Investigation of plant-derived products with the *in vitro* comet assay

L. Verschaeve  
WIV-ISP, Toxicology Unit  
& Univ. Antwerp, Dept. Biomedical Sciences
TRADITIONAL MEDICINAL PLANTS
Medicinal plants (South Africa)

>80% of medication in SA = traditional medicinal plants
Plants were never tested the way our medicines are tested. Harmful effects cannot be excluded (e.g., Hypoxis).

Search for new medicines, e.g., against cancer (e.g., Rotheca).

Traditional medicinal plants offers many opportunities.

Also for veterinary use (e.g., plant extracts against mycotoxins).

Study of toxicity (NRU), genotoxicity (micronucleus test, Ames assay, ...) and antigenotoxicity.
Example of test strategy

**Gene mutations**
- Ames test
- *Hprt* test
- MLA
- *MN* \textit{vitro} test
- *CA* \textit{vitro} test

**Chromosomal aberrations**
- *MN* \textit{vivo} test
- *CA* \textit{vivo} test

\textit{in vitro} tests
- positive

\textit{in vivo} tests
- comet assay
- positive

- Carcinogenicity test
- positive
Plant extracts

Different plant extraction protocols exist, e.g.,

MeOH
DCM
Screening of large numbers of extracts/fractions, …

Bacterial Vitotox test (corr. Ames assay)

Vitotox test

**Genox/Cytox ratio Extract 1 MeOH**

- 0 mM
- 0.02 mg/ml
- 0.1 mg/ml
- 0.5 mg/ml

**Genox/Cytox ratio + S9 Extract 1 MeOH**

- 0 mM
- 0.02 mg/ml
- 0.1 mg/ml
- 0.5 mg/ml

**Genox Extract 33 + 1 µg/ml AFB1**

- 0 mM
- 0.02 mg/ml
- 0.1 mg/ml
- 0.5 mg/ml

**Cytox Extract 33 + 1 µg/ml AFB1**

- 0 mM
- 0.02 mg/ml
- 0.1 mg/ml
- 0.5 mg/ml
Vitotox test:

- Rapidity of first screening
- Sometimes difficulties in interpretation
- Based on SOS induction/gene mutations only
- Other tests to confirm and complete an evaluation

- Ames assay ....
  histidine content & antibacterial properties of many plants
- Comet assay can be a good alternative
## EXAMPLES COMET ASSAY OF PLANT EXTRACTS

<table>
<thead>
<tr>
<th>Plant</th>
<th>Plant part used</th>
<th>Extraction procedure</th>
<th>Control 250 ppm</th>
<th>500 ppm Kruskall-Wallis ANOVA test</th>
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<td>MeOH</td>
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<td>1.91*</td>
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</table>

†The median tail DNA content is given for each of the concentrations tested. Statistical significance as evaluated with the Mann-Whitney U-test (each dose compared separately with the corresponding control) and Kruskall-Wallis ANOVA test (all groups compared), and indicated as follows: *p < 0.05; **p < 0.01; ***p < 0.001. L, leaves; T, twigs; R, roots; B, bark; ANOVA, analysis of variance.
Vitotox first (concordance with Ames assay)
Then comet assay:
- Few concentrations (0.1 & 0.5 mg/ml)
- C3A cells (largely conserved their biotransformation capacities)
large number of extracts can be tested in a ‘relative’ short period of time

<table>
<thead>
<tr>
<th>Plant</th>
<th>Vitotox &amp; Ames test –S9</th>
<th>Vitotox &amp; Ames test + S9</th>
<th>Comet assay (C3A)</th>
<th>Micronucleus test –S9</th>
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Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants

L. Verschaeve, J. Van Staden

*Scientific Institute of Public Health, Department of Epidemiology and Toxicology, J. Wytensinstraat 14, B-1050 Brussels, Belgium
University of Antwerp, Department of Biomedical Sciences, B-2610 Antwerp, Belgium
Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3201, South Africa

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Mutagenicity

ABSTRACT

Aim of the study: The aim of this paper was to summarize the results of our investigations on the in vitro genotoxic as well as antigenotoxic effects of a great number of selected South African traditional medicinal plants.

Materials and methods: Investigations of methanol and dichloromethane extracts of selected plants were conducted with the bacterial Ames, Umu-C and VITOTOX® tests, and with the cytochalasin B micronucleus test and alkaline comet assay in human white blood cells.

Results: A number of extracts were found to have genotoxic properties. Amongst the genotoxic plant extracts, especially methanol extracts of Helichrysum simillimum DC. (Asteraceae) should be highlighted. On the other hand, some plant extracts also showed antimutagenic potential. Here Bauhinia galpinii N.E.Br. (Fabaceae) and especially Chlerodendrum myricoides (Hochst.) Vatke (=Rotheca myricoides (Hochst.) Steane & Mabb.; Lamiaceae) appear to have antimutagenic properties.

Conclusion: The safe use of Helichrysum simillimum should be questioned and further investigations on its mutagenicity and overall biological properties should be encouraged. Antimutagenic properties of especially Bauhinia galpinii and Rotheca myricoides are considered of particular interest as it may be assumed that these antimutagenic natural substances are able to lower the cancer risk from everyday exposures to environmental mutagens as well as to mutagenic pharmaceuticals.

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Vitotox and comet assays are complementary and allow a relatively rapid screening of many plant extracts and fractions.

We used it for genotoxicity and antigenotoxicity testing of traditional medicinal plant extracts, but also for other studies on natural products:

→ Novel TB chemotherapeutic drugs
Search for new anti-tuberculosis agents

Original article

Straightforward palladium-mediated synthesis and biological evaluation of benzo[j]phenanthridine-7,12-diones as anti-tuberculosis agents

Davie Capponen a,1, Jan Jacobs b,1, Tuyen Nguyen Van b, Sven Claessens b, Gaston Diels c, Roel Anthonissen d, Thorbjorg Einarsdottir a, Maryse Fauville d, Luc Verschaeve e, Kris Huygen a,**, Norbert De Kimpe b,*

a Service Immunology, O.D. Communicable & Infectious Diseases, Scientific Institute of Public Health (Site Ukkel), Engelststraat 642, B-1180 Ukkel, Belgium
b Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium
\( ^* \) Johnson & Johnson Pharmaceutical Research & Development, Synthesis Enabling Technologies, Turnhoutseweg 30, B-2340 Beerse, Belgium
c ServiceBacterial Diseases, O.D. Communicable & Infectious Diseases, Scientific Institute of Public Health (Site Ukkel), Engelststraat 642, B-1180 Ukkel, Belgium
d ServiceBacterial Diseases, O.D. Communicable & Infectious Diseases, Scientific Institute of Public Health (Site Ukkel), Engelststraat 642, B-1180 Ukkel, Belgium
\( ^* \) O.D. Public Health and Surveillance, Scientific Institute of Public Health (Site Elsele), J. Witsmanstraat 14, B-1050 Brussels, Belgium

ABSTRACT

In 1991, WHO recognized the resurgence of tuberculosis as a global health problem. Although modern chemotherapy is effective against the causative pathogen Mycobacterium tuberculosis, the current drug regimens have failed to eradicate the disease. The success of the pathogen, partially attributed to drug resistance, necessitates the development of novel anti-tuberculosis drugs. Benzo[j]phenanthridine-7,12-diones, tetracyclic derivatives of the natural product benz[g]isoquinoline-5,10-dione, were conveniently synthesized via palladium-catalyzed intramolecular cyclization of N-methanesulfonyl-3-bromo-2-(arylamino)methyl-1,4-naphthoquinones. Here we report on the bioactivity of eight benzo[j]phenanthridine-7,12-dione derivatives as candidate drug molecules against M. tuberculosis and on their cytotoxicity on C3A human hepatocytes. The strongest antimicrobial activity (as detected by growth inhibition of bacteria, using luminometry and BACTEC 460-TB) and lowest cytotoxicity was found for 3-methylbenzo[j]phenanthridine-7,12-dione 5e, which was also effective in targeting intracellular M. tuberculosis (in murine J774 macrophages) and was not genotoxic for C3A hepatocytes.
Synthesis and antimycobacterial activity of analogues of the bioactive natural products sampangine and cleistopholine

Pieter Claes a,1, Davie Cappoen b,1, Blaise Mavinga Mbala a, Jan Jacobs a, Birgit Mertens d, Vanessa Mathys c, Luc Verschaeve d,e, Kris Huygen b,*, Norbert De Kimpe a,**

a Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium
b Scientific Service Immunology, O.D. Communicable & Infectious Diseases, Scientific Institute of Public Health (Site Ukkel), Engelandstraat 642, B-21180 Ukkel, Belgium
c Program Tuberculosis and Mycobacteria, O.D. Communicable & Infectious Diseases, Scientific Institute of Public Health (Site Ukkel), Engelandstraat 642, B-1180 Ukkel, Belgium
d Program Toxicology, O.D. Public Health and Surveillance, Scientific Institute of Public Health (Site Elsene), J. Wytsmanstraat 14, B-1050 Brussels, Belgium
e Department of Biomedical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium

ABSTRACT

Identification and investigation of novel classes and compounds for the treatment of tuberculosis remains of utmost importance in the fight against the disease. Despite many efforts, the weakly gram positive Mycobacterium tuberculosis keeps demanding its toll in human lives. For this reason a small library of substituted and unsubstituted aza analogues of cleistopholine and sampangine were synthesized in a short and straightforward manner and tested in vitro against M.tuberculosis. The compounds showed promising activity against the M.tuberculosis H37Rv strain and Minimal Inhibitory Concentrations (MIC) could be observed as low as 0.88 μM. Accompanied by moderate acute toxicity against C3A hepatocytes, the therapeutic index showed an acceptable range. Further tests confirmed the inhibition by up to 74% of intracellular growth of M.tuberculosis inside macrophages conferred by 1-hydroxybenzo[g]isoquinoline-5,10-diones. Activity of the library against other clinically relevant mycobacterial species such as Mycobacterium bovis, Mycobacterium avium and Mycobacterium ulcerans was confirmed. Furthermore the activity against a multi-drug-resistant MDR LAM-1 M.tuberculosis strain was tested and the MIC value situated around 1 μM. The lacking genotoxicity of a group of enamine substituted cleistopholine analogues indicates this series to be promising targets for further development.

Keywords:
Sampangine
Cleistopholine
Pyridinium ylids
Tuberculosis
Antibiotics
Acute toxicity
SMOKE AND SMOKE COMPOUNDS
SMOKE AND SMOKE COMPOUNDS
Tests of:
- smoke water
- crude extracts
- isolated active compounds
- *in vivo* testing in the plants (comet assay)
Genetic toxicity testing of 3-methyl-2H-furo[2,3-c]pyran-2-one, an important biologically active compound from plant-derived smoke

Luc Verschaeve a, Jef Maes a, Marnie E. Light b, Johannes van Staden b,*

a VITO, Expertise Centre of Environmental Toxicology, Boeretang 200, B-2400 Mol, Belgium  
b Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

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Abstract

Aerosol smoke, aqueous smoke solutions and 3-methyl-2H-furo[2,3-c]pyran-2-one isolated from plant-derived smoke stimulate seed germination and enhance seedling vigour of many species. Consequently, smoke technology has important potential applications in agriculture, horticulture and other environmental sectors. However, use of 3-methyl-2H-furo[2,3-c]pyran-2-one as a germination promoter and/or growth stimulant is only possible provided it can be shown that it is not hazardous, i.e. not toxic or genotoxic. We therefore performed an investigation to evaluate the genotoxic effects of this compound in five strains of Salmonella typhimurium in the Ames test (1.5 μg/plate to 7.5 ng/plate), and in the VITOTOX® test (1500–0.045 ppb). In all tests performed, no mutagenic activity was induced by 3-methyl-2H-furo[2,3-c]pyran-2-one, with or without S9 metabolic activation. Due to the potential wide use of this smoke-derived compound, it is of importance that no toxic and genotoxic effects were observed at the concentrations tested.

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Effect of smoke-water and a smoke-isolated butenolide on the growth and genotoxicity of commercial onion

M.G. Kulkarni\textsuperscript{a}, G.D. Ascough\textsuperscript{a}, L. Verschaeve\textsuperscript{b, c}, K. Baeten\textsuperscript{b, c}, M.P. Arruda\textsuperscript{d}, J. Van Staden\textsuperscript{a,*}

\textsuperscript{a} Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa
\textsuperscript{b} Laboratory of Toxicology, Scientific Institute of Public Health (IPH), B 1050 Brussels, Belgium
\textsuperscript{c} Department of Biomedical Sciences, University of Antwerp, B 2610 Antwerp, Belgium
\textsuperscript{d} Departamento de Plantas de Lavoura, Faculty of Agronomy, Federal University of Rio Grande do Sul, Av. Bento Gonçalves 7712, Caixa Postal 15.100, CEP 91501-970 Avenida, Porto Alegre, Rio Grande do Sul, Brazil

\textbf{ABSTRACT}

Smoke-water and a biologically active butenolide compound (3-methyl-2H-furo[2,3-c]pyran-2-one) derived from burning plant material, show stimulating effects on a number of agricultural and horticultural crops. In these trials, onion (\textit{Allium cepa} L.) plants were treated (drenched) with either a 1:500 (v/v) smoke-water solution or a butenolide solution of 10^{-10} M under greenhouse conditions. Onion plants supplied with smoke-water and butenolide solution exhibited a significantly greater number of leaves, increased leaf length, and a higher fresh and dry leaf weight than untreated plants at 175 days after seed sowing (DASS) (third harvest). In addition, smoke-water and butenolide-treated onion plants exhibited a significantly higher bulb diameter and bulb weight than untreated plants, when these plants were harvested at 175 DASS. Overall, smoke-water was more effective than butenolide and achieved the highest harvest index. Genotoxicity was not detected in the bulbs of onion when they were treated with either smoke-water or butenolide.

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Short communication

Genotoxicity testing of 3,4,5-trimethylfuran-2(5H)-one, a compound from plant-derived smoke with germination inhibitory activity

Marnie E. Light a,*, Roel Anthonissen b, Annemarie Maes b, Luc Verschaeve b, c, Martin Pošta d, Johannes Van Staden a

a Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, South Africa
b Laboratory of Toxicology, O.D. Public Health and Surveillance, Scientific Institute of Public Health, Brussels, Belgium
c Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium
d Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Flemingovo nám. 2., 166 10, Prague 6, Czech Republic

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ABSTRACT

Plant-derived smoke and certain smoke compounds improve seed germination and enhance seedling growth of many species. Thus, smoke-infused water and the active smoke-derived compounds have the potential to be used in different agricultural and horticultural applications. However, despite these interesting and potentially practical properties, it should also be ascertained whether such compounds may pose a health risk, particularly if they are to be used in the production of food or fodder crops. Amongst some of the aspects that would be important to understand are any possible genotoxic properties that the compounds may possess due to potential carry-over effects. Here, we report on a genotoxicity study of 3,4,5-trimethylfuran-2(5H)-one, a compound from plant-derived smoke previously shown to have germination inhibitory activity. Using two in vitro tests, namely the bacterial VITOTOX® test (with/without S9 metabolic activation) and the cytome assay on human C3A cells, no genotoxicity or toxicity was found. Furthermore, these results support a previous study where a related smoke-derived compound with germination promoting properties was investigated.

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ATTENTION SHOULD BE PAID TO:

1. Histinine content of plants (Ames assay)
2. Antibacterial properties of plants (Ames assay)
3. Janus mutagens/carcinogens

An agent (extract/fraction) can be antimitageneic against a given mutagen but not or even comutagenenic with another mutagen.

→ Against which mutagen(s) should we test antimitageneic or antigenotoxic effects?
ATTENTION SHOULD BE PAID TO:

4. Plants may have different properties according to the location where they grow. For example, in polluted regions: uptake and accumulation of toxic metals may result in physiological changes such as inactivation of certain enzymes and blocking functional groups of metabolically important molecules.

5. Plant metabolism should be taken into account. Smokers metabolise foreign compounds with their own plant metabolic systems that may differ from those in mammals. Therefore, we most probably need to test a compound (smoke compound) also with for example S10 (from plant origin) rather than S9.