Research articles

# Photochemical Analysis, Antioxidant Activity and Cytotoxicity of *Adansonia digitata* L. Seeds Ethanol Extract

Huiam Salah Eldeen IzzEldeen AbdAlaziz [1] and Mona A M Mageed[2]
1 and 2: Omdurman Islamic University, Faculty of Pharmacy, Department of Pharmacognosy, Omdurman, Sudan

corresponding author: Email: mona.mageed@gmail.com Tel: 249129747440

## Abstract

*Adansonia digitate* Linn (Malvaceae) is a native plant to the west Sudan, locally named Tabaldy. *Adansonia digitate* seeds are believed to have nutritional and medicinal benefits in Sudan. The aims of this study are to screening the phytochemicals of *Adansonia digitate* L seeds ethanol extract and to assess the antioxidant activity and cytotoxicity. The phytochemical screening revealed the presence of glycosides, saponins, steroids, flavonoids, and protein, but alkaloids, tannins and resins were not detected. The antioxidant test of baobab seeds ethanol extract was performed by using the DPPH scavenging test which revealed a moderate activity (53.04±0.067) with IC50 value 0.092± 0.006. The cytotoxicity was tested using three cancer cell line: lung cancer cell (A549), breast cancer cell (MCF7), hepatic cancer cell (HEPG2) and tow normal cell line melanocytes cell (HFB4) and fibrinocytes cell (BHK). The cytotoxicity result of the seed aqueous ethanol extract was exhibited a cytotoxic effect against the normal cell tested (HFB4), (BHK) with IC50 11.6, and 24.5 µg/ml respectively and no effect on cancer cell tested except (MCF7) cell with IC50 11.5 µg/ml. This study does not support the use of seeds powder as nutrition due to the cytotoxic effect.

***Keyword***: *Adansonia digitate*, Tabaldy, Baobab, Phytochemical Screening, Antibacterial, Antioxidant, Cytotoxicity.

## I. INTRODUCTION

The vernacular name for Adansonia digitata (Bombacaceae), baobab, which is means ‘fruit with many seeds(1). The plant is widespread throughout the hot drier region of tropical Africa, being native to the arid parts of central Africa and widely spread in the savannah regions (2). In Sudan it is distributed in the west region, and locally named Tabaldy(3).

The tree is up to 21 meter in height with spreading branches; bark is smooth grayish, often with purplish tinge or brown, leaves digitate, leaflets 3 in young plants 5or 7 in older plants. 5 cm×12.5 cm obviates oblong or lanceolate; flowers solitary, one of the longest-lived trees of the world. It can tolerate well the high temperature up to 40-42°C. The tender roots, tubers, twigs, fruits, seeds, leaves and flowers are all edible and they are common ingredients in traditional dishes in Sudan [(4)].

The literature review revealed that the Leaves consist of protein, lipids, carbohydrates, ash, and vitamin-c, traces of calcium, phosphorus, and mucilage[(4)]. The fruit consist of protein, lipids, ash, calcium, vitamin B1[(5)]. The Phytochemicals of the seed are protein, lipids, ash, calcium, vitamin B1, fatty acids (palmitic acid, oleic, stearic, linoleic acid)[(3,5, 6)].

Young shoot, and stem bark consist of a large quantity of semi fluid white gum, have acidic reaction[(3)]. The presence of flavonoids, phytosterols, amino acids, fatty acids, vitamins and minerals has been reported [(2)].

Baobab tree has multi-purpose uses and every part of the plant is reported to have a nutritional value as a protein and minerals source.

Processing influences the nutritional quality. Seeds are used as a thickening agent in soups. The fermented seeds used as a flavoring agent and the roasted seeds were eaten as snacks or used as a substitute for coffee [(5)]. Fermentation of powdered de-hulled seeds is known to increase protein digestibility[(6)]. It also reduces the trypsin inhibition activity but increasing tannin content[(6)]. Baobab seeds are ground with peanuts, water and sugar to make a sauce used with porridge[(5)]. Seed pulp is sometimes known as monkey bread and is eaten and traded in the different regions. Traditionally it is used in scurvy related diseases, laxative purpose, anti-diabetic, anti-diarrheal, anti- trypanosoma [(3, 7 and 8)]. The powder of raw seeds is used as hiccoughs in infants and children [(8)].

## II. Materials and Methods

### Reagents

All solvents, chemicals and reagent used were manufactured in Germany.

***Collection and preparation of Adansonia digitata* L. seeds**

Mature edible fruits of *Adansonia digitata* L were collected from west Sudan and authenticated by Botany Department, Faculty of Science, Khartoum University. The seeds were separated from the authenticated fruits manually and crushed using mortar and pestle to coarse powder. The coarse powder was macerated with 80% ethanol for 24 hours at room temperature then filtered and concentrated using rotary evaporator and kept in a dry brown container at 4°C until used for further investigations.

**Phytochemical Screening**

Phytochemical analysis of *Adansonia digitata* seed 80% ethanol extract was performed using the methods described [9, 10] with some minor modifications.

**Test for Alkaloids:** 0.5g of extract was diluted to 10ml with acidic alcohol, boiled and filtered. To 5 ml of the filtrate, 2 ml of dilute ammonia was added. 5 ml of chloroform were added and the mixture was shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid. This was divided into three portions. Mayer's reagent was added to one portion, Draggendortf's reagent to the second and Hager's to the third portion. The formation of a cream with Meyer's reagent or reddish-brown precipitate with Draggendortf's reagent and the formation of yellow precipitate with Hager's reagent was regarded as positive for the presence of alkaloids.

**Test for Flavonoids**: 0.5g of the extract diluted with 3ml distilled water and filtered. Dilute ammonia (5ml) was added to the filtrate followed by 1ml of concentrated sulphuric acid. A yellow coloration that disappears on standing indicates the presence of flavonoids.

**Test for Saponins**: 0.5g of the extract was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

**Test for Tannins**: About 0.5g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration.

**Test for Anthraquinones**: 0.5g of the extract was boiled with 10 ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes.

**Test for Terpenoids** (Salkowski Test): 0.5g each of the extract was added to 2ml of chloroform. Concentrated $H_2SO_4$ (3 ml) was carefully added at the tube wall. A reddish-brown coloration layer of the interface indicates the presence of terpenoids.

**Test for Cardiac glycosides** (Keller Killiani Test): 0.5g of extract diluted with 5ml water was added to 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer

**Test for Carbohydrates**: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

**Test for Phytosterols**

**Salkowski's Test:** Extract was treated with chloroform and filtered. The filtrate was treated with few drops of Conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

**Liebermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

**Test for Proteins and Amino Acids**: The extracts were treated with few drops of concentrated nitric acid. Formation of yellow color indicates the presence of proteins.

**Test for Diterpenes:** the extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

**Antioxidant Assay**

The free radical scavenging activity was measured using 1,1diphenyl-2picryl-hydroxyl (DPPH) assay [12,13] with minor modifications. The extracts and vitamin C (positive control), 1000 µg/ml (20 µl), were added in the first three wells of a 96 well plate containing 200 µl distilled water to make up a final concentration of 100 µg/ml. The remaining wells were filled with 110 µl distilled water. The 100 µg/ml extracts and vitamin C in the first rows were serially diluted by adding 20 µl to the wells (which had been dispensed with 110 µl distilled water), followed by 90 µl DPPH (90 ml) methanolic solution to obtain final concentrations of the extracts (which ranged from 100 to 0.8 µg/ml). The plates were

incubated at $37^0$C for 30 minutes and the absorbance was measured at 517 nm, using the enzyme-linked immune sorbent assay (ELISA) plate reader. The percentage radical scavenging activity in the extracts was determined through comparison with ethanol (blank).

The inhibition ratio was calculated as follows: % DPPH radical scavenging = (AC-AS)/AC x 100, where AC is the absorbance of the control solution (containing only DPPH solution) and AS is the absorbance of the sample in the DPPH solution. The percentage of DPPH radical scavenging was plotted against the plant extract/compound concentrations (µg/ml) to determine the concentration of extract/compound required to scavenge DPPH by 50% (EC50).

**Cytotoxicity using SRB Assay**

The cytotoxicity of extracts was measured against normal human skin cells (Normal fibroblast (BKH), and Normal melanocytes (HFB4)) and three different cancer human cell ((Hepatic carcinoma cell line (HEPG2), Breast carcinoma cell line (MCF7), and Lung carcinoma cell line (A549)). Sulfo-Rhodamine-B stain method [14] was used. In a microtiter plate, the outer wells were filled with 200 µl of incomplete medium (without FBS or PS) and the inner wells were filled with 100 µl cell suspension and incubated for 24 h in a humidified atmosphere, with 5% $CO_2$ at 37°C. The plant extract was serially diluted at different concentrations 100, 250 ,500 and 1000 µg/ml and added to the micro titer plate containing human cells, and incubated for 72 hours. Each extract was tested in triplicate. Medium control and DMSO control were included in triplicate for each sample that was tested. Sulfo Rhodamine-B stain reagent was prepared to make a final concentration of 0.3 mg/ml, which was added to the cells in the microtiter plate and incubated for two to three hours. Included in the assay was positive drug controls used are actinomycin-D at 10 µg/ml for fibroblast cell (BKH), breast cancer cell (MCF7), lung cancer cell (A549). Cisplastin at 10 µg/ml was used for breast cancer cells (MCF7) and lung cancer cells (A549), paclitaxel at concentration 5µg/ml for (HEPG2), hydroquinone and kojic acid at concentrations at 100µg/ml and 10µg/ml were used respectively for melanocytes. After incubation, the absorbance was measured at 490 nm with a reference wavelength of 690 nm.

## III. RESULTS

*Adansonia digitate* L. seeds 80% ethanol extract was viscous, brown, and insoluble in water either hot or cold and yielded 4.2%.

The Phytochemical screening of *Adansonia digitata* seeds 80% ethanol extract revealed the presence of alkaloids, carbohydrates, anthraquinone glycosides, phytosterols, and phenolics as tannins and flavonoids. Saponins and diterpenes were not detected (Table 1).

**Table 1: The Phytochemical screening of *Adansonia digitata* seeds 80% ethanol extract**

| Phytochemical group | *Adansonia digitata* seeds 80% ethanol content |
|---|---|
| Alkaloids | + |
| Carbohydrates | + |
| Anthraquinone glycoside | + |
| Saponin | - |
| Phytosterols | + |
| Tannins | + |
| Flavonoids | + |
| Diterpens | - |
| Proteins | + |

**Key: + = detected - = not detected**

The antioxidant activity of *Adansonia digitata* L. seeds 80% ethanol extract exhibited 50% RSA radical scavenging activity with $IC_{50}$ 0.093 µg/ml (Table 2).

**Table 2: The Antioxidant activity *Adansonia digitata* seeds 80% ethanol extract**

| Sample | RSA% ±SD | $IC_{50}$(µg/ml)±SD |
|---|---|---|
| Seed extract | 50.66±0.034 | 0.093± 0.004 |
| Vitamin C | 98.94 ± .051 | 0.0167 ±0.195 |

**Key: RSA= Radical Scavenging Activity, SD = Standard Deviation**

The cytotoxicity effect of *Adansonia digitata* seeds 80% ethanol extract against the five selected cell lines (two are normal and three are cancer cells) was determined. The $IC_{50}$ value revealed no cytotoxic effect against the cancer cell tested except the breast cancer cell with $IC_{50}$ 11.5 µg/ml. the normal cell line was affected by the ethanol extract with $IC_{50}$ 11.5 µg/ml and 24.5µg/ml against normal fibroblasts cell and normal melanocytes cell respectively (Table 3)

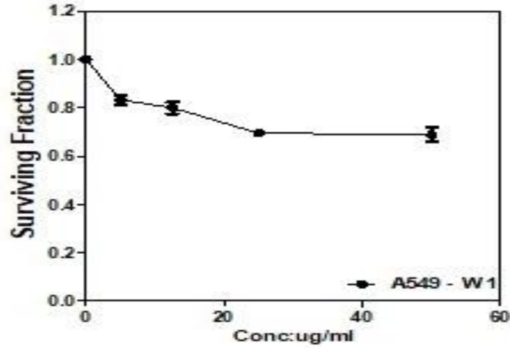
**Table 3: In vitro cytotoxic effect of *Adansonia digitata* seeds 80% ethanol extract at 100 µg /ml against two normal cell line and three carcinoma cell line**

| Sample | The seed extract $IC_{50}$ (µg/ml) | | | | |
|---|---|---|---|---|---|
| | Cell line | | | | |
| | Normal cell line | | Carcinoma cell line | | |
| | BKH | HFB4 | MCF7 | HEPG2 | A549 |
| Seed extract | 11.6 | 24.5 | 11.5 | < 50.5 | < 50.5 |
| Control 1 | 1.0 | NT | 3.8 | NT | 0.6 |
| Control 2 | NT | NT | 1.4 | NT | 7.6 |
| Control 3 | NT | NT | NT | 3.5 | NT |
| Control 4 | NT | 6.0 | NT | NT | NT |

**Key: BKH** = Normal Fibroblasts Cell, **HFB4** = Normal Melanocytes Cell, **MCF7** = Breast Carcinoma Cell, **HEPG2** = Hepatic Carcinoma Cell Line, **A549** = Lung Carcinoma Cell Line, NT = not tested, control 1= Actinomycin-D (5 µg/ml), control 2= Ciplastin (10 µg/ml), control 3= Paclitaxel (5 µg/ml), control 4 = Kojec Acid (100 µg /ml).

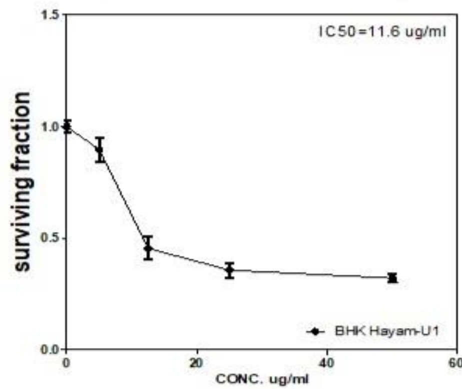
**DRUG CYTOTOXICITY**

| Conc: ug/ml | A549 - W1 | | |
|---|---|---|---|
| | Mean | SD | N |
| 0.000 | 1.000 | 0.029 | 6 |
| 5.000 | 0.832 | 0.045 | 6 |
| 12.500 | 0.799 | 0.062 | 6 |
| 25.000 | 0.695 | 0.036 | 6 |
| 50.000 | 0.688 | 0.071 | 6 |



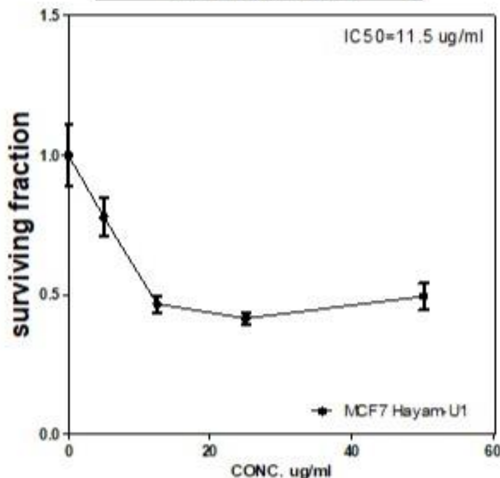

**Drug Cytotoxicity**

| CONC. ug/ml | BHK Hayam-U1 | | |
|---|---|---|---|
| | Mean | SD | N |
| 0.000 | 1.000 | 0.029 | 6 |
| 5.000 | 0.897 | 0.054 | 6 |
| 12.500 | 0.454 | 0.050 | 6 |
| 25.000 | 0.356 | 0.032 | 6 |
| 50.000 | 0.320 | 0.020 | 6 |



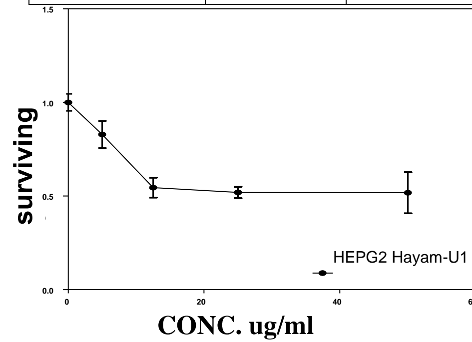

**Drug Cytotoxicity**

| CONC. ug/ml | MCF7 Hayam-U1 | | |
|---|---|---|---|
| | Mean | SD | N |
| 0.000 | 1.000 | 0.109 | 6 |
| 5.000 | 0.779 | 0.067 | 6 |
| 12.500 | 0.466 | 0.029 | 6 |
| 25.000 | 0.415 | 0.021 | 6 |
| 50.000 | 0.494 | 0.047 | 6 |




**Drug Cytotoxicity**

| CONC. ug/ml | HEPG2 Hayam-U1 | | |
|---|---|---|---|
| | Mean | SD | N |
| 0.000 | 1.000 | 0.046 | 6 |
| 5.000 | 0.829 | 0.072 | 6 |
| 12.500 | 0.545 | 0.053 | 6 |
| 25.000 | 0.519 | 0.030 | 6 |
| 50.000 | 0.518 | 0.110 | 6 |




**CONC. ug/ml**

**Drug Cytotoxicity**

| CONC. ug/ml | HFB4 Hayam- U1 | | |
|---|---|---|---|
| | Mean | SD | N |
| 0.000 | 1.000 | 0.019 | 6 |
| 5.000 | 0.931 | 0.026 | 6 |
| 12.500 | 0.690 | 0.060 | 6 |
| 25.000 | 0.492 | 0.046 | 6 |
| 50.000 | 0.413 | 0.013 | 6 |

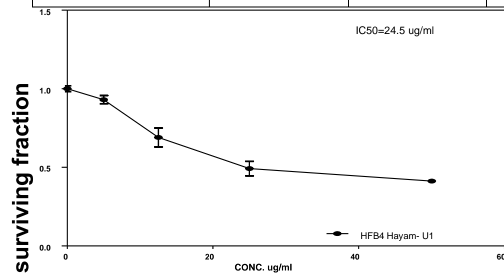



Figure1. The cytotoxicity effects of *Adansonia digitata* seeds 80% ethanol extract on the growth of human cell (two normal cell line and three carcinoma cell line)

## IV. DISCUSSION

Several phytochemical groups were detected in the seed aqueous alcohol extract of *Adansonia digitata* as anthraquinone glycoside, alkaloids, phenol compounds proteins, fats, and carbohydrates this agree well with [15,16]. The presence of these constituents was supported the purposes of its traditional use and agree with [17] who reported that baobab seed flour is an important source of energy and protein, also this finding agree with [18]. The extract revealed the presence of the flavonoids and this result support the significant antioxidant activity of the tested extract. The cytotoxicity of the aqueous ethanol extract revealed significant effects against the normal cell indicating that the soluble compounds in aqueous ethanol are toxic to the dermal cell IC50 for extract on melanocytes and fibroblast was 24.5µg/ml and 11.6µg/ml respectively hydroquinone and kojic acid were used as standard in test of melanocytes cell line their results were 72 and 6µg/ml respectively. Hydroquinone has highly cytotoxicity although kojic acid has less effect. IC50 for extract was less than hydroquinone and more than kojic acid. For fibroblast standard used was doxorubicin IC50 value was

1µg/ml this value is less than that of extract. On the other hand the extract polar compounds revealed a cytotoxic effect against the breast carcinoma cell with IC50 = 11.5 µg/ml. This agrees with [19]. Standards used in test doxorubicin, cisplatin have result of 3.8 and 1.4±0.54 µg/ml respectively. These value were less than that of extract indicating that the standards are cytotoxic than extract.

## V. CONCLUSION

*Adansonia digitata* seeds 80% ethanol extract contain a lot of benefit phytochemicals such as glycosides, alkaloids flavonoids, terpene, carbohydrates, phytosterols. This finding suggest that compound could serve as anew lead for development of novel synthetic with enhancement of anticancer activity. The aqueous ethanol extract showed antioxidant and cytotoxic effect This finding suggest that compound could serve as anew lead for development of novel synthetic with enhancement of anticancer activity. lead to discover another anticancer effect on skin cell. Further biological study is required carbohydrates, phytosterols.

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