South African Journal of Botany 2002, 68: 408–410
Printed in South Africa — All rights reserved

SOUTH AFRICAN JOURNAL
OF BOTANY
ISSN 0254–6299

Short Communication

The use of plants in traditional medicine: potential genotoxic risks

EE Elgorashi[1,2,3], JLS Taylor[1,2,3], A Maes[1], N de Kimpe[2]*, J van Staden[3] and L Verschaeve[1]*

[1] Division of Environmental Toxicology, Flemish Institute for Technological Research (VITO), Boeretang 200, B-2400 Mol, Belgium
[2] Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, Ghent University, B-9000 Gent, Belgium
[3] Research Centre for Plant Growth and Development, University of Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa
** Corresponding authors, e-mail: luc.verschaeve@vito.be, norbert.dekimpe@rug.ac.be*

Received 20 December 2001, accepted in revised form 1 May 2002

Dichloromethane and 90% methanol extracts of different parts of *Antidesma venosum*, *Balanites maughamii, Chaetacme aristata, Croton sylvaticus, Gardenia volkensii*, *Plumbago auriculata* and *Spirostachys africana* which are commonly used in South African traditional medicine were evaluated for their mutagenic potential. The genotoxicity tests used were the Ames test, micronucleus test, comet assay and VITOTOX® test. All species showed mutagenicity or DNA damage in at least one test. The species, organ extracted, extraction solvent and the type of test used, (whether based on bacterial or human cells), could affect the induction of genotoxicity.

Man has, for centuries, used plants as the primary therapeutic agent in medicine. The discovery of antibiotics, however, led to the development of a pharmaceutical industry in the second half of the last century that relied heavily on pure single active natural compounds and synthetic drugs (Eloff 2000). As a result, the use of traditional medicine has declined or almost disappeared in many industrialised countries. At the same time, professional medicine practitioners and the pharmaceutical companies publicly dismissed traditional medicine as unsafe, backward, medieval and linked with magic and quackery (Walker 1999). Despite these campaigns, traditional medicine has recently been proved to have a solid scientific basis, with increasing numbers of publications appearing on work related to the screening for, and isolation of, bioactive compounds from plants used by traditional healers. Moreover, ethnomedicine has been one of the approaches used for the selection of plants for the isolation of natural compounds for use as pharmaceuticals, pesticides, foodstuffs, flavours, scents and as industrial feedstocks.

In developing countries traditional medicine is still widely used and is incorporated in almost 65% of the world's population primary health care systems (Fabricant and Farnsworth 2001). In addition, the increasing demand for natural products in industrialised countries has added to the popularity of traditional medicines. The plants used in traditional medicine are assumed to be safe, due to their long-term use by humans. Recent research has shown potential mutagenicity of some medicinal plants. Many mutagens of plant origin have been identified, some of which are capable of inducing or promoting tumors in man (Schimmer *et al*. 1994, Kassie *et al*. 1996).

In this study, different parts of seven plant species used in South African traditional medicine namely, *Antidesma venosum*, *Balanites maughamii, Chaetacme aristata, Croton sylvaticus, Gardenia volkensii*, *Plumbago auriculata* and *Spirostachys africana* were investigated for potential mutagenic effects (Table 1). The plants were selected on the basis of their ethnobotanical use and availability (Hutchings *et al.* 1996, Van Wyk *et al.* 1997). The dried plant material was extracted using a sonication bath (40°C) for 30min. Sequential extractions with dichloromethane (CH_2Cl_2) and 90% methanol (CH_3OH) (10mlg^{-1}) were performed. The crude extracts were filtered and the filtrates were dried under vacuum. The CH_2Cl_2 and 90% CH_3OH extracts, after suspension in 10% dimethyl sulfoxide (10% DMSO), were tested in a battery of bacterial and mammalian cell assays. These assays included the bacterial *Salmonella typhimurium* (TA98) Ames test (Maron and Ames 1983), VITOTOX® (Verschaeve *et al*. 1999), comet assay (Singh *et al.* 1988) and micronucleus test (Van Hummelen and Kirsch-Volders 1990, Fenech 2000). Dilutions tested were 5 000µgml^{-1}, 500µgml^{-1} and 50µgml^{-1} for Ames and VITOTOX® tests while, 2 500µgml^{-1}, 500µgml^{-1}, and 250µgml^{-1} were used in the micronucleus and comet assays. 10% DMSO was included as a control.

The Ames test using *S. typhimurium* strain TA98 detects frame shift mutations involving the *histidine* operon, whereas the VITOTOX® test detects DNA damage in *S. typhimuri-*

Table 1: Induction of genotoxic effects in four different genotoxicity tests by CH_2Cl_2 and 90% CH_3OH plant extracts

Plant Species	Use in Traditional Medicine	Plant Part	Family	Dichloromethane Extracts				Methanol Extracts (90%)			
				Ames[1] Test (TA98)	Vitotox[1]	Comet Assay	MN[2] Test	Ames[1] Test (TA98)	Vitotox[1]	Comet Assay	MN[2] Test
Antidesma venosum E. Mey. ex Tul.	Leaves, twigs: Abdominal pain,enema	Leaf	Euphorbiaceae	-/-	-/-	-	+	-/-	-/-	-	-
		Twigs		-/-	-/-	+	+	-/-	-/-	-	-
Balanites maughamii Sprague	Roots, bark: Mulluscicidal properties	Leaf	Balanitaceae	-/-	-/-	+	+	-/-	-/-	-	+
		Twigs		-/-	-/-	+	+	-/-	-/-	+	-
Chaetacme aristata Planch.	Bark, roots: Haemorrhoids	Leaf	Ulmaceae	+/+	-/-	-	-	-/-	-/-	-	-
		Twigs		-/-	-/-	-	-	-/-	-/-	-	+
Croton sylvaticus Hochst.	Bark, roots: Abdominal, internal inflammation, uterine disorders, tonic, fabrile conditions, purgative, pleurisy, indigestion, TB, rheumatism	Leaf	Euphorbiaceae	-/-	-/-	+	+	-/-	-/-	-	-
		Twigs/bark		-/-	-/-	+	+	-/-	-/-	-	+
Gardenia volkensii K. Schum.	Fruit/roots: Emetics, sore eyes, headache, asthma, dysmenorrhoea, infertility, epilepsy, convulsion, earache	Leaf	Rubiaceae	-/-	-/-	-	+	-/-	-/-	-	-
		Twigs/bark		-/-	-/-	-	- (t)	-/-	-/-	+	-
Plumbago auriculata Lam.	Roots, leaves: Headache, emetics, warts, fractures, scrofula, oedema, malaria, skin lesions	Foliage	Plumbaginaceae	-/-	-/-	-	+	-/-	-/-	-	-
		Twigs		+/+	-/-	-	-	-/-	-/-	+	+
Spirostachys africana Sond.	Wood: Stomach ulcers, acute gastritis, eye washes, headaches, rashes, boils, emetics, renal ailments, purgative, bloodpurifiers, diarrhoea, dysentry	Leaf	Euphorbiaceae	-/-	-/-	-	-	-/-	-/-	+	-
		Twigs/bark		-/-	-/-	+	+	-/-	-/-	+	+

(+): positive genotoxic response, (-): negative genotoxic response, (t): toxic.
[1] : With/without S9.
[2] : Micronucleus test

um TA104 resulting in SOS-induction (which is an attempt of the cell to stay alive when other repair mechanisms have failed). The alkaline comet assay detects single strand DNA damage and alkali labile sites, whereas structural and/or numerical chromosome aberrations are detected by the micronucleus test.

The genotoxic response of CH_2Cl_2 and 90% CH_3OH extracts of different parts of each plant species is summarised in Table 1. All plant species tested showed genotoxicity in at least one of the assays used.

All plant extracts showed a negative genotoxic response in the VITOTOX® test. Only CH_2Cl_2 extracts from the leaves of *C. aristata* and twigs of *P. auriculata* were mutagenic in the Ames test. In the micronucleus test, all plant species were genotoxic, although, the positive response differed with the plant part tested and the extracting solvent. The CH_2Cl_2 extracts of *A. venosum*, *B. maughamii* and *C. sylvaticus* showed genotoxic activity irrespective of the plant part tested, whereas, the mutagenic response of the 90% CH_3OH extracts were plant part specific.

The results of the present study indicated that CH_2Cl_2 extracts of *C. aristata* and *P. auriculata* induce frame shift mutations in the bacterium species used in the Ames test. Extracts of almost every species caused either DNA damage detected by the comet assay, or chromosomal aberations and/or non-disjunction or chromosome lagging in human white blood cells detected in the micronucleus test.

The plant extracts tested in this study were crude extracts comprising a complex mixture of organic compounds. Further research, including bioassay-guided fractionation of these extracts, is necessary to identify the compounds responsible for the genotoxic response of the plant extracts. Compounds such as isothiocyanates and quercetin, present in some plant species, do act as genotoxins (Schimmer *et al*. 1994, Kassie *et al*. 1996).

It is well known that genotoxicity, especially mammalian DNA damage, is repairable by self-DNA repair systems. Despite this, the results of this study raise concern about the safety of the long-term use of these plants in traditional medicine. It is also possible that these plants are good candidates for anticancer drug research as many cancer chemotherapeutic agents are mutagenic for example in the *Salmonella*/microsome test (Benedict *et al.* 1977, Senio *et al*. 1978).

Aknowledgements — The authors thank Ms M Light, Research Centre for Plant Growth and Development, University of Natal Pietermaritzburg, South Africa for plant collection, Mr U van Gorp, Flemish Institute for Technological Research (VITO), Belgium, for help with the comet assay. The research was supported by the Bilateral Scientific and Technological Cooperation Project between Flanders (Belgium) and South Africa (BIL project 98/77).

References

Benedict WF, Baker MS, Haroun E, Choi E, Ames BN (1977) Mutagenicity of cancer chemotherapeutic agents in

Salmonella/microsome test. ***Cancer Research* 37**: 2209–2213
Eloff JN (2000) On expressing the antibacterial activity of plant extracts — a small first step in applying scientific knowledge to rural primary health care. ***South African Journal of Science* 96**: 116–118
Fabricant TS, Farnsworth NR (2001) The value of plants used in traditional medicine for drug discovery. ***Environmental Health Perspective* 109**: 69–75
Fenech M (2000) The *in vitro* micronucleus technique. ***Mutation Research* 455**: 81–95
Hutchings A, Scott AH, Lewis G, Cunningham A (1996) Zulu Medicinal Plants. University of Natal Press, Pietermaritzburg. ISBN 0 86980 893 1
Kassie F, Parzefall W, Musk S, Johnson I, Lamprecht G, Sontag G, Knasmuller S (1996) Genotoxic effects of crude juices from *Brassica* vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells. ***Chemico-Biological Interactions* 102**: 1–16
Maron DM, Ames BN (1983) Revised methods for the *Salmonella* mutagenicity test. ***Mutation Research* 113**: 173–215
Schimmer O, Kruger A, Paulini H, Haefele F (1994) An evaluation of 55 commercial plant extracts in the Ames mutagenicity test. Pharmazie: 448–451
Senio Y, Nagao M, Yahagi T, Hoshino A, Kawachi T, Sugimura T (1978) Mutagenicity of several classes of antitumor agents to *Salmonella typhimurium* TA98, TA100 and TA92. ***Cancer Research* 38**: 2148–2156
Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. ***Experimental Cell Research* 175**: 184–191
Van Hummelen P, Kirsch-Volders M (1990) An improved method for the *in vitro* micronucleus test using human lymphocytes. ***Mutagenesis* 5**: 203–204
Van Wyk B-E, Van Oudtshoorn B, Gericke N (1997) Medicinal Plants of South Africa. Briza Publication, Pretoria. ISBN 1 875093 09 5
Verschaeve L, Van Gompel J, Thilemans L, Regniers L, Vanparys P, Van der Lelie D (1999) VITOTOX® bacterial genotoxicity and toxicity test for the rapid screening of chemicals. ***Environmental and Molecular Mutagenesis* 33**: 240–248
Walker MJ (1999) The resurgence of natural medicine. ***The Ecologist* 29**: 226–228

Edited by AK Jäger