Available online at www.sciencedirect.com

ScienceDirect

South African Journal of Botany 73 (2007) 435–440

SOUTH AFRICAN JOURNAL OF BOTANY

www.elsevier.com/locate/sajb

In vitro pharmacological investigation of extracts from some trees used in Sudanese traditional medicine

I.M.S. Eldeen, J. Van Staden *

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

Received 14 December 2006; received in revised form 8 March 2007; accepted 26 March 2007

Abstract

Extracts obtained from seven tree species used in Sudanese traditional medicine were screened for antibacterial, anti-cholinesterase activities and investigated for potential mutagenic effects using the Ames test. Antibacterial activity was detected using the micro-dilution assay. The extracts were tested against Gram-positive: *Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative: *Escherichia coli* and *Klebsiella pneumoniae*. Of the plant extracts investigated, 75% showed minimum inhibitory concentration (MIC) values less than/or around 1.5 mg/ml. Extracts obtained from *Acacia seyal* (ethanolic leaf extract) and *Combretum hartmannianum* (ethanolic leaf and root extracts), inhibited bacterial growth of both Gram-positive and Gram-negative bacteria at a concentration less than/or around 0.39 mg/ml. The lowest MIC value (less than/or around 0.1 mg/ml) was observed with the ethanolic (leaf, bark and root) and dichloromethane (bark) extracts of *A. seyal*, dichloromethane root extract of *Capparis decidua*, ethyl acetate (bark and root) and ethanolic (root) extracts of *Erythrina latissima* against Gram-negative bacteria *Klebsiella pneumoniae*. In the acetylcholinesterase inhibitory test, 58% of the plant extracts were active at a concentration of/or below 1 mg/ml using the micro-dilution assay. The lowest IC_{50} value was 0.09 mg/ml observed with the ethanolic bark and root extracts of *E. latissima* and *Kigelia africana*. No potential mutagenic effects was shown by the investigated plant extracts in the Ames assay.

Keywords: Acetylcholinesterase inhibitory activity; Antibacterial activity; Genotoxicity

1. Introduction

Plants continue to play a major role as therapeutic remedies in primary health care in developing countries (Tshikalange et al., 2005). Sudan is situated in tropical Africa and has remarkable plant diversity and a cosmopolitan population. As with other developing countries, traditional medicine plays a major role especially in rural areas due to both economic and cultural factors (Ali et al., 2002). Information about Sudanese folk medicine was documented during comprehensive ethnobotanical investigations by El Ghazali et al. (1994, 1997), and El Kamali and El Khalifa (1999).

There is an increasing number of diseases, including bacterial infections which are exhibiting various levels of drug resistance. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Eldeen et al., 2005). Higher plant extracts lend themselves for medicinal applications due to the ability of plants to produce compounds which confer antimicrobial defenses against microbes in their own environment. This ecological rationale justifies potential exploitation of this resource for new therapeutic agents (Gibbons, 2003).

Acetylcholine is an organic molecule liberated at nerve endings as a neurotransmitter. Neurotransmitter disturbances and insufficient cholinergic functions are identified among the pathological features in central nervous system disorders. The most important changes observed in the brain are a decrease in cortical levels of the neurotransmitter acetylcholine and associated enzyme choline transferase. Inhibition of acetylcholinesterase therefore can restore the level of acetylcholine (Greenblatt et al., 1999; Personeni et al., 2001; López et al.,

Abbreviations: AChE, acetylcholinesterase; ATCI, acetylthiocholine iodide; BSA, bovine serum albumin; DTNB, 5,5-dithiobis-2-nitrobenzoic acid; INT, iodonitrotetrazolium violet; MH, Mueller–Hinton agar; MIC, minimum inhibitory concentration; TLC, thin layer chromatography.

* Corresponding author.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

0254-6299/$ - see front matter © 2007 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2007.03.009

2002; Howes and Houghton, 2003). Plants have been used traditionally to enhance cognitive function and to alleviate other symptoms associated with Alzheimer's disease (Howes and Houghton, 2003).

Chemicals that produce DNA damage leading to mutations or cancer are described as genotoxic. Plants commonly used in traditional medicine are assumed to be safe based on their long usage in the treatment of diseases according to knowledge accumulated over centuries. However, recent research has shown some substances present in these medicinal plants to be potentially toxic and carcinogenic (Fennell et al., 2004). It is therefore important to investigate potential mutagenic effects in order to validate safety for the continued use of medicinal plants.

In this study, extracts from seven tree species used in Sudanese folk medicine were screened for antibacterial and anti-cholinesterase activities and investigated for their potential mutagenic effects.

2. Materials and methods

2.1. Plant material

Selection of the tree species investigated in this study was based on their uses in Sudanese traditional medicine (Table 1). Plant materials (leaves and/or twigs, roots, bark) were collected from Shambat, Khartoum north-Sudan in December 2005. The plants were authenticated at the Department of Silviculture, Faculty of Forestry University of Khartoum, Sudan and voucher specimens deposited in the Herbarium (Table 1).

The collected materials were dried, powdered and extracted sequentially using dichloromethane, ethyl acetate and ethanol (10 mg/ml) by sonication for 1 h. The extracts were filtered using Whatman No. 1 filter paper and dried under a fan at room temperature.

2.2. Micro-dilution antibacterial assay

The serial dilution technique described by Eloff (1998), using 96-well micro-plates was used to determine the minimum inhibitory concentration (MIC) of extracts for antibacterial activity. Two ml cultures of four bacterial strains: Gram-positive: *Bacillus subtilis* (ATCC No. 6051) and *Staphylococcus aureus* (ATCC No. 12600) and two Gram-negative bacteria: *Escherichia coli* (ATCC No. 11775) and *Klebsiella pneumoniae* (ATCC No. 13883) were prepared and placed in a water bath overnight at 37 °C. The overnight-cultures were diluted with sterile MH broth (1 ml bacteria/50 ml MH). The extracts were resuspended using ethanol to a concentration of 50 mg/ml. For each of the four bacteria used, 100 µl of redissolved extract were two-fold serially diluted with 100 µl sterile distilled water in a 96-well micro-plate. A similar two-fold serial dilution of neomycin (Sigma) (0.1 mg/ml) was used as a positive control against each bacterium. One hundred µl of each bacterial culture were added to each well. The plates were covered and incubated overnight at 37 °C. To indicate bacterial growth, 50 µl of 0.2 mg/ml *p*-iodonitrotetrazolium violet (INT) was added to each well and the plates incubated at 37 °C for 30 min. Bacterial growth in the wells was indicated by a red colour, whereas clear wells indicated inhibition by the tested substances.

2.3. Acetylcholinesterase enzyme inhibitory activity

Inhibition of acetylcholinesterase biosynthesis by plant extracts was investigated using thin layer chromatography (TLC) and

Table 1
Botanical name, family, voucher specimen and traditional uses of tree species used in Sudanese traditional medicine

Plant name	Family	Voucher specimen	Traditional uses in Sudan and other African countries
Acacia seyal Del.	Mimosaceae	Ibra 1	Smoke of the wood is used for treatment of arthritis rheumatism and rheumatic fever in Sudan. Leaves are used for inflammation and stomach disorders El Ghazali et al. (1997).
Balanites aegyptiaca (L.) Del.	Balanitaceae	Ibra 2	Bark is used for venereal diseases and rheumatism. Fruits are used for digestion problems, dysentery and bilharzias in Sudan. The bark is reported to be applied in the form of cutaneous implantations to strengthen the body and as an abortifacient. The tree features prominently in Hausa ethnomedicine and has been found most useful for other household and for religious rituals Iwu (1993), El Ghazali et al. (1997), Van Wyk et al. (1997).
Capparis decidua (Forssk.) Edgew.	Capparidaceae	Ibra 3	Fresh twigs are used for jaundice, rheumatic arthritis and to treat swells. Different plant parts are used in Sudan for stomach disorders El Ghazali et al. (1997).
Combretum hartmannianum Schweinf.	Combretaceae	Ibra 4	Smoke of the bark and wood is used for arthritis rheumatism and to treat dryness of skin in Sudan. Leaves are used as an ingredient of a medication for jaundice. Various members of the genus are employed for bacterial diseases and as an anthelmintic in many parts of Africa Iwu (1993), Neuwinger (1996), El Ghazali et al. (1997).
Erythrina latissima E. Mey.	Papilionaceae	Ibra 5	Bark is used as a purgative and for the treatment of bronchial infections, coughs, wounds, abscesses, arthritis and throat inflammation in Sudan. In Nigeria, aqueous extract of the bark is used for the treatment of jaundice Iwu (1993), Hutchings et al. (1996), Van Wyk et al. (1997).
Kigelia africana (Lam.) Benth.	Bignoniaceae	Ibra 6	Bark is used for stomach problems and bilharzias. In Sudan, Nigeria, Senegal, Ghana, Benin and Kenya, bark is used for cough, dysentery and venereal diseases. A decoction of the leaves is drunk for jaundice Neuwinger (1996), El Ghazali et al. (1997).
Ziziphus spina-christi (L.) Willd.	Rhamnaceae	Ibra 7	In Zambia, leaf preparations are used for ulcers and gonorrhea. Leaves are used to slow balding and also for dry hair. Fruits are used for sore throat. The tree is also reputable from a religion perspective in Sudan. Bark is used for chest pain, while root infusion is administered for dysentery in Sudan and some African countries. Roots are used for psychiatric medicine in Sudan and Mali. Hausa in Niger used the roots and the leaves for gastric infections, diarrhea, venereal diseases, wounds, chest pain, constipation and nervousness Neuwinger (1996), El Ghazali et al. (1997), Dafni et al. (2005).

microplate assays. These assays are based on Ellman's method (Ellman et al., 1961) with modifications. The enzyme activity is measured by observing the increase of a yellow colour produced from thiocholine when it reacts with the dithiobisnitrobenzoate ion. Acetylthiocholine iodide (ATCl), acetylcholinesterase (AChE), from electric eels (type VI-S lypophilized powder), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), and galanthamine were obtained from Sigma-Aldrich. The following buffers were used; Buffer A:50 mM Tris–HCl, pH 8; Buffer B: 50 mM Tris–HCl, pH 8 containing 0.1% bovine serum albumin (BSA); Buffer C:50 mM Tris–HCl, pH 8, containing 0.1 M NaCl and 0.02 M $MgCl_2 \cdot 6H_2O$.

For the TLC assay, 10 μl plant extract and 5 μl 0.2 mM galanthamine hydrobromide were spotted on TLC plates and developed in chloroform:methanol (8:2). The TLC plate was sprayed with 5 mM ATCl and 5 mM DTNB in 50 mM Tris–HCl, pH 8 until the silica was saturated with the solvent. Enzyme inhibitory activity of the plant extracts was detected by spraying the TLC plate with substrate (1 mM ATCl in buffer A), dye (1 mM DTNB in buffer A) and enzyme (3 U/ml AChE in buffer A). After 2–5 min a yellow background appeared with white spots for AChE inhibiting compounds. These were observed and recorded within 5 min. False-positive reactions were eliminated by mixing enzyme with the substrate before spraying onto the TLC plate.

The microplate assay utilised an Opsys MR 96-well microplate reader. In the 96-well plates, 25 μl of 15 mM ATCl in water, 125 μl of 3 mM DTNB in buffer C, 50 μl of buffer B and 25 μl of sample were added. The absorbance was measured at 405 nm every 45 s (five times). Then 25 μl of 0.2 U/ml of enzyme were added, the absorbance was measured again every 45 s (eight times). The rate of reaction was calculated. Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the ratio of reaction before adding the enzyme from the rate after adding the enzyme. Percentage of inhibition was calculated by comparing the reaction rates for the sample to the blank (methanol in buffer A).

2.4. Genotoxicity test

The potential mutagenic effects of the investigated plants were determined using the Ames test. The Ames assay was performed with *Salmonella typhimurium* (strain TA98) using the plate incorporation procedure described by Maron and Ames (1983). One hundred μl of bacterial stock were incubated in 20 ml of Oxoid Nutrient for 16 h at 37 °C on an orbital shaker. The overnight culture (0.1 ml) was added to 2 ml top agar (containing traces of biotin and histidine) together with 0.1 ml test solution (plant extract, solvent control or positive control) and 0.5 ml phosphate buffer (for exposure without metabolic activation). The top agar mixture was poured over the surface of the agar plate and incubated for 48 h at 37 °C. After incubation, the number of revertant colonies (mutants) were counted. All cultures were made in triplicate (except the solvent control where five replicates were made) for each assay. The assays were repeated twice. The positive control used was 4-nitroquinoline 1-oxide (4-NQO) at a concentration of 2 μg/ml.

3. Results and discussion

3.1. Antibacterial activity

The MIC values of the plant extracts obtained using the micro-dilution assay are shown in Table 2. Of the plant extracts tested, 75% showed MIC values less than/or around 1.5 mg/ml. The

Table 2
Antibacterial activity (MIC) of plant extracts obtained from trees used in Sudanese traditional medicine as determined by the micro-dilution assay

Plant species	Plant part analyzed	Dichloromethane extract				Ethyl acetate				Ethanol extract			
		Bacteria tested				Bacteria tested				Bacteria tested			
		B. s	S. a	E. c	K. p	B. s	S. a	E. c	K. p	B. s	S. a	E. c	K. p
Acacia seyal	Leaf	**0.39**	1.5	1.5	**0.39**	**0.39**	1.56	**0.78**	**0.2**	**0.2**	**0.39**	**0.39**	**<0.1**
	Bark	**078**	1.56	1.56	**<0.1**	3.13	3.13	6.25	1.56	**0.39**	**0.36**	1.56	**<0.1**
	Root	3.13	3.13	3.13	**0.78**	1.56	3.13	3.13	**0.78**	**<0.1**	1.56	1.56	**<0.1**
Balanites aegyptiaca	Leaf	3.13	3.13	3.13	**0.78**	3.13	1.56	3.13	**0.39**	3.13	3.13	3.13	**0.78**
	Bark	**0.78**	3.13	3.13	**0.39**	**0.78**	3.13	3.13	3.13	6.25	3.13	3.13	12.5
	Root	**0.78**	3.13	3.13	**0.39**	**0.39**	3.13	3.13	**0.2**	6.25	3.13	3.13	12.5
Capparis decidua	Twigs	3.13	1.56	1.56	**0.78**	3.13	3.13	3.13	**0.78**	6.25	3.13	3.13	1.56
	Root	1.56	1.56	1.56	**<0.1**	1.56	1.56	1.56	**0.78**	3.13	3.13	3.13	1.56
Combretum hartmannianum	Leaf	**<0.1**	1.56	1.56	**0.2**	**0.39**	1.56	1.56	**0.78**	**0.2**	**0.2**	**0.39**	**0.39**
	Bark	3.13	3.13	3.13	**0.39**	**0.39**	3.13	3.13	**0.78**	1.56	3.13	1.56	**0.78**
	Root	**0.1**	3.13	3.13	**0.78**	**0.39**	3.13	3.13	**0.78**	**0.39**	**0.2**	**0.2**	**0.2**
Erythrina latissima	Bark	**<0.1**	1.56	1.56	**<0.1**	1.56	1.56	1.56	**<0.1**	**<0.1**	**0.39**	**0.39**	**0.2**
	Root	6.25	3.13	3.13	6.25	1.56	1.56	1.56	**<0.1**	1.56	1.56	1.56	**<0.1**
Kigelia africana	Leaf	1.56	3.13	1.56	1.56	**0.2**	1.56	**0.78**	**0.2**	**0.78**	1.56	1.56	1.56
	Bark	**0.39**	1.56	**0.78**	**0.78**	**0.1**	1.56	**0.78**	**0.78**	6.25	6.25	6.25	6.25
Ziziphus spina-christi	Leaf	3.13	1.56	3.13	1.56	**0.2**	1.56	1.56	**0.2**	1.56	3.13	3.13	1.56
	Bark	3.13	6.25	6.25	3.13	1.56	1.56	1.56	1.56	**0.39**	1.56	1.56	**0.78**
Neomycin (μg/ml)	Bs:1.9×10^{-1}			Sa: 7.8×10^{-1}				Ec: 6.25			Kp: 1.56		

Values given are in mg/ml.
Bacteria: B. s = *Bacillus subtilis*; E. c = *Escherichia coli*; K. p = *Klebsiella pneumoniae*; S. a = *Staphylococcus aureus.*

ethanolic leaf extract of *A. seyal*, root and leaf of *Combretum hartmannianum* (ethanolic extracts), inhibited growth of both Gram-positive and Gram-negative bacteria at a concentration less than/or around 0.39 mg/ml. The ethanolic (leaf, bark, root) and dichloromethane (bark) extracts of *A. seyal*, dichloromethane root extract of *Capparis decidua*, ethyl acetate (bark and root) and ethanolic (root) extracts of *Erythrina latissima* inhibited growth of Gram-negative bacteria *Klebsiella pneumoniae* at a concentration less than/or around 0.1 mg/ml while the ethanolic root extracts of *C. hartmannianum* inhibited growth of *E. coli* with an MIC value of 0.2 mg/ml.

Different parts of the investigated plants are administered in Sudanese traditional medicine for the treatment of various ailments including microbial infections. Bacteria species such as *E. coli*, *S. aureus*, *Bacillus* and *Klebsiella* species possess potent enterotoxins. It can be life-threatening during gastroenteritis infections (Lewis and Elvin-Lewis, 2003).

Previous work from our laboratory showed high levels of antibacterial activities of extracts obtained from *Acacia nilotica* and *A. sieberiana* (Eldeen et al., 2005). The previously reported biologically activity of *Acacia* species was attributed to the presence of some bioactive agents such as ethyl galate and the flavonoids, octasanol, β-amyrin and α-betulin (Ayoub, 1984; Abdelnabi et al., 1992; Neuwinger, 1996). Not much information is available about the biological activity of *A. seyal*. The activity shown by the species may well be due to the presence of similar compounds.

Several bioactive compounds such as the drug 'kinkeliba" (isolated from leaves of *C. micranthum*), stilbenes, hydroxystilbenes and acidic triterpenoids amongst many others are present in *Combretum* species (Iwu, 1993; Bruneton, 1995; Hutchings et al., 1996; Rogers and Verotta, 1996; McGaw et al., 2000). While little pharmacological data is available for *C. hartmannianum*, the high antibacterial activity shown by the plant extracts in this study is interesting and may well be attributed to the presence of similar or related bioactive constituents.

The relatively weak antibacterial activities (with some exceptions) showed by some extracts obtained from *Balanites aegyptiaca*, *C. decidua* and *Ziziphus spina-christi* does not necessarily mean absence of bioactive constituents. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed. Lack of activity can thus only be proven by using large doses. Extracts may also be active against other bacterial species which were not tested. (Farnsworth, 1993; Jäger et al., 1996; Shale et al., 1999; Taylor et al., 2001).

The leaf and bark of *Kigelia africana* showed antibacterial activity against both Gram-positive and Gram-negative bacteria. This supports previous findings of Kwo and Craker (1996). These results may justify the intensive use of this tree in traditional medicine throughout its distribution range (Retief and Herman, 1997).

The low MIC values detected by some of the investigated plant extracts against both Gram-positive and Gram-negative bacteria (less than/or around 0.39 mg/ml) give an indication of the presence of promising antibacterial compounds which need to be isolated and identified.

3.2. Acetylcholinesterase enzyme inhibitory activity

Results of acetylcholinesterase enzyme inhibitory activity of the tested plant extracts are shown in Table 3. A minimum inhibition of 50% is required for the tested plant extracts to be considered active. Based on this criterion, 58% of the extracts showed activity against the acetylcholinesterase enzyme in the TLC and micro-dilution assays. At a concentration of 1 mg/ml, dichloromethane (bark) and ethanolic (leaf and bark) extracts of

Table 3
Inhibition of acetylcholinesterase activity by plant extracts (1 mg/ml) obtained from trees used in Sudanese traditional medicine as determined by the micro-plate assay

Plant species	Plant part analyzed	Inhibition (%) and IC_{50} values (mg/ml)							
		Plant extracts tested at concentration of 1 mg/ml							
		Dichloro-methane	IC_{50}	Ethyl acetate	IC_{50}	Ethanol	IC_{50}	Galanthamine (20 μM)	IC_{50} (μM)
Acacia seyal	Leaf	55	0.37	77	0.37	92	0.37	90	2.0
	Bark	89	0.18	40	–	90	0.37		
	Root	50	1.0	56	0.37	56	0.76		
Balanites aegyptiaca	Leaf	47	–	47	–	42	–		
	Bark	48	–	22	–	40	–		
	Root	48	–	13	–	24	–		
Capparis decidua	Twigs	67	0.37	55	0.76	42	–		
	Root	70	0.37	50	1.0	44	–		
Combretum hartmannianum	Leaf	6	–	33	–	79	0.25		
	Bark	50	1.0	45	–	66	0.37		
	Root	44	–	11	–	71	0.37		
Erythrina latissima	Bark	50	1.0	20	–	68	0.187		
	Root	27	–	22	–	91	0.093		
Kigelia africana	Leaf	22		66	0.76	78	0.25		
	Bark	56	0.5	75	0.37	87	0.09		
Ziziphus spina-christi	Leaf	53	0.76	56	0.37	67	0.37		
	Bark	57	0.78	50	1.0	74	0.37		

Results given are percentage inhibition and IC_{50} values.
Inhibition (%) of acetylcholinesterase enzyme by galanthamine (20 μM) was 90% with an IC_{50} value of 2.0 μM.

A. seyal, ethanolic root extract of *E. latissima* and ethanolic bark extract of *K. africana* showed the highest level of activity (>85%) against the acetylcholinesterase enzyme. The range of IC_{50} value was 0.3 to 0.09 mg/ml. Moderate activities (<60%) were observed with the dichloromethane (leaf) and all extracts from the root of *A. seyal*, dichloromethane bark extract of *C. hartmannianum*, *E. latissima*, *K. africana* and both dichloromethane and ethyl acetate (leaf and bark) extracts of *Z. spina-christi* (range of IC_{50} value was 1.0–0.3 mg/ml). The lowest IC_{50} value was observed with the ethanolic bark and root extracts of *E. latissima* and *K. africana* (0.09 mg/ml). The percentage inhibition of galanthamine (positive control) at a concentration of 20 μM was 90% and the IC_{50} value was 2 μM. This was comparable to a previous report in the literature where 1.9 μM was recorded (Elgorashi et al., 2004).

With the exception of twigs and roots of *C. decidua* and roots of *A. seyal*, ethanolic extracts exhibited strong activity when compared to ethyl acetate and dichloromethane extracts.

The plant extracts that possessed activity against acetylcholinesterase in this study were reported to contain different types of alkaloids. Alkaloids are known to exhibit a number of pharmacological properties, amongst them inhibition of acetylcholineseterase enzyme activity. *Combretum* species are reported to have saponaretine and quaternary amino bases comprising two major alkaloids, combretines A and B, stereoisomers of betonicine (Iwu, 1993; Bruneton, 1995). *E. latissima* was reported to produce ery-thrina-type alkaloids such as (+)-ery-thraline, (+)-11-hydroxyerysodine, (+)-erythratidine and (+)-erysosalvine (Majinda et al., 2001). The presence of alkaloids and/or other similar compounds might explain the inhibition of acetylcholinesterase activity observed by the plant extracts.

Z. spina-christi is a "Holy" tree in Arab and Middle eastern cultures. It has been used traditionally for treatments of various ailments related to central nervous system disfunction (Iwu, 1993; Dafni et al., 2005). The present findings support such usage in the traditional remedies.

In general, leaf extracts of some of the investigated plants showed different levels of biological activities compared to the other plant parts (*A. seyal*, *C. hartmannianum, E. latissima* and *K. africana*). Presence of such activities in the leaf extracts implies that there could be possibilities of substituting leaves for roots and bark while utilising the particular plant species (Matu and Van Staden, 2003).

3.3. Mutagenic effects

In the mutagenicity testing of different plant parts using the *Salmonella* strain (TA98), results are based on the number of induced revertant colonies. Plant extracts were considered active if the number of the induced revertant colonies was double the revertant colonies of the blank (negative control). None of the investigated plants showed any potential mutagenic effects.

Although no potential mutagenic effects have been shown by the plants in this study, this does not necessarily mean absence of adverse effects that justifies their use as a safe traditional medicine. Due to complexity of mutation mechanisms, accurate evaluation of mutagenic activity of medicinal plants using developed and sophisticated tools are needed (Debnath et al., 1991).

Generally, the results obtained in this study are in line with the traditional uses of the plants as crude antibacterial drugs. However, traditional healers seldom use a single plant in their extracts. In many cases the therapeutic benefits are attributed to the consumption of plant mixtures in which different plant parts are prepared and/or consumed in combination or in sequence (Etikin, 1986; Taylor et al., 2001).

There is not much detailed documentation on the pharmacological properties of Sudanese medicinal plants. Such studies therefore can contribute positively towards the formulation of traditional knowledge by providing a scientific basis with respect to raw materials and methods of production. It would also enable local knowledge of medicinal plants to be compiled and conserved (Anand and Nityanand, 1984; Taylor et al., 2001).

The results obtained from this study strongly support further investigation into pharmacological properties of secondary metabolites of higher plants as a promising source of bioactive compounds (Balandrin et al., 1993; Lewis and Elvin-Lewis, 2003). Higher plants represent an extraordinary reservoir based on the fact that trees resist attack by fungi and/or other organisms by producing antimicrobial agents in their wood, bark and leaves. It is reasonable then to assume that these bioactive compounds may also be inhibitory to closely related human pathogens (Lewis and Elvin-Lewis, 2003).

Acknowledgements

Dr. Essam Warrag and Dr. Ashraf Abdellah from the Faculty of Forestry, University of Khartoum, Sudan are thanked for their assistance with plant collection and primary preparations. This project was supported financially by the University of KwaZulu-Natal Research Fund and the National Research Foundation (NRF), Pretoria.

References

Abdelnabi, O.M., Reisinger, E.C., Reinthaler, F.F., Still, F., Fibel, U., Kreis, G.J., 1992. Antimicrobial activity of *Acacia nilotica* (L.) Willed ex Del. Var. *nilotica* (*Mimosaceae*). Journal of Ethnopharmacology 37, 77–79.

Ali, H., König, G.M., Khalid, S.A., Wright, A.D., Kaminsky, R., 2002. Evaluation of selected Sudanese medicinal plants for their *in vitro* activity against hemoflagellates, selected bacteria, HIV-1-RT and tyrosine kinase inhibitory, and for cytotoxicity. Journal of Ethnopharmacology 83, 219–228.

Anand, N., Nityanand, S., 1984. Integrated approach to development of new drugs from plants and indigenous remedies. In: Krogsgaard, P., Christensen, S., Kofod, H. (Eds.), Natural Products and Drug Development. Munksgaard, Copenhagen, pp. 78–93.

Ayoub, S.M.H., 1984. Polyphenolic molluscicides from *Acacia nilotica*. Planta Medica 50, 532–540.

Balandrin, M.F., Kinghorn, A.D., Farnsworth, N.R., 1993. Plant-derived natural products in drug discovery and development. An overview. In: Kinghorn, A.D., Balandrin, M.F. (Eds.), Human Medicinal Agents from Plants. ACS Symposium Series, vol. 534. American Chemical Society, Washington, USA.

Bruneton, J., 1995. Pharmacognosy, Phytochemistry, Medicinal Plant. Intercept, Hampshire, UK. ISBV:2-7430-0028-7.

Dafni, A., Levy, S., Lev, E., 2005. The ethnobotany of Christ's Thorn jujube (*Ziziphus spina-christi*) in Israel. Journal of Ethnobiology and Ethnomedicine 1, 8.

Debnath, A.K., Lopez de Compadre, R.L., Debnath, G., Shusterman, A.J., Hansch, C., 1991. Structure–activity relationship of mutagenic aromatic and heteroaromatic nitro compounds. Correlation with molecular orbital energies and hydrophobicity. Journal of Medicinal Chemistry 34, 786–797.

El Ghazali, G.B., El Tohami, M.S., El Egami, A.B., 1994. Medicinal Plants of the Sudan. Part III. Medicinal Plants of the White Nile Province. Khartoum University Press, Sudan.

El Ghazali, G.B., El Tohami, M.S., El Egami, A.B., Abdalla, W.S., Mohammed, M.G., 1997. Medicinal Plants of the Sudan. Part IV. Medicinal Plants of Northern Kordofan. Omdurman Islamic University Press, Khartoum, Sudan.

Eldeen, I.M.S., Elgorashi, E.E., Van Staden, J., 2005. Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. Journal of Ethnopharmacology 102, 457–464.

Elgorashi, E.E., Stafford, G.I., Van Staden, J., 2004. Acetylcholinesterase enzyme inhibitory effects of Amaryllidaceae alkaloids. Planta Medica 70, 258–260.

El Kamali, H.M., El Khalifa, K.F., 1999. Folk medicinal plants of riverside forests of the southern Blue Nile district, Sudan. Fitoterapia 70, 493–497.

Ellman, G.L., Courtney, D., Andies, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7, 88–95.

Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711–713.

Etikin, N.L., 1986. Multidisciplinary Perspectives in Interpretation of Plants used in Indigenous Medicine and Diet. Redgrave Publishing Company, New York, pp. 2–29.

Farnsworth, N.R., 1993. Biological approaches to the Screening and evaluation of natural products. In: Rasoanaivo, P., Ratsimamanga-Urverg, S. (Eds.), Biological Evaluation of Plants with Reference to the Malagasy Flora, Madagascar, pp. 35–43.

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., Van Staden, J., 2004. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology 94, 205–217.

Gibbons, S., 2003. An overview of plant extracts as potential therapeutics. Expert Opinion on Therapeutic Patents 13, 489–497.

Greenblatt, H.M., Kryger, G., Lewis, T., Silman, I., Sussman, J.L., 1999. Structure of acetylcholinesterase complexed with (-)galanthamine at 2.3 Å resolution. Federation of European Biochemical Societies Letters 463, 321–326.

Howes, M.R., Houghton, P.J., 2003. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacology, Biochemistry and Behavior 75, 513–527.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg.

Iwu, M.M., 1993. Handbook of African Medicinal Plants. CRC Press, Boca Raton, Florida, USA.

Jäger, A.K., Hutchings, A., Van Staden, J., 1996. Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. Journal of Ethnopharmacology 52, 95–100.

Kwo, V.T., Craker, L.E., 1996. Screening Cameroon medicinal plant extracts for antimicrobial activity. Acta Horticulturae 426, 147–155.

Lewis, W.H., Elvin-Lewis, M.P., 2003. Medical Botany: Plant Affecting Human Health, 2nd edn. Wiley & Sons, Inc., Hoboken, New Jersey, USA.

López, S., Bastida, J., Viladomat, F., Codina, C., 2002. Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and *Narcissus* extracts. Life Sciences 71, 2521–2529.

Majinda, R.R.T., Abegaz, B.M., Bezabih, M., Ngadjui, B.T., Wanjala, C.C.W., Mdee, L.K., Bojase, G., Silayo, A., Masesane, I., Yeboah, S.O., 2001. Recent results from natural product research at the University of Botswana. Pure and Applied Chemistry 73, 1197–1208.

Maron, D.M., Ames, B.N., 1983. Revised methods for the *Salmonella* mutagenicity test. Mutation Research 113, 173–215.

Matu, E.N., Van Staden, J., 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. Journal of Ethnopharmacology 87, 35–41.

McGaw, L.J., Jäger, A.K., Van Staden, J., 2000. Anti-bacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. Journal of Ethnopharmacology 72, 247–263.

Neuwinger, H., 1996. African Ethnobotany Poisons and Drugs Chemistry (Germany). Chapman and Hall Gm bH, D-69469 Weinheim (Bundes Republik Deutschland), ISBN 3-8261-0077-8.

Personeni, C.D., Bentley, P.D., Fletcher, R.J., Kinkaid, A., Kryger, B.P., Taylor, A., Taylor, R., Taylor, J., Viner, R., Silman, I., Sussman, J.L., Greenblatt, H.M., Lewis, T., 2001. A structure-based design approach to the development of novel, reversible AChE inhibitors. Journal of Medicinal Chemistry 44, 3203–3215.

Retief, E., Herman, P.P.J., 1997. Plants of the Northern Provinces of South Africa: Keys and Diagnostic Characters. National Botanical Institute, Pretoria, South Africa.

Rogers, C.B., Verotta, L., 1996. Chemistry and biological properties of the African Combretaceae. In: Hostettmann, K., Chinyanganya, F., Maillard, M., Wolfender, J-L. (Eds.), Chemistry, Biological and Pharmacological Properties of African Medicinal Plants. Proceedings of the First International IOCD-Symposium Victoria Falls, Zimbabwe.

Shale, T.L., Stirk, W.A., Van Staden, J., 1999. Screening of medicinal plants used in Lesotho for antibacterial and anti-inflammatory activity. Journal of Ethnopharmacology 67, 347–354.

Taylor, J.L.S., Rabe, T., McGaw, L.J., Jäger, A.K., Van Staden, J., 2001. Towards the scientific validation of traditional medicinal plants. Plant Growth Regulation 34, 23–37.

Tshikalange, T.E., Meyer, J.J.M., Hussein, A.A., 2005. Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. Journal of Ethnopharmacology 96, 515–519.

Van Wyk, B-E., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa, 1st edn. Briza Publications, Pretoria.

Edited by JN Eloff