4	GC, GC-MS, O
humulene oxide	0.3	GC, GC-MS
nerolidol	0.3	GC, GC-MS, O
β -farnesene	0.3	GC, GC-MS, O
β -bisabolene	0.3	GC, GC-MS
pinocarveol	0.3	GC, GC-MS, O
δ -cadinol	0.3	GC, GC-MS, O
β -elemene	0.3	GC, GC-MS, O
α -terpineol	0.2	GC, GC-MS, O
β -farnesol	0.2	GC, GC-MS, O
octanol	0.2	GC, GC-MS, O
caryophyllene oxide II	0.2	GC, GC-MS
α -bisabolene	0.2	GC, GC-MS
phytol	0.2	GC, GC-MS
β -bisabolol	0.1	GC, GC-MS
germacrene A	0.1	GC, GC-MS
ledol	0.1	GC, GC-MS
2-octanol	0.1	GC, GC-MS
hexyl acetate	0.1	GC, GC-MS, O
thymol	0.1	GC, GC-MS, O
indole	0.1	GC, GC-MS, O

β -phellandrene	0.1	GC, GC-MS, O
tetradecanol	0.1	GC, GC-MS
5 β H,7 β ,10 α -selina4(14),11-diene	0.1	GC-MS, R [8]
β -pinene	0.1	GC, GC-MS, O
borneol	tr ⁴	GC, GC-MS, O
terpinene-4-ol	tr	GC, GC-MS, O
β -selinene	tr	GC, GC-MS
hexanol	tr	GC, GC-MS, O
(12S)-7,12-secoishwaran-12-ol	tr	GC-MS, R [18]
camphene	tr	GC, GC-MS, O
tricyclene	tr	GC, GC-MS
Fatty acids and their esters (totally 6 compounds)	4.2	
Higher hydrocarbons (more than 16C; totally 4 compounds)	2.3	

¹in order of their concentrations

²concentrations as %-peak-area calculated by GC-FID analysis

³Identification by: GC=retention time, GC-MS=mass spectra, O=olfactometry, R=reference

⁴trace compound less than 0.1%

The correlation of gas chromatographic-spectroscopic data with olfactoric ones can be shown as follows: The identified main compounds β -caryophyllene, α -humulene, caryophyllene oxide I, linalool and α -terpinolene are responsible especially for the side-notes (green-caryophyllene-terpinene as well as floral-fruity), while the characteristic smoky, leather-like and woody-earthy odor can be attributed to further sesquiterpenes, octane-derivatives and N-heterocycles (in non-detectable concentrations by using the above mentioned methods).

Some therapeutic effects of the essential oil of the aerial parts of *Aristolochia indica* from South-India may be possible by the comparison of identified constituents with published applications:

1.) Some main or minor compounds of the investigated *A. indica* sample are ishwarane-derivatives with reported effects on fertility, especially in animal

experiments [1,5-8,16-18]. Therefore, also these activities can be expected by the use of the essential oil sample of these *Aristolochia* species in similar testings.

2.) The identified main compounds β -caryophyllene, α -humulene, caryophyllene oxide I, linalool and α -terpinolene are well-known natural products possessing antimicrobial effects [9,12,14]. So this essential oil of *A. indica* from South-India may be used as a powerful medicine in this field.

3.) Additional β -caryophyllene and linalool (concentration of both compounds together about 60%) show sedative effects [12] and an application as mild sedative seems to be effective.

In conclusion we can report, that the essential oil of the aerial parts of *Aristolochia indica* Linn. from South-India is rich in sesquiterpenes (and monoterpenes) with known effects on fertility in animal experiments and on the field of antimicrobiology as well as on the treatment of sedation problems. Additionally, this essential oil possesses a very characteristic smoky, leather-like, woody-earthy, green-caryophyllene-terpinene-like and floral-fruity odor.

Experimental

Plant Material:

The aerial parts of *Aristolochia indica* Linn. were collected at the Calicut University campus in summer 1999 and the plant material identified by Dr.A.K. Pradeep, Department of Botany, Calicut University of Kerala. A voucher specimen was deposited in the specially maintained Herbarium of the Department of Chemistry at Calicut University (voucher-no. 28).

Essential Oil Extractions:

The aerial parts of *Aristolochia indica* (100g) were cut into small pieces and ground into a paste using an electric grinder. This paste was distilled by steam for 2.5 hours. The distillate was extracted with diethyl ether (100mL for 2-times) and

dried over anhydrous sodium sulphate. After the removal of the solvent a yield of 0.15g light green essential oil of *A. indica* was obtained.

Olfactoric evaluations:

10µl of a solution of the essential oil in dichloromethane were placed on a commercial odor-strip (Dragoco Co.) and its odor characterized by professional perfumers.

Gas Chromatography:

GC analyses were carried out using a Shimadzu GC-14A with FID and the integrator C-R6A-Chromatopac and a Varian GC-3700 with FID and the integrator C-R1B-Chromatopac (Shimadzu Co.). As columns a 30mx0.32mm bonded unpolar FSOT-RSL-200 fused silica (film-thickness: 0.25µm, Biorad Co.) and a 30mx0.32mm bonded polar Stabilwax (film-thickness: 0.50µm; Restek Co.) were used. Additional parameters as follows: Carrier gas: hydrogen; injector-temperature: 250°C; detector-temperature: 320°C; temp.-programs: 40°C/ 5min. to 280°C/ 5min. with a heating-rate of 6°C/ min.; quantifications by %-peak-area-calculations (unpolar column);

Some single components could be identified by co-injection of pure compounds and correlation of their retention-times (using Kovats indices) with published data [3,10,13,19].

Gas chromatography - Mass spectrometry:

The samples were analyzed by the GC-MS systems Shimadzu GC-17A with QP5000 and the data system Compaq-ProLinea (class5k-software), Shimadzu GC-17A with QP5050 and data system Pentium-II (Böhm Co., class5k-software), Hewlett-Packard GC-HP5890 with HP-5970MSD and PC-Pentium (Böhm Co., ChemStation-software) and Finnigan MAT GCQ with data system Gateway-2000-PS75 (Siemens Co., GCQ-software). Additional parameters are as

follows: Carrier gas: helium; injector-temperature: 250°C; interface-heating: 300°C; ion-source-heating: 200°C; EI-mode; scan-range: 41-450amu; other parameters see GC-FID-part.

For compound identifications Wiley-, NBS- and NIST-library spectra (on-line) as well as reference MS-spectra data [2,4,10,11,15,20-22] were used.

Acknowledgments

We acknowledge the olfactoric evaluations by Mr. W. Höppner and Mr. V. Hausmann, chief-perfumers of Dragoco Co., the botanical identification of the plant material by Dr.A.K. Pradeep, Department of Botany, Calicut University Kerala and the financial support (scholarship for Ch. Puschmann) by the University of Vienna.

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