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Genotoxicity and Antigenotoxicity of selected South African indigenous plants

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ABSTRACT

Twenty-two plant species extracted with dichloromethane and 90% methanol were investigated for their genotoxicity as well as antigenotoxicity against aflatoxin B_1 induced-mutagenicity using the Ames (*Salmonella typhimurium* strains TA98 and TA100) and Vitotox assays in the presence of S9 rat liver fraction. The results obtained from Ames assay for some plant extracts correlated well with the results obtained from the Vitotox assay. Dichloromethane and methanolic extracts of *Helichrysum petiolare*, *Protea hybrid*, *Protea roupelliae*, *Artabotrys brachypetalus* (*leaves*), *Friesodielsia obovata*, *Hexalobus monopetalus*, *Monanthotaxis caffra*, *Monodora junodis*, *Uvaria caffra*, *Xylopia parviflora*, *Podocarpus henkellii*, *Rhoicissus sekhukhuniensis*, *Podocarpus elongatus* and *Agapanthus praecox* had moderate to strong antimutagenic activities in both Ames and Vitotox assays. The methanolic extract of *Annona senegalensis* and dichloromethane extract of *Podocarpus falcutus* also showed antigenotoxic potentials against aflatoxin B_1 induced mutagenicity. Methanolic extracts of *Xylopia* sp., showed a co-mutagenic effect with aflatoxin B_1 in the Ames assay (strain TA100). All extracts were not genotoxic in the Vitotox assay in the absence of S9. Plant extracts with promising antimutagenic effects could be used in the form of feed and food supplements as a preventative strategy against aflatoxin B_1 induced mutagenicity and carcinogenicity.

1. Introduction

Deoxyribonucleic acid (DNA) damage in living organisms occurs spontaneously or could be induced by genotoxins and can lead to gene mutations, chromosomal aberrations and rearrangement of the chromosomes through translocation, deletion and inversion (Sloczynska et al., 2014). Mutagenicity plays a crucial role in carcinogenesis and it may lead to different types of cancers and genetic diseases, which are increasing at an alarming rate in human beings and animals (Nagarathna et al., 2013). Globally, cancer is one of the leading diseases and is expected to become the leading cause of morbidity and mortality in the next decades (Canceratlas.cancer.org, 2014).

Aflatoxins, a class of mycotoxins, contaminate various foodstuffs including animal feeds and foods such as nuts, corn, cereals, oilseeds, and dehydrated foods during production, harvest, storage and food processing (Bennett and Klich, 2003; Madrigal-Santillan et al., 2010). They are the most common known mutagens and linked with the incidences of genetic diseases, especially hepatocellular cancer and other liver diseases such as aflatoxicosis. Aflatoxins consist of four major groups namely, B_1, B_2, G_1 and G_2 (Zain, 2011). However, aflatoxin B_1 is the most potent genotoxin, highly mutagenic and carcinogenic metabolite known so far. They are recognized as human carcinogens (class 1) by the international agency for research on cancer (IARC). Aflatoxin B_1 is metabolized in the liver cells by cytochrome P450 enzyme into a highly reactive aflatoxin B_1-8, 9-epoxide, which binds to the guanine residues forming G to T transversion mutation. This biotransformation of aflatoxin B_1 induces DNA adducts which leads to mutation, genetic and oxidative damage, thus resulting in cancer (Tiemersma et al., 2001; Bhat et al., 2010; Ferrante et al., 2012).

Various strategies have been employed in the control and prevention of contamination with aflatoxins, but most of them have major drawbacks that limit their use, starting from limited efficacy due to limitless reservoir to loss of essential nutrients and high costs. Therefore,

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potential strategies that will detoxify aflatoxins without altering the nutritional value of food and feed are needed. Scientists today are exploring the plant kingdom to search for antimutagens or anticarcinogens that are capable of decreasing or inhibiting the mutagenic effects of aflatoxins (Alabi et al., 2011; Sloczynska et al., 2014). Plants contain many bioactive compounds with promising activity against many diseases including genetic diseases such as cancer that could be explored for drug discovery and development (Palombo, 2011; Street and Prinsloo, 2013).

This study focused on the screening of South African indigenous plants for their antimutagenic or antigenotoxic potentials against aflatoxin B_1 induced mutagenicity. These plant extracts were also evaluated for their mutagenicity to confirm that they were not mutagenic. The plants were selected based on results from preliminary screening in our laboratory (unpublished results). The antigenotoxicity of the plant extracts was tested using the *Salmonella* microsome and Vitotox assays. These two assays are genotoxicity bioassays commonly used in the screening of genotoxic substances (Verschaeve et al., 1999; Sloczynska et al., 2014).

2. Materials and methods

2.1. Sample collection and processing

Twenty-two plant species collected from South African National botanical gardens (Lowveld, Walter Sisulu and Pretoria) and in the university of Pretoria botanical garden (Manie Van der Schijff Botanical Garden) are listed in Table 1. The table also shows the common names, plant part used as well as the accession number for the plants. The plant material (leaves, seeds or fruits) was dried in an oven set at 45 °C. Thereafter, the plant material was ground to a fine powder and stored in airtight containers in the dark at room temperature until use. Voucher specimens for the collected plant species were deposited in the H.G.W.J. Schweickerdt herbarium of the University of Pretoria.

2.2. Sample extraction and preparation

Ten grams of ground powder of each plant material was sequentially extracted with 100 mL of dichloromethane (Merck) followed by 90% methanol (Merck) by vigorous shaking for 2 h in a rotary shaker. Thereafter, the crude extracts were filtered under vacuum using Whatman No.1 filter paper (Merck). Organic solvents were concentrated using a rotary evaporator (Buchi) and then dried under a stream of cold air. Stock solutions of 100 mg/mL extracts were prepared and dissolved in dimethyl sulfoxide (DMSO; Merck) or methanol.

2.3. Genotoxicity and antigenotoxicity assay

2.3.1. Ames assay

The Ames assay was performed using the pre-incubation test. Two *S. typhimurium* tester strains were used in the Ames test, including the frame shift mutation detecting strain TA98 and the base-pair substitution detecting strain TA100 (Moltox) as described by Maron and Ames (1983). Hundred microliters of stock bacterium (kept at −80 °C) were added to 20 mL of Oxoid nutrient broth No.2 and incubated on a rotary shaker at 37 °C for 16 h. An aliquot of 0.1 mL was added to 0.1 mL test solution or the solvent (negative control), 0.5 mL of 4% (*v*/v) S9 mixture from Sprague Dawley rat liver (Moltox) and 2 mL of top agar containing biotin (Sigma Aldrich) and histidine (Sigma Aldrich). For mutagenicity screening, the test solution contained 50 μL test sample and 50 μL solvent control. For antimutagenicity screening, the test solution contained 50 μL test sample and 50 μL Aflatoxin B_1 (2 μg/mL, Sigma Aldrich). The top agar mixture was poured over the surface of the minimal glucose agar plates and incubated at 37 °C for 48 h. The number of revertant colonies (mutants) in each plate were counted following incubation. All cultures were done in triplicate for all concentrations of plant extract (5, 0.5 and 0.05 mg/mL) with the exception of controls where five replicates were used. The positive control was 1 μg/mL aflatoxin B_1 and 10% (v/v) DMSO/methanol (Merck) was used as negative control. Antimutagenicity of the test sample was expressed as percentage inhibition of mutagenicity and calculated as follows:

$$\%\text{inhibition} = [(1\text{-}T/M)\ X\ 100]$$

where T is the number of revertants per plate in the presence of mutagen and the test solution and M is the number of revertants per plate in the positive control (Ong et al., 1986). Absence of toxicity was confirmed by the presence of a background layer of bacterial growth in the plate.

Table 1
Plant species investigated for antimutagenicity and mutagenicity.

Sample no.	Specie name	Common name	Family name	Plant part	Accession No.
1	*Helichrysum petiolare* Hilliard & B.L. Burtt	Silverbush everlasting	Asteraceae	Leaves	122,773
2	*Protea cynaroides* (L.) L.	King protea	Proteaceae	Leaves	122,756
3	*Protea hybrid*		Proteaceae	Leaves	122,758
4	*Protea roupelliae* Meisn.subsp. hamiltonii Beard ex Rourke	Silver protea	Proteaceae	Leaves	122,757
5	*Artabotrys* brachypetalus Benth.	Hook berry	Annonaceae	Leaves	122,766
6	*Annona senegalensis* Pers.ssp. senegalensis	Wild custard apple	Annonaceae	Leaves	122,755
7	*Friesodielsia obovata* (Benth.) Verdc	Dwaba berry	Annonaceae	Leaves	122,759
8	*Hexalobus monopetalus* (A.Rich.) Engl. & Diels	Baboons breakfast	Annonaceae	Leaves	122,760
9	*Monanthotaxis caffra* (Sond.) Verdc	Dwaba berry	Annonaceae	Leaves	122,761
10	*Monodora junodii* Engl. & Diels		Annonaceae	Leaves	122,768
11	*Uvaria caffra* E. Mey. Ex Sond	Small cluster pear	Annonaceae	Leaves	122,764
12	*Xylopia parviflora* (A. Rich.) Benth	Bushveld bitterwood	Annonaceae	Leaves	122,765
13	*Xylopia* sp.		Annonaceae	Leaves	122,763
14	*Artabotrys brachypetalus* Benth	Hook berry	Annonaceae	Fruits	122,762
15	*Podocarpus henkelii* Stapf ex Dallim. & A.B. Jacks.	Henkel's yellow wood	Podocarpaceae	Seeds	122,771
16	*Rhoicissus sekhukhuniensis* Retief, Siebert & A.E. Van Wyk	Sekhukhune grape	Vitaceae	Leaves	122,774
17	*Podocarpus elongatus* (Aiton) L'Her.ex Pers	Breede river yellow wood	Podocarpaceae	Seeds	122,772
18	*Agapanthus praecox* Willd.	Blue lily	Agapanthaceae	Leaves	122,767
19	*Podocarpus falcutus* (Thunb) R.Br.ex Mirb.	Outeniqua yellow wood	Podocarpaceae	Seeds	122,770
20	*Rhoicissus rhomboidea* (E.Mey ex Harv.) Planch	Glossy forest grape	Vitaceae	Leaves	[a]NV
21	*Ledebouria revoluta* (L.f.) Jessop 1970	Bushveld grape	Asparagaceae	Leaves	[a]NV
22	*Rhoicissus laetans* Retief		Vitaceae	Leaves	122,769

[a] NV – not voucher specimen due to lack of plant material.