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Research Article

Antioxidant Activity and Cytotoxicity of some Sudanese Medicinal Plants

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Abstract

Many species of plants have been shown effective antioxidant activities. This study is carried out to investigate the antioxidant and cytotoxic activities of six Sudanese medicinal plants commonly used as anti-inflammatory. Azadiracta indica leaves, Ziziphus spina-christi leaves , Matricaria chmomilla flower, Ricinus communis leaves, Acacia arabica leaves and Trigonella foenum seads were extracted with methanol 80% and screened for their free radical scavenging properties using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) and iron metal chelating, while propyl galate was used as standard antioxidant. MTT colorimetric assay was used against vero cell line for evaluation the cytotoxicity of all above plant extracts. All the above extracts showed strong anti-DPPH with IC_{50} values 0.49, 4.74, 9.91, 2.14, 17.19 and 4.24 µg/ml respectively, the inhibition percentage of propylgalate levels was found 88 % at concentrations 0.5 mM.
The inhibition percentage of radical scavenging activity against iron metal chelating 38.44, 26.88, 8.28, 17.05, 15.23, 27.73 % for all of them at concentration 50 µg/ml respectively. None of the above mentioned extracts revealed cytotoxic activity against vero cell line. The results obtained support the ethnobotanical uses of the above plants as anti-inflammatory.

Key Words: *Medicinal plants, DPPH, Metal chelating, Antioxidant, MTT*

1. Introduction

Reactive oxygen species (ROS) have been implicated in the induction of various types of oxidative damage to biomolecules that results against, cancer, neurodegenerative diseases, atherosclerosis, malaria, several pathological events in living organisms and different other diseases associated with our life-style (Shahidi and Naczk,1995 ; Halliwell *et al*., 1992). These molecules can induce changes in different biological tissues and cell biomolecules such as lipids, proteins, DNA or RNA. Free radicals can also affect food quality; reducing its nutritional content and promoting the development of food deterioration (Nickavar and Aboalhasani, 2009).

Recently in many African countries comprehensive research was conducted on medicinal plants for the treatment of different diseases and conditions, such as diabetes, malaria, anaemia ,and cancer. The availability and relatively cheaper cost of medicinal plants in sub-Saharan Africa, makes them more attractive as therapeutic agents when compared to 'modern' medicines (Agbor *et al*., 2005)

High-throughput screening methods for toxicology evaluation in the early phases of drug discovery are highly desired. In the early 1980´s a novel assay for cell survival determination was reported by Mosmann, (1983) which has been frequently used to study the biological activity of a variety of potential cytostatic drugs. The assay was presented as a rapid, precise and simple method to detect living cells in mammalian cell cultures, using the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and a microtiter plate reader.

The following plants *Azadiracta indica*, *Ziziphus spina-christi*, *Matricaria chmomilla*, *Ricinus communis*, *Acacia arabica* and *Trigonella foenum* were found of multiple biological activities and broad traditional uses against infectious and non infectious diseases (Zillur Rahman and Jairajpuri, 1996; Bhaskaran *et al*., 2010; Tayel *et al*., 2009; El Ghazali *et al.*, 1998 Embaby and Mokhtar , 2011 and Thorbe, 2011)

This paper is conducted to study the antioxidant activities of the above mentioned plants by two different methods and evaluating their cytotoxicity against vero normal cell line by using MTT spectrophotometric assay.

2-Materials and methods

2-1 Collection of tested plant parts

Tested plant parts of the *Trigonella foenum* seeds (TR fo) were obtained from the local market. *Acacia Arabica* leaves (AC ar) were collected from Elsunut Forest,

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Khartoum State. *Azadiracta indica* leaves (AZ in) and *Matricaria chmomilla* flowers (MA ch) were collected from the farm of Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan. *Ricinus communes* leaves (RI co) were collected from Umdorman area, Khartoum State. While *Ziziphus spina-christi* leaves (ZI sp) were collected from Algezira State, central Sudan. All plant materials were collected during the period June and July 2010 and authenticated by the taxonomist team of MABRI where the voucher specimens were deposited (**Table 1).**

Table (1) List of Sudanese medicinal plants selected to be investigated for their antioxidant and cytotoxiciy.

Scientific name	**Family name**	**Part used**	**Yield %**	**Traditional medicine**
Azdiracta indicia	Meliaceae	Leave	19.66	Antiviral-antibacterial-antifungal
Ziziphus spina-christi	Rhamnaceae	Leave	18.58	Anti inflammatory-filter the blood
Matricaria chmomilla	Asteraceae	Flowe r	14.92	Ant proliferative-apoptotic effect
Ricinus communes	Euphorbiace ae	Leave	14.1	Nerve pain-rheumatism-tumors
Acacia Arabica	Fabaceae	Leave	31.38	Cough-headache-antibacterial
Trigonella foenum	Fabaceae	Seed	8.16	Anti inflammatory-anemia-stomach-infection in the lung

This table indicates the scientific names, families, parts used, yield% of methanol (80%) extract and traditional uses of above selected Sudanese medicinal plants

2-2 Preparation of crude plant extracts

100 gram of each plant sample was coarsely powdered using a mortar and pistil and extracted with 80% methanol by soaking for 18 hours using a shaker (Stuart scientific, flash shaker, S F 1, U K). The extracts were filtered and evaporated using a rotary evaporator (Buchi, 461, Switzerland) at 40°C.

3-1 DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method of Shimada *et al.*, (1992) with some modification. In 96-wells plate, the test samples (6.25 – 50 µg/mL) were allowed to react with 2.2 Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate. Propylgalate was used as standard drug at concentrations 0.5 mM.

3-2 Iron chelating activity assay

The iron chelating ability was determined according to the modified method of Dinis *et al.*, (1994). The Fe^{+2} were monitored by measuring the formation of ferrous ion-ferrozine complex. The experiment was carried out in 96 micro titer plates. The plant extracts (50 µg/mL) were mixed with $FeSO_4$. The reaction was initiated by adding 5mM ferrozine. The mixture was shaken and left at room temperature for 10 min., the absorbance was measured at 562 nm. EDTA was used as standard, and DMSO as control. All tests and analysis were run in triplicate.

4-MTT assay

Serial dilutions of extract 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 2µl of sterile 0.5% Triton X. 50 µl/wells complete culture medium (CCM) were added and 30 µl more were added to second column wells (B – G) that were used as first extract dilution wells. To the first dilution wells in the row, 500 µg of c suspension extract were added to the 80 µl. extract were then serially diluted by two-fold dilution from well B3 till B11 by transferring 250 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 X 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 three-five days (72 hours-120 hours). On the third/fifth day, the supernatant was removed from each well without detaching cells. MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) stock (5 mg/ml) was prepared earlier in 100 ml PBS (phosphate buffer saline). MTT suspension was vortexed and kept on magnetic stirrer until all MTT dissolved. The clear suspension was filter sterilized with 0.2 µ Millipore filter and stored at 4 °C or – 20 until use. MTT was diluted (1:3.5) in a culture medium and brought to room temperature. To each well of the 96 well plate, 50 µl of diluted MTT were added. The plate was incubated further at 37° C for 2 to 3 hours 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 minutes then read at 540 nm using micro plate reader.

%Inhibition = [(A Control – A Sample) / A Control] × 100

Where A Control is the absorbance of the negative control and A Sample the absorbance of tested samples or standard (the same equation was used for calculation %inhibition of all antioxidant assays). All data are an average of triplicate analyses.

5- Statistical analysis

All data were presented as mean ± standard deviation of the mean. Linear regression equations were used for calculation of IC_{50}.

6-Results and discussion

Fig. (1) indicates the anti DPPH activity of all examined plant extracts. *A indica* leaves extract was the most potent it gave inhibition percentage range between 92 – 94% for all tested concentrations 6.25 – 50 µg/mL.

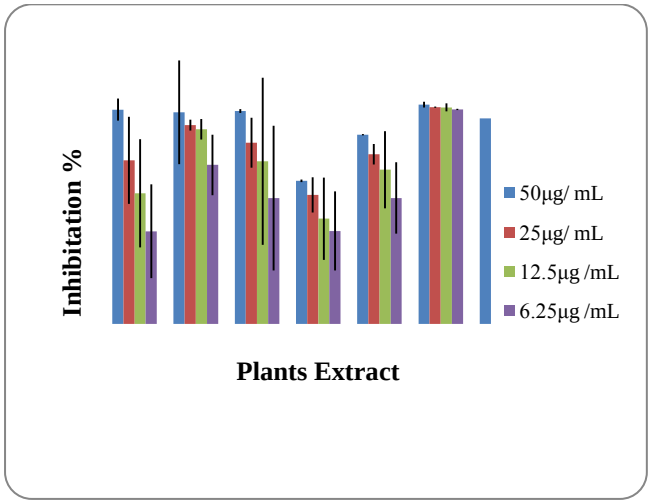

Figure (1): Antioxidant properties assay against DPPH of methanol extract of six Sudanese medicinal plants.

This figure indicate the anti DPPH of 80% methanol extract of six Sudanese medicinal plants the reading in triplicate for different concentration from 6.25 -50 µg/mL propyl galate was used as standard drug level and gave 88% inhibition.

The other plant extracts which are *M. chmomilla* flowers, *R. communis* leaves, *Z. spina-christi* leaves, *A. arabica* leaves and *T. foenum* seeds had shown 91.69, 90.57, 91.13, 61.20 and 90.94 inhibition percentage for higher concentration (50µg/mL). While they were revealed 55.89, 39.66, 53.87, 45.03 and 58.61 inhibition percentage for the lower concentration (6.25 µg/mL) respectively.
The results for antioxidant assay against chelating activity is shown in Fig (2). The following inhibition% values recorded were 8.28, 17.05, 26.87, 15.22, 27.73,and 38.43 for the following plant extracts *M. chmomilla* flowers, *R. communis* leaves, *Z. spina-christi* leaves , *A. Arabica* leaves, *T. foenum* seeds and *A. indica* leaves respectively by using single concentration 50µg/mL.

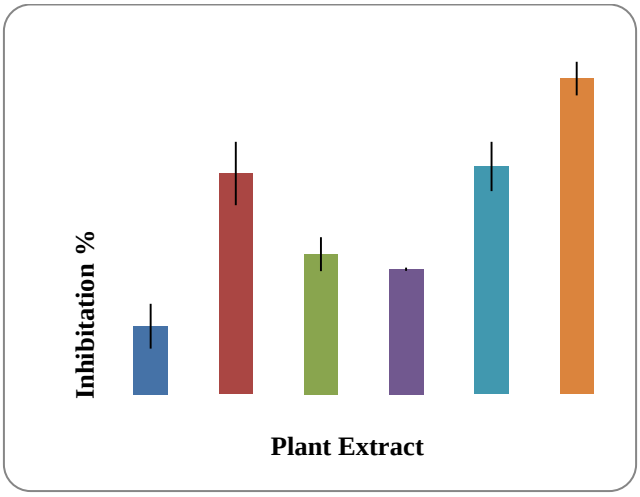

Figure (2): Antioxidant Properties assay against chelating activity of methanol extract of Six Sudanese medicinal plants.

This figure indicates the Antioxidant properties assay against chelating activity of 80 % methanolic extract for the six Sudanese medicinal plants the reading at concentrations of 50 µg/mL.

All the above plant extracts tested for their cytotoxicity against vero cell line. Fortunately they revealed inhibition% less than 50% for all examined concentrations 125 – 500 µg/mL. Although many of them showed negative results i.e. they have nutritional value (Table 2).
Natural antioxidants discovery will help to develop new drug candidates for antioxidant based therap. So the present research is conducted to investigate the anti-oxidative properties of six selected Sudanese plants *A. indica* leaves, *Z. spina-christi* leaves, *M. chmomilla* flowers, *R. communis* leaves, *A. Arabica* leaves *and T. foenum* seeds.

In the first experiment the anti DPPH assay indicated that all the examined plant extracts posses antioxidant activity their IC_{50} was found 9.91, 2.14, 4.74, 17.19, 4.24 and 0.49 µg/mL for extracts *M. chmomilla* flowers, *R. communis* leaves, *Z. spina-christi* leaves , *A. Arabica* leaves, *T. foenum* seeds and *A. indica* respectively however none of the above plant mange to give potent antichelating but *A. indica* was found the most potent among all examined plant extracts that is may be due to many medicinal properties. Neem products are believed to be anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative. It is considered a major component in Ayurvedic and Unani medicine and is particularly prescribed for skin diseases (Zillur Rahman and Jairaipuri, 1993). Siddiqui was the first scientist to bring the anthelmintic, antifungal, antibacterial and antiviral constituents of the neem tree to the attention of natural products chemists. In 1942 he extracted three bitter compounds from neem oil, which are nimbin, nimbinin, and nimbidin respectively (Ganguli, 2002).

Table (2) Cytotoxic activity of selected medicinal plant extracts

Extract	**Concentrations µg/mL**	**Inhibition % ±SD**
Matricaria chmo milla	500	38.83 ±10.8
	250	28.59 ±17.3
	125	- 10.44 ±14.2
Ziziphus spina–Christi	500	40.68 ±13.4
	250	37.40 ±7.5
	125	20.83 ±5.04
Ricinus communis	500	33.19 ±18.1
	250	30.27 ±13.1
	125	6.05 ±2.2
Acacia arabica	500	- 69.27 ±23.6
	250	- 37.26 ±30.8
	125	-17.32 ±33.5
Trigonella foenum greacum	500	- 48.03 ±30.2
	250	- 36.22 ±18.3
	125	3.76 ±21.5
Azadiracta indica	500	- 85.80 ±33.8
	250	- 29.63 ±21.9
	125	3.84 ±29.3

This table indicates the % inhibition of vero cell line growth in vitro by 80% methanolic extract of the selected Sudanesemedicinal plants. MTT colorimetric assay was used. Reading in triplicate for different concentrations 125-500 µg/mL.

The process involved extracting the water insoluble components with ether, petroleum ether, ethyl acetate and dilute alcohol. Recently these above compounds were found of highly potential anti-inflammatory activities (non steroidal anti-inflammatory) (Gadekar *et al*., 2010). Previously neem leaves were reported to contain group of flavonoids, such as quercetin and isorhamnetin (Zielińska *et al.,* 2001). Hence may be these compounds played an important for the antioxidant activities obtained in this study. Also these results totally agree with the ethonbotanical uses of these plants as anti-inflammatory and antirheumatoid. So further investigation for isolation and identification of active ingredients are highly recommended.

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