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Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, Vol. 8, Núm. 5, septiembre-sin mes, 2009, pp. 402-411
Sociedad Latinoamericana de Fitoquímica
Chile

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Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas
ISSN (Versión impresa): 0717-7917
blacpma_editorial@hotmail.com
Sociedad Latinoamericana de Fitoquímica
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BLACPMA ISSN 0717 7917

Artículo Original | Original Article

Antioxidant and antiglycation potential of some Sudanese medicinal plants and their isolated compounds

[Potencial antioxidante y antiglicosidación de algunas plantas medicinales sudanesas y de sus compuestos aislados]

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Abstract

Free radicals or reactive oxygen species (ROS) appear to be associated with a number of human neurodegenerative disorders, inflammation, diabetes, viral infections, autoimmune pathologies and digestive system disorders. In the present work twenty three ethanolic extracts of 20 medicinal plants, commonly used in Sudanese folk medicines against infectious diseases were investigated for their potential scavenging of superoxide free radicals. 14 extracts showed significant activity $P < 0.05$ for the scavenging of superoxide free radicals. *Acacia nilotica* bark, *Balanites aegyptiaca* barks, *Khaya senegalensis* leaves were the most active with 75, 72, 71% inhibition, respectively. However, the rest revealed moderate inhibition activity. Only *A. nilotica* barks (78%), *K. senegalensis* barks (74%), *A. nilotica* fruits (66%) and *Tinspora bakis* (61%) showed over 50% inhibition of glycation production assay, while the rest were less effective. Bioassay-guided phytochemical investigation proved catechin was the most active isolated secondary metabolite for both scavenging of superoxide free radicals and inhibition of glycation production assay. MTT cytotoxicity against 3T3 cell line indicates the safety of all plant ethanolic extracts as well as isolated compounds.

Keywords: Antioxidant; Antiglycation; Cytotoxicity; Superoxide scavenging; Sudanese medicinal plants.

Resumen

Los radicales libres o especies reactivas del oxígeno (ROS) parecen estar asociados con un número de desórdenes neurodegenerativos, inflamación, diabetes, infecciones virales, patologías autoinmunes y desórdenes del sistema digestivo. En el presente trabajo fueron investigados 23 extractos etanólicos de 20 plantas medicinales, usadas comúnmente en la medicina tradicional sudanesa contra enfermedades infecciosas por sus potenciales como secuestradores del radical libre superóxido. 14 extractos mostraron una actividad significativa $P < 0.05$ como secuestradores de radicales libres. Las cortezas de *Acacia nilotica y Balanites aegyptiaca* y las hojas de *Khaya senegalensis* fueron las más activas con 75, 72, 71% de inhibición, respectivamente. El resto mostró una actividad inhibitoria moderada. Solamente *A. nilotica* corteza (78%), *K. senegalensis* corteza (74%), *A. nilotica* frutos (66%) y *Tinspora bakis* (61%) mostraron una inhibición por encima del 50% en el ensayo de producción de glicosidación, mientras el resto fue menos efectivo. En una investigación fitoquímica guiada por bioensayo se obtuvo que la catequina fue el metabolito secundario aislado más activo tanto como secuestrador de radicales libres como inhibidor de la producción de glicosidación. En el ensayo de citotoxicidad, con el uso de MTT, contra células 3T3 indicó la seguridad de todos los extractos etanólicos así como de los compuestos aislados.

Palabras Clave: Antioxidante; Antiglicosidación, Citotoxicidad; Secuestro de supeóxido, Plantas medicinales sudanesas.

Recibido | Received: May 14, 2009.
Aceptado en Versión Corregida | Accepted in Corrected Version: July 10, 2009.
Publicado en Línea | Published Online: September 30, 2009.
Declaración de intereses | Declaration of interests: Authors have no competing interests.
Financiación | Funding: This work was financed by Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan.
This article must be cited as: Waleed S. Koko, Elbadri E. Osman, Mohamed Galal. 2009. Antioxidant and antiglycation potential of some Sudanese medicinal plants and their isolated compounds. Bol Latinoam Caribe Plant Med Aromat 8(5):402 – 411. {EPub September 30, 2009}.

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INTRODUCTION

Considerable evidence has accumulated to implicate cellular damage arising from reactive oxygen species (ROS), at least in part, in the etiology and pathophysiology of human diseases such as neurodegenerative disorders (e.g. Alzeimer disease, Parkinson disease, multiple sclerosis, Down's syndrome), inflammation, viral infections, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and ulcer (Repetto and Llesuy, 2002; Aruoma, 2003). In living systems, free-radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, by bacterial leucocytes, through xanthine oxidase activity, atmospheric pollutants, and from transitional metal catalysts, drugs and xenobiotics. For instance, in diabetes, increased oxidative stress which co-exists with reduction of the antioxidant status has been postulated. Oxygen free radicals can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and plays a role in the long-term complication of immune-related diseases (Boynes, 1991; Sabu and Kuttan, 2002). Similarly, in carcinogenesis, reactive oxygen species are responsible for initiating multistage carcinogenesis (Atawodi 2005).

From time immemorial, people have used herbal medicine for the treatment of various types of diseases (Pezzuto, 1977 and Mahomoodally et al., 2005). Up today, ethnomedicine still play the axial role in the search and development of new drugs (Heinrich, 2000). As in many developing countries, herbal drugs are of major importance in Sudanese folk medicine (Ali et al., 2002). Traditional medical practices play an important role in Sudan, and 90% of population particularly those who are living in frontiers and rural areas depend mainly on herbal medicine for the treatment of various types of diseases (Koko et al., 2000).

Little has been done to evaluate Sudanese plants of folk medicine for their antioxidant and antiglycation properties. In the present work, 23 ethanolic extracts of some Sudanese medicinal plants were tested for their potential antioxidant and antiglycation activity and their cytotoxicity activity against 3T3 cells.

MATERIALS AND METHODS

Plant Material

The selected plant species (Table–1) were collected between January and April 2005 from their natural habitats in the central part of Sudan. The voucher specimens were identified by Dr. Wai'l S. Abdalla and Mr. Haidar Elsidig of Herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan, where the specimens were also deposited.

Preparation of the Crude Extract

One hundred grams of each plant material were air-dried under the shed, ground, and extracted by triple soaking in 80% ethanol at room temperature for 3 days. Extracts were obtained by removing the organic solvent under reduced pressure, followed by the calculation of the % yield obtained (Table 1).

Isolation of secondary metabolites

Spectroscopic techniques

UV spectra were recorded in methanol or chloroform on a Hitachi UV 3200 spectrophotometer. IR spectra were recorded in $CHCl_3$ or KBr on a Jasco A-302 IR spectrophotometer. The EI MS spectra were recorded on a double focusing mass spectrometer (Varian MAT 311A). HREI MS measurements were performed on a Jeol HX 110 mass spectrometer. The ^{1}H-NMR spectra were recorded on Bruker AC-300, AM-400 and AMX-500 MHz instruments, while ^{13}C-NMR spectra were recorded at 100 and 125 MHz. Multiplicities of the carbon signals were determined by DEPT 90° and 135° experiments. ^{1}H-NMR and ^{13}C-NMR chemical shifts were reported in δ (ppm) and coupling constants (*J*) were measured in Hz.

Chromatographic techniques

Column chromatography was performed on silica gel 60 (Merck, Darmstadt, Germany, 70-230 and 240-300 mesh sizes), Diaion HP-20 Resin and Sephadex LH-20 were used. Final purification of some compounds was achieved by preparative recycling HPLC (model LC-908) (JAI) and the columns used were L-80 or H-80 (YMC, Co. Ltd) at appropriate flow rates (4 mL/min) using equal

amounts of MeOH and H_2O. Pre-coated silica gel TLC plates (E. Merck, F_{254}) were used to evaluate the compound's purity. TLC plates were viewed under ultraviolet light at 254 nm for fluorescence quenching spots and at 366 nm for fluorescent spots. Ceric-sulphate in 10% H_2SO_4 spraying reagent was used for staining the compounds on TLC.

Bioassay Techniques

Superoxide anion radical scavenging activity

The experiment was carried out in 96 microtiter ELISA plates with a final volume volume of 200 µL/ well. 10 µL of tested samples were dissolved in DMSO (final concentration of 1 mM for pure compounds and 1 µg of extracts). In this experiment, for the generation of the superoxide radicals, 90 µL of phosphate buffer (0.1 M, pH 7.5) were added followed by the addition of 40 µl of NBT (80 mM) solution and 40 µl NADH (280 mM) and the reaction was started by adding 20 µL of PMS solution (8 mM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min and the absorbance at 560 nm was measured against blank samples. Propyl gallate was used as a positive control and DMSO as negative control. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The inhibition percentage (%I) of superoxide anion generation was calculated by dividing the difference of the absorbance from the sample and control, divided by the control absorbance, multiplied by 100.

Inhibition of nonenzymatic protein glycation assay

Experiments were performed in 96 ELISA micro-plates as described by Ahmed (2005) with slight modification. Briefly, 30 µL/well of bovine serum albumin (BSA, 10 mg/mL) in sodium phosphate buffer (76 mM, pH 7.4) containing 0.02% (w/v) sodium azide was preincubated with equivalent amount (30 µL) of glucose (50 mg/mL), then 30 µg of test samples were added (final concentration 1 µg/mL for crude extracts and 1 mM for pure compounds). Rutin was used as control positive, the glycated group only glucose with BSA and buffer were used with 30 µL of each, 60 µL of buffer were added to 30 µL BSA and kept as blank (control negative). The mixtures were incubated at 37 °C for 1 week. After one week, 90 µL trichloroacetic acid (TCA) 100% were added to each well for precipitation of glycation followed by centrifugation at 15000 rpm at 4 °C for 4 min. The supernatant was removed gently from each well and 90 µL of PBS (phosphate buffer saline pH = 10) were added to dissolve the pellet. Fluorescence was determined using a Gemini Fluorescence (Molecular Devices) with an excitation wavelength of 370 nm and an emission wavelength of 440 nm. Results were expressed as percentage inhibition of formation of the glycated protein by using the following formula: Percentage inhibition = {1 – Flu. of sample/ Flu. of glycated} X 100

All data were an average of triplicate analysis.

MTT cytotoxicity test

Serial dilutions of compound were prepared in a 96 well flat bottomed plate (Nalge Nunc, Inter.). The outer wells of the plate were filled with 250 µL of in-complete culture medium except the last row 6 middle wells (B - G), which were used for the negative control receiving 50 µL of culture medium and 2 µL of sterile 0.5% Tritonx.

To the rest of the plate, 50 µL/wells (CCM) were added and 30 µL more were added to second column wells (B–G) that were used as first compounds dilution wells. To the first dilution wells in the row, 20 µg of compound suspension were added to the 80 µl. Compounds were then serially diluted by two-fold dilution from well B3 till B11 by transferring 50 µL to the next well after proper mixing. From the last dilution wells (B-11), 50 µL were discarded. Each compound was tested in triplicate. Cell suspension in a complete culture medium containing 2.5×10^5/mL was properly mixed, and 150 µL of it were transferred into each well of the plate. The plate was covered and placed in 5% CO_2 incubator at 37 °C for 72 -120 h. On the third/fifth day, the supernatant was removed from each well without detaching the cells. MTT stock (5 mg/mL) was prepared earlier in 100 mL PBS. The clear suspension was filter sterilized with 0.2 µm Millipore filter and stored at 4 °C or –20 ºC until use. MTT was diluted (1:3.5) in a culture medium and brought to room temperature. To each well of the 96 well plates, 50 µL of diluted MTT were added. The plate was incubated further at 37 °C for 2 to 3 h in CO_2 incubator. MTT was removed carefully without detaching cells, and 200 µl of DMSO were added to each well. The plate was agitated at room temperature for 15 min then read at 540 nm using microplate reader.

Statistical analysis

All data are presented as means ± standard deviation of the mean. Statistical analyses for all the assays results were done using Student's t-test. Significance was attributed to probability values $P \leq 0.05$ or $P \leq 0.005$ in some cases.

RESULTS

Effects of extracts on superoxide radicals scavenging

All the plant ethanolic extracts were screened for their superoxide radicals scavenging by using single concentration of 1 μg/mL (Table 1). The results obtained indicate that *A. nilotica* barks, *B. aegyptiaca* barks, *K. senegalensis* leaves and *A. nilotica* barks are the more active inhibitors of superoxide generation. They showed 75, 72, 71 and 68% inhibition, respectively. The moderate activity was observed from *O. brasilicum, K. senegalensis* barks, *H. abyssinica, S. oleraceus* and *T. bakis* (66, 61, 59, 56, 55 and 54% inhibition, respectively). On the other hand, the rest of remaining extracts revealed less than 50%. Propyl gallate was used as reference drug. It had shown 85% inhibitory activity. However, *B. aegyptiaca* fruits increased superoxide generation (Fig. 1).

Antiglycation activity of selected medicinal plants

In the last set of experiments, all the 15 ethanolic extracts were screened for their properties in preventing advanced gylcation end product formation. Fig. 1 indicated that only four extracts revealed over 50% antiglycation properties which are *A. nilotica* barks, *K. senegalensis* barks, *A. nilotica* fruits and *T. bakis*. Their %I was 78, 74, 66 and 61% respectively. While the rest of the examined plant extracts were found to show less activity. However, on the other hand, *K. senegalensis* leaves and *C. colocynthis* revealed a remarkable increase in accumulated glycation in comparison to the negative control. Rutin was used as reference drug. It showed 84% inhibition of glycation end product formation (Fig. 1).

Cytotoxicity of selected medicinal plants

All the ethanolic extracts of twenty medicinal plants selected for this study were evaluated for cytotoxicity using the MTT test system. It is a spectrophotometeric assay which measures the cell death caused by the incubation of test extracts with 3T3 mouse fibroblast cells (Dimas et al., 1998). The assay is based on the reduction of the yellow dye, 3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by the mitochondrial dehydrogenase of lives cells.

Table 2 indicate the %I of cell growth i.e. the toxicity of three different doses (100, 25 and 6.25 μg/mL) as well as the IC_{50} after 3 days of incubation with 23 ethanolic extracts of the selected medicinal plants. Tritonex was used as a positive control in this experiment with dose of 0.2 μg/ mL. It showed 69% inhibition of cell growth while all plant extracts tested revealed less toxicity. *A. mexicana* was the most toxic plant (IC_{50} = 44.6 μg/mL), followed by *A. nilotica* leaves, *A. anthelmintica, T. apollinea, B. aegyptiaca* bark, and *A. bracteolata* which showed IC_{50} of 65.2, 67.9, 74.5, 78.0, and 80.9 μg/mL, respectively. However, the rest of plants tested were found to produce less than 50% growth inhibition at 100 μg/mL (the highest concentration tested).

Isolated compounds

Bioassay-guided phytochemical investigation of two Sudanese plants with proven activity was carried out to isolate secondary metabolites. Four compounds were isolated from *Hydnora abyssinica*: tetradecanoic acid, 2-hydroxyhexadecyl ester, catechin, tyrosol and benzoic acid, 3, 4, dihydroxy-, ethyl ester, while the phytochemical analysis of *T. bakis* led to isolation of columbin, przewalskinone B, 1-tetracontanol and sitosterol. Their structures were elucidated only on the basis of spectroscopic analysis and in comparison with previously authenticated compounds.

Superoxide radicals scavenging of isolated compounds

All isolated compounds were subjected to the superoxide radical scavenging assay at a single dose of 1 mM from each (Fig. 2). Catechin was the most potent, with 68.5% inhibition of superoxide production, followed by benzoic acid, 3, 4, dihydroxy-, ethyl ester (59%). The other compounds showed inhibition of less than 50%, specifically, tetradecanoic acid, 2-hydroxyhexadecyl ester (37 %), sitosterol (33%), przewalskinone B (29%), 1-tetracontanol (26 %), tyrosol (25%) and columbin (20%). Propanyl gallate was used as the reference inhibitor (85%).

Table 1: Sudanese medicinal plants screened for their potential antioxidant properties, their major classes of secondary metabolites previously isolated and some of reported bioactivities.

Scientific names	Family	Herbarium code	Secondary metabolites										Bioactivity	Part used	% Yield	S. No.
			Alk	Anth	Coum	Flav	Lig	Phe	Sap	Terp	Tan	Oth				
Acacia nilotica L.	Mimosaceae	AH112-07	-	-	-	6	-	10	5	4	2	-	antiinflammatory, antimicrobial anthelmentic	Fruits	35.56	1
														Barks	21.30	2
Albizzia anthelmintica Broongn.	Mimoscasea	MO7-07	-	-	-	-	-	-	4	6	-	-	anthelmintic	Barks	07.87	3
Argemone mexicana L.	Papaveraceae	AH55-07	15	-	-	8	-	4	4	7	-	5	-	Whole plant	15.33	4
Aristolochia braceolata Lam.	Aristolochiaceae	WE35-07	-	-	-	-	-	-	-	-	-	-	antiinflammatory, estrogenic	Roots	03.95	5
Balanites aegyptiaca L.	Balanitaceae	GG32-07	-	-	2	5	-	5	11	17	-	3	antiinflammatory, antimicrobial	Fruits	17.50	6
														Barks	10.10	7
Cassia nigricans Vahl.	Caesalpinaceae	AH53-07	-	-	-	-	-	-	-	-	-	-	-	Whole plant	13.46	8
Citrullus colocynthis L.	Cucurbitaceae	MO2-07	-	-	-	5	-	4	2	13	-	3	laxative, antitoxic	Fruits	02.39	9
Geigeria alata D. C.	Asteraceae	AH14-07	-	-	-	-	-	-	-	2	-	-	-	Whole plant	08.43	10
Hydnora abyssinica A. Braun.	Hydnoraceae	MO22-07	-	-	-	-	-	-	-	-	-	-	-	Whole plant	14.52	11
Hyphaene thebaica L. Mart.	Arecacea	AH44-07	-	-	-	-	-	-	-	-	-	-	-	Fruits	34.06	12
Khaya senegalensis Desr A.	Meliaceae	AH9-07	-	-	-	2	-	11	6	25	-	4	antimicrobial, antiprotozoal	Barks	07.56	13
														Leaves	18.74	14
Nigella sativa L.	Ranunculaceae	MO16-07	18	-	-	-	3	7	4	14	-	9	antimicrobial, antihistaminic, antiinflammatory	Seeds	14.84	15
Ocimum basilicum L.	Lamiaceae	WE43-07	-	-	-	-	-	2	-	4	-	2	insecticidal	Arial part	07.19	16
Peganum harmala L.	Zygophyllaceae	GG11-07	33	4	2	12	-	16	9	19	-	8	antimicrobial, CNS stimulant	Seeds	05.22	17
Sonchus oleraceus L.	Asteraceae	WE105-07	-	-	-	-	-	-	1	4	-	-	-	Whole plant	10.77	18
Tephrosia apollinea D. C.	Papilionaceae	WE89-07	-	-	-	7	-	4	-	5	-	2	-	Whole plant	08.25	19
Tinospora bakis A. Rich. Miers	Menispermaceae	GG6-07	-	-	-	-	-	-	-	-	-	-	-	Roots	05.48	20
Vernonia amygdalina Del.	Asteraceae	GG64-07	-	-	-	-	-	4	1	12	-	3	antimicrobial	Leaves	06.70	21
Xanthium brasilicum Waller.	Asteraceae	WE27-07	-	-	-	6	-	10	5	4	2	-	antioxidant	Leaves	21.73	22
Ximenia americana L.	Olacaceae	WE8-07	-	-	-	-	-	-	4	6	-	-	-	Leaves	05.93	23

* **Source:** Dictionary of Natural Products (2005) Chapman and Hall CRC FL 33487 USA.**Table key (secondary metabolite classes):** Alk = alkaloids, Anth = Anthraquinones, Coum = Coumarins, flav = flavonoids, Lig = Lignins, Phe = Phenols, Sap = Saponins, Terp = Terpenes, Tan = Tannins, Oth = Others.**The active plants were written in bold The S.** No. used as code for the above mentioned plant ethanolic extract in all coming tables and figures.

Figure 1. Antiglycation and antioxidant inhibitory properties for the tested plant ethanolic extract

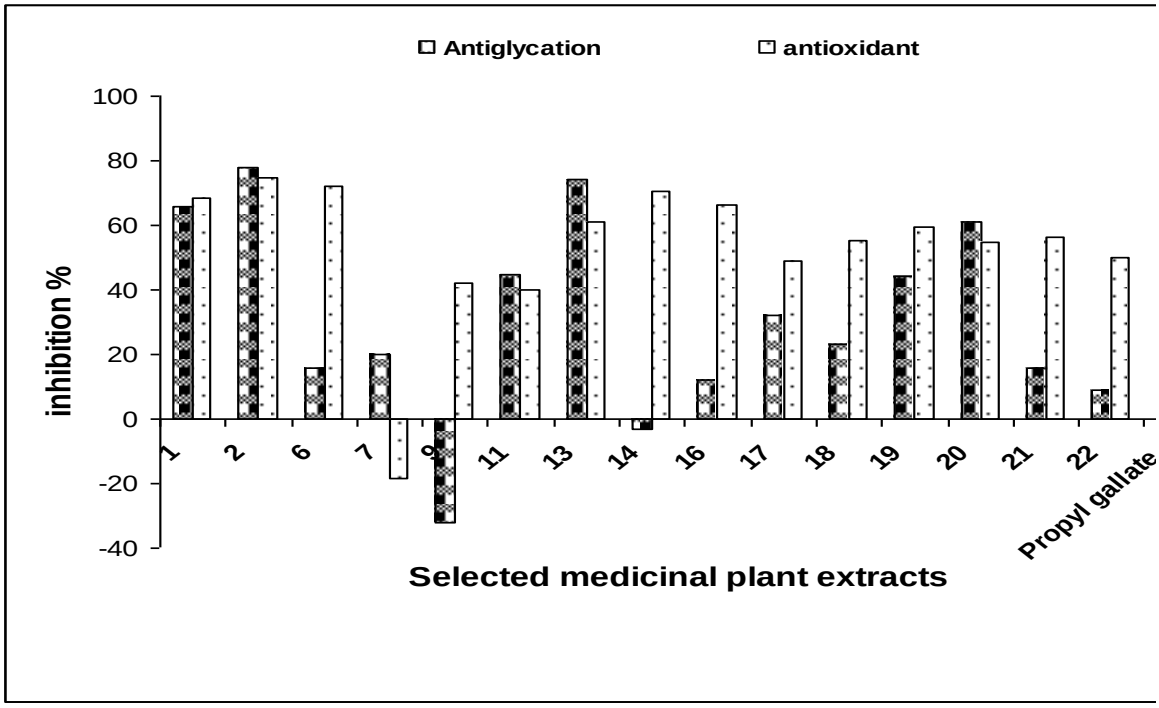

Figure 2. Antiglycation and antioxidant properties of isolated compounds

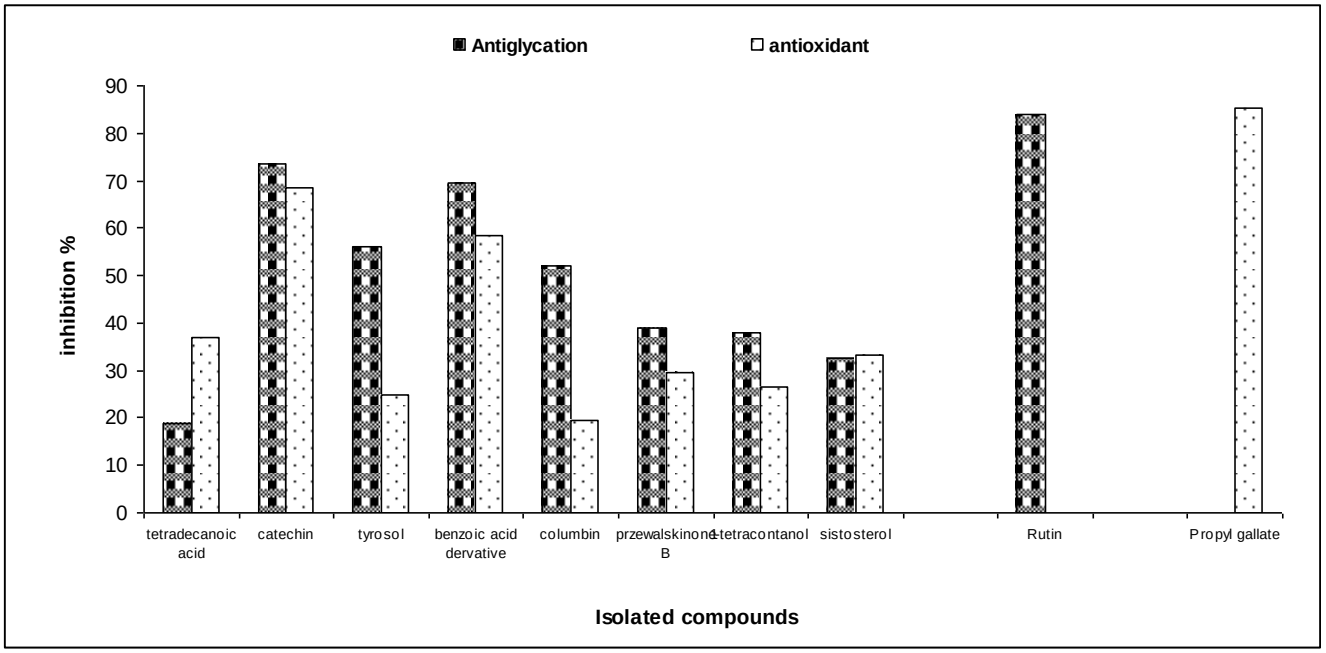

Table 2. Screening of the selected Sudanese medicinal plant extracts for their cytotoxicity against 3T3 cell line. MTT assay.

S. No.	Con.	Growth inh%	IC_{50} µg/mL	S. No.	Con.	Growth inh%	IC_{50} µg/mL
1	100	59.1	65.2	13	100	29.2	> 100
	25	34.2			25	-3.1	
	6.25	33.1			6.25	12.2	
2	100	37.7	> 100	14	100	-17.2	> 100
	25	24.0			25	-33.4	
	6.25	33.1			6.25	-12.8	
3	100	59.3	67.9	15	100	-21.0	> 100
	25	32.3			25	-48.4	
	6.25	43.6			6.25	-22.9	
4	100	65.7	44.6	16	100	43.4	> 100
	25	37.7			25	-10.9	
	6.25	39.4			6.25	6.4	
5	100	56.3	80.9	17	100	9.5	> 100
	50	21.2			25	-14.0	
	25	13.1			6.25	-4.7	
6	100	34.6	> 100	18	100	45.2	> 100
	25	29.0			25	-9.1	
	6.25	16.2			6.25	-2.2	
7	100	54.9	78.0	19	100	60.4	74.5
	25	20.2			25	18.1	
	6.25	10.4			6.25	3.2	
8	100	15.2	> 100	20	100	13.3	> 100
	25	20.8			25	8.4	
	6.25	28.1			6.25	-3.4	
9	100	33.1	> 100	21	100	17.6	> 100
	25	24.4			25	-0.7	
	6.25	8.7			6.25	9.3	
10	100	5.8	> 100	22	100	-11.9	> 100
	25	-5.6			25	-10.7	
	6.25	5.4			6.25	1.3	
11	100	14.1	> 100	23	100	36.7	> 100
	25	-9.1			25	25.7	
	6.25	-12.7			6.25	15.8	
12	100	-2.3	> 100		•Control	67.9	
	25	-7.9					
	6.25	11.8					

Con. = Concentration. Inh.% = Inhibition%,

•Control = Tritonex was used as the control positive at 0.2 µg/mL.

Note: The maximum concentration used was 100 µg/mL. When this concentration produced less than 50% inhibition, the IC_{50} cannot be calculated.

Antiglycation properties of isolated compounds

The 8 compounds isolated from *H. abyssinica* and *T. bakis* were investigated for their inhibition of glycation end product formation at a single dose of 1 mM. As shown in Fig. 2, catechin was found to be the most potent (73%), followed by benzoic acid, 3, 4, dihydroxy-, ethyl ester (69%), tyrosol (56%) and columbin (52%). The other 4 compounds were less active, przewalskinone B (39%), 1-tetracontanol (38%), sitosterol (33%) and the least potent tetradecanoic acid, 2-hydroxyhexadecyl ester (19%). Rutin was used as the reference inhibitor (84%).

Cytotoxicity of isolated compounds

Fig. 3 shows the cytotoxic properties of the isolated compounds. Tritonex at 0.2 μg/mL was used as the positive control (67% inhibition). None of the examined compounds showed cytotoxic activity over 50% at the tested doses (3.13 - 50 μg/mL). However, tyrosol and compounds (4) and (5) did show significant activity ($P < 0.05$) at a higher dose of 50 μg/mL, with 36, 26, 25% inhibition respectively (Table 3).

Figure 3. MTT reduction cytotoxic assay for evaluation of isolated compounds. 3T3 cell line were incubated for 3 days with these compounds.

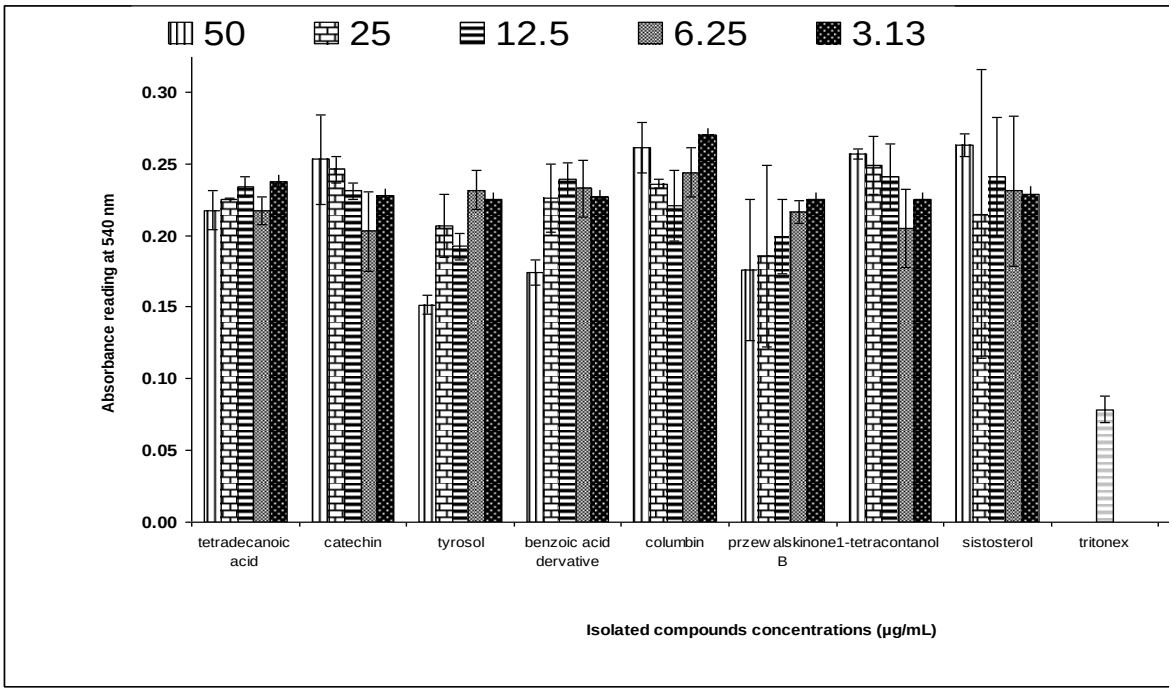

Table 3. Inhibition (%) of isolated compounds on MTT reduction cytotoxic assay against 3T3 cells.

Conc. in μg/mL	cpd1	cpd2	cpd3	cpd4	cpd5	cpd6	cpd7	cpd8
50	7.8	-7.2	35.8	26.3	-10.6	25.4	-8.9	-11.4
25	4.4	-4.2	12.5	4.2	0.0	21.4	-5.5	8.9
12.5	0.8	2.1	18.4	-1.3	6.4	15.7	-2.1	-2.1
6.25	8.1	14.0	1.9	1.3	-3.4	8.3	13.1	2.1
3.13	-0.4	3.4	4.7	3.8	-14.4	4.7	4.7	3.0
Tritonex				66.7				

DISCUSSION

Of these extracts, only 14 revealed significant superoxide inhibition activity (40 – 75% inhibition), with eleven of them showing inhibitory activity above 50% among them *H. abyssinica* (59%) to which, in a previous study, we attributed a more potent inhibitory activity using lucigenin (which reacts mainly with superoxide) rather than luminal (Koko, et. al., 2008) Glycation formation is also accompanied by formation of free radicals via autoxidation of glucose and glycated proteins. Compounds with combined antiglycation and antioxidant properties may offer therapeutic potential (Ahmed and Ahmed, 2006). For these reasons, all the above extracts were evaluated for their potential antiglycation properties at 1 μg/mL. Thirteen were found with antiglycation activity ($P < 0.05$) in the range of 9–78% inhibition. Four showed over 50% inhibition *A. nilotica* bark, *K. senegalensis* bark, *A. nilotica* fruit and *T. bakis* (78, 74, 66 and 61% inhibition respectively). On the other hand, *C. colocynthis* was found to increase glycation production about 33% above the negative control.

The plant extracts were found to show no important toxic activity, with that of *A. mexicana* being the most toxic (IC_{50} = 44.6 μg/mL). Hence, toxicity would not affect the therapeutic uses of their antioxidant and antiglycation properties.

Phytochemical investigation of two semi-endemic (endogenous) Sudanese plants revealed potent activity and characterized by no previous phytochemical studies from the literature. Four compounds were isolated from *H. abyssinica:* tetradecanoic acid 2-hydroxyhexadecyl ester is a new plant compound while the others are three known phenolic derivatives, catechin, tyrosol and benzoic acid, 3, 4, dihydroxy-, ethyl ester. Phytochemical analysis of *T. bakis* led to isolation of 1-tetracontanol as a new plant compound as well as three well known secondary metabolites, columbin, zewalskinone B and sitosterol. Their structures were elucidated only on the basis of spectroscopic analysis and comparison with previously authenticated compounds.

These 8 compounds can be classified into 3 major groups of plant secondary metabolites, the phenol derivatives catchen, tyrosol, benzoic acid, 3, 4, dihydroxy-, ethyl ester and przewalskinone B, the terpenes columbin and sitosterol and the long chain aliphatic compounds tetradecanoic acid, 2-hydroxyhexadecyl ester and 1-tetracontanol. The average inhibition of superoxide scavenging was 45% for the phenolic compounds, 31% for the long chain compounds and 26% for the terpenes. The corresponding inhibitory values for antigylication were 59% for the phenols, 42% for terpenes and 28% in the case of the long chain compound groups. These results absolutely agree with the previous observation of Wang et al. (1987). They indicated that aromatic compounds are extremely active in suppressing immune responses in both *in vitro* and *in vivo* assays. None of the above compounds indicated toxic effects against 3T3 fibroblast cells at the doses tested.

Catechin was found the most potent compound in general, showing 68 and 73% inhibition for both superoxide and glycation production, respectively. Previous studies indicated that catechin is the major polyphenol component of green tea which has attracted considerable attention during the last years. It is a powerful antioxidant and shows significant activities against all diseases associated with ROS production (Lee et al., 2004; Ban et al., 2006).

CONCLUSION

We can conclude that the inhibitory activity of these extracts is probably associated with the large amount of phenolic compounds. We recommend that *A. nilotica* and *B. aegyptiaca* be subjected to further studies for their active ingredients. The isolation of their most potent compounds for *in vivo* pharmacological studies is highly recommended.

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