Antioxidant and Cytotoxic Activities of Sterculia setigera Del.

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Abstract

Antioxidant evaluation of Sterculia setigera plant, used in Sudan traditional medicine, was carried out using 2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) assay on both ethanolic and petroleum ether extracts of seed, fruits, leaves, stem bark, and roots in comparison to propyl gallate as standard level. The ethanolic extract of the fruits showed the highest scavenging activity 91.81% ±0.02 followed by ethanolic extract of stem bark 86% ±0.09 then ethanolic extract of leaves 85%±0.03 at 500 µg mL^{-1}.While petroleum ether extracts of seeds, fruits and leaves exhibit low scavenging activity. In the ethanolic extract of the leaves, stem bark, and fruits, the results of the DPPH scavenging activity indicate a concentration dependent antioxidant activity at 500, 250, 125, 62.50, and 31.25 µgml^{-1} with those of the standard propyl gallate. The tested extracts reduced DPPH free radical in a concentration dependent manner. They showed maximum activities at highest concentrations. The cytotoxic effect of ethanolic extracts was determined against African green monkey kidney cells (Vero cells). The extracts were non-toxic to Vero cells.

Key words: Sterculia setigera, Cytotoxicity, Antioxidant, Vero cell

{**Citation:** Nahla M. M. Taha, Barakat M. Mudawi. Antioxidant and cytotoxic activities of Sterculia setigera Del. American journal of Research Communication, 2018, 6(5): 41-49} www.usa-journals.com, ISSN: 2325-4076.

Introduction

The plant Sterculia setigera Del., (family: Sterculiaceae), is a deciduous savanna tree. It grows up to 15m high characterized by pale purplish bark with thin scales which peel off to expose yellowish patches; slash crimson, exuding a gummy sap.

It distributes in central Sudan in the Blue Nile province and in Kassala and Northern Darfur (Jabal Marra), southern Kordofan and Red Sea hills. It is locally referred to as Terter, Baroot, Um Telaih, Raraiba but its English name karaya gum tree. In Sudan tradition, dried stem bark hot water extract is used for treatment of jaundice, bilharzia and diarrhea. Dried stem bark for treating wounds.

The current study was designed to evaluate the antioxidant activity of petroleum ether and ethanolic extracts from five parts of Sterculia setigera including root, stem bark, leaf, seed, and fruit by using DPPH scavenging assay and to determine cytotoxicity of ethanolic extracts on Vero cell line at different concentrations.

Materials and Method

Sampling of Plant materials

The fresh fruits, seeds, stem barks, leaves and roots of Sterculia setigera Del. were collected from Umabdalla village which located in Southern Kordofan state, western of Sudan in November 2010 except leaves in August. The plant authenticated by a Plant Taxonomist Dr. Haidar Abd Algadir, Medicinal and Aromatic plant research Institute (MAPRI), national research centre, Khartoum, Sudan. Voucher specimens were deposited at the herbarium of the institute. Collected plant parts were cleaned, chopped into pieces, air dried under shade for 4 weeks, and then coarsely powdered using a clean electric blender then carefully stored.

Methods

Preparation of plant extracts

The powder of each part from Sterculia setigera (500 g) were successively extracted with petroleum ether and 80 % ethanol using shaker extractor apparatus. Solvents were

evaporated under reduced pressure using rotary evaporator apparatus. Finally extracts allowed to air in petri dishes till complete dryness to produce 90g, 1.5, 3.5, 5, and 4.5g for seed, fruits, roots, leaves and stem bark respectively for petroleum ether extracts and give 17.5, 37.5, 20, 50, and 49g for seed, fruits, roots, leaves and stem bark respectively regarding ethanol extracts.

Antioxidant activity:

The DPPH radical scavenging assay was determined according to the method of Shimada et al. 1992, with a slight modification.

In 96-wells plate, the test samples were allowed to react with 2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl stable free radical (DPPH). 5mg of each extract is dissolved in 1ml DMSO then 10μl sub-extract is added to 90 μl DPPH for half an hour at 37℃. The concentration of DPPH was kept as (300 μM).The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiple reader spectrophotometer (Thermo Scientific version v1.2). Propyl gallate was used as comparative antioxidant standard, and the concentration used were the same as with the sample. All determinations were run in triplicate.

Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group using the following formula:

The DPPH scavenging effect (%) = 100 - [(A_s / A_c) × 100], where: A_s was the absorbance in the presence of the sample and A_c was the absorbance of negative control.

IC_{50} values for the samples that exhibit RSA% >80% was determined by preparing various concentrations of 500, 250, 125, 62.5, and 31.25µgml^{-1} of sample extracts in DMSO, and the above method was done for each concentration. Then the concentration of each extract required to scavenge 50% of DPPH (IC_{50}) was calculated using EZ-Fit Enzyme Kinetic Program.

Cytotoxicity screening

Cell toxicity was monitored on Vero cell. Vero (Normal, African green monkey kidney) cells were grown in RPMI-1640 medium, supplemented with 10% inactivated fetal bovine serum and 50 µg /ml gentamycin. The cells were maintained at 37˚C in a humidified atmosphere with 5% CO_2 and were sub-cultured two to three times per week.

Cultures were viewed using an inverted microscope to assess the degree of confluence and to confirm the absence of bacterial and fungal contaminants. Cell monolayer washed with phosphate buffer solution (PBS). Trypsin/EDTA was added on to the washed cell monolayer using 1 ml per 25 cm^2 of surface area. The culturing flask was rotated to merge the monolayer with Trypsin/EDTA. The flask was returned to the incubator and left for 2 - 10 minutes. The cells were examined using an inverted microscope (CKX41; Olympus, Japan) to ensure that all the cells were detached and floated.

Crystal violet assay was applied to demonstrate the viability of Vero cell lines. The assay was carried out using 100 µl of cell suspension, containing 10,000 cells seeded in each well of a 96-well micro titre plate for 24 h. Then 100 µl aliquots of the test samples which prepared in DMSO and diluted in fresh media were added. The micro titre plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48 h. Three independent experiments were performed each containing six replicates for each concentration from 2-fold serial concentrations of the tested samples (15.6, 31.25, 62.5,125,250, 500 µg/mL). Untreated cells served as controls. Rifampin was used as a positive standard drug and the concentration used were 3.125, 6.25, 12.5, 25……., 400 µg/mL in DMSO.

After the incubation period, media were aspirated and the cells were fixed with 10% formalin solution for at least 20 min. The fixed cells were rinsed with PBS then stained with a 1% crystal violet for 1 hour. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Finally, the dye was extracted from the cells by adding glacial acetic acid (33%) to the contents of each well. The absorbance was measured

in triplicates at 590 nm using a microplate ELISA Reader (SunRise TECAN, Inc., USA). The percentage cell viability was calculated as follows:

The percentage of cell viability = $[(A_s/A_c)] \times 100\%$

where A_s is the mean absorbance of wells treated with the tested sample and A_c is the mean absorbance of untreated cells.

% **Cytotoxicity = 100 - % cell survival**

The 50% Cytotoxic concentration (CC_{50}), the concentration required to kill or cause visible changes in 50% of intact Vero cells, was estimated from graphic plots.

Statistical analysis

All data were expressed as means ± S.D. Statistical analysis for all assays results were done using the Microsoft Excel program. Student's t-test was used to determine significant difference between control and plant extracts at level of $P < 0.05$.

Results

Antioxidant assay was carried out using DPPH assay on both ethanolic and petroleum ether extracts for seeds, fruits, leaves, stem bark, and roots in comparison to propyl gallate level and presented in Table1. The ethanolic extract of the fruits was found to be the highest scavenging activity 91.81%, followed in descending order by ethanolic extract of stem bark 86% then ethanolic extract of leaves 85%, ethanolic extracts of root 45.38% and ethanolic extracts of root 33 % at 500 µg mL^{-1}. We can conclude that the scavenging effects of fruit, stem bark, and leaves extracts on DPPH radicals were excellent. Table 2 shows antioxidant activity with IC_{50} values for fruits, stem bark and leaves which determined by preparing various concentrations. The tested extracts reduced DPPH free radical in a concentration dependent manner. Figure 1 showed maximum activities at 500µg$ml^{-1}$and minimum activity at 31.25µgml^{-1}. All IC_{50} values are less than100 µg/mL. Their decreasing order of parts

tested was fruit >leaves>stem bark. IC_{50} value of bark was equal to that of propyl gallate so antioxidant properties of bark were similar to the positive standard (IC_{50} value =54 µg/mL).

Table (1): DPPH scavenging effect of five parts of Sterculia setigera

Plant part	Extracting solvent	RSA%* ±SD
Seed	Pet. eth.*	6±0.24
Seed	80%ethanol	33±0.19
Fruit	Pet. eth.	9±0.13
Fruit	80%ethanol	91±0.02
Leaves	Pet. eth.	3.96±0.009
Leaves	80%ethanol	85±0.03
Stem bark	Pet. eth.	37.48±0.27
Stem bark	80%ethanol	86±0.09
Roots	80%ethanol	45.38±0.02
STD, PG		87±0.03

Key: RSA%* = Radicals scavenging activity; STD* = PG = Propyl gallate used as standard level, Pet. eth.* = petroleum ether

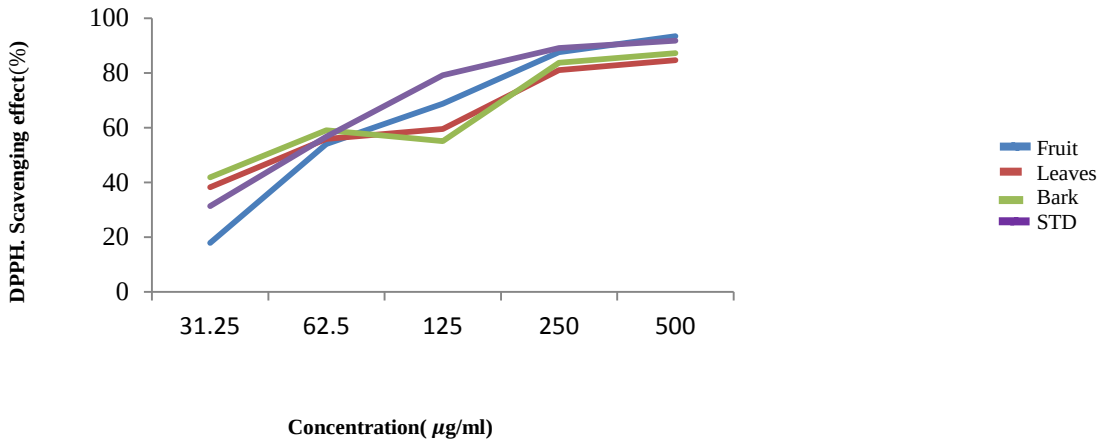

Figure 1. The percentage scavenging of DPPH radical in the presence of different concentrations of Sterculia setigera extracts.

Table (2): The IC_{50} values of DPPH scavenging effect of Sterculia setigera extracts & propyl gallate(µg/mL)

Plant Parts	$IC_{50}\pm$ SD
Fruit	61±0.001
Leaves	58±0.0197
Bark	54±0.007
STD PG	54±0.004

Using crystal violet assay the in vitro cytotoxicity of ethanolic extracts of target parts of Sterculia setigera was evaluated against Vero cells at different concentrations to determine CC_{50}. Results of % viability are represented graphically in Fig. 1. The percentage viability was found to be decreasing with increasing concentration of test compound. CC_{50} (50% growth inhibition) values summarized in Table 3. Cytotoxicity study shows that examined parts of Sterculia setigera do not have any significant cytotoxicity on the normal Vero cell line.

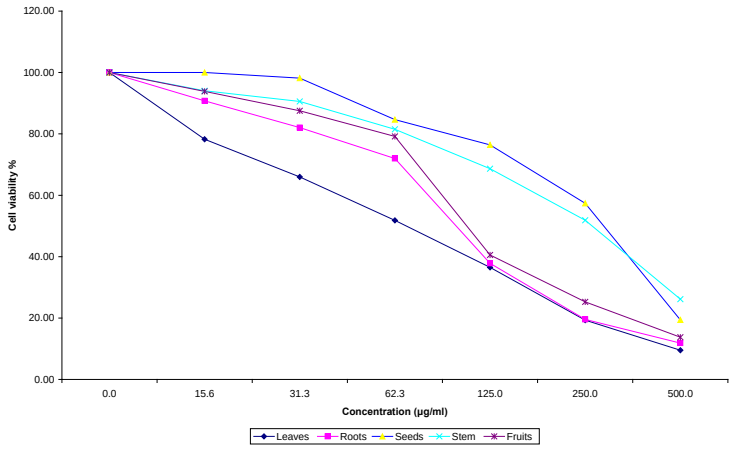

Figure 2. % viability of Vero cell line of alcoholic extracts from five parts of Sterculia setigera.

Table (2): CC50 values of ethanolic extracts from Sterculia setigera parts.

Sample	CC50$\mu g/mL$
Fruit	110
Leaves	69.9
Stem bark	268
Root	103
Seed	298
STD *	115.9

Key: STD* = Rifampin was used as standard drug level.

Conclusions

This study shows that Sterculia setigera extracts exhibit great potential for antioxidant activity and may be useful for their nutritional and medicinal functions.

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