



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 776-781
Received: 04-09-2019
Accepted: 06-10-2019

**Huiam Salah Eldeen Izz Eldeen
Abd Alaziz**
Department of Pharmacognosy,
Faculty of Pharmacy,
Omdurman Islamic University,
Omdurman, Sudan

**Mona Abdelmoneim Mohamed
Abdelmageed**
Department of Pharmacognosy,
Faculty of Pharmacy,
Omdurman Islamic University,
Omdurman, Sudan

Phytochemical analysis, antioxidant, cytotoxicity and antibacterial activity of *Adansonia digitata* L. seeds ethanol extract

Huiam Salah Eldeen Izz Eldeen Abd Alaziz and Mona Abdelmoneim Mohamed Abdelmageed

Abstract

Adansonia digitata Linn (Malvaceae) is a native plant to the west Sudan, locally named Tabaldy. *Adansonia digitata* seeds are believed to have nutritional and medicinal benefits in Sudan. The aims of this study are to screening the phytochemicals of *Adansonia digitata* L seeds ethanol extract and to test its antimicrobial activity, antioxidant and cytotoxicity. The phytochemical screening reveals the presence of glycosides, saponins, steroids and flavonoids, protein, but alkaloids, tannins and resins are not detected. The antioxidant test of baobab seeds ethanol extract by using DPPH scavenging test revealed a moderate activity (53.04 ± 0.067) with IC_{50} value 0.092 ± 0.006 . The cytotoxicity was tested using cancer cell line : lung cancer cell(A549), breast cancer cell (MCF7), hepatic cancer cell (HEPG2) and 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 IC_{50} 11.6, and 24.5 $\mu\text{g/ml}$ respectively and no effect on cancer cell tested except (MCF7) cell with IC_{50} 11.5 $\mu\text{g/ml}$. the antibacterial activity tested against three standard bacterial strains *Staphylococcus aureus*, *Eschericia coli*, and *Bacillus subtilis* using cup diffusion method. The antibacterial activity result revealed a moderate activity against *Eschericia coli* (17mm), and high activity against both *Staphylococcus aureus*, and *Bacillus subtilis* with inhibition zone diameter 28mm, and 21mm respectively. this study dose not support the used of seeds powder as nutrition due to cytotoxic effect.

Keywords: *Adansonia digitata*, tabaldy, baobab, phytochemical, antioxidant, cytotoxicity

Introduction

The vernacular name for *Adansonia digitata* (Bombacaceae), baobab, means 'fruit with many seeds' [1]. It is widespread throughout the hot drier region of tropical Africa, 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 the locally named Tabaldy [3].

The tree is Up to 21 meter in height and spreading branches, bark – smooth grayish, often with purplish tinge (or) brown, leaves digitate, leaflets 3 in young plants 5(or) 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 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 review revealed that the Leaves consist of protein, lipids, carbohydrates, ash, and vitamin-c, traces of calcium, phosphorus, and mucilage. The fruit consist of protein, lipids, ash, calcium, vitamin B1. The Phytochemical 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. the presence of flavonoids, phytosterols, amino acids, fatty acids, vitamins and minerals also 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; they are also fermented and used as a flavoring agent or roasted and eaten as snacks when they are roasted, they are sometimes used as a substitute for coffee. In some cases, seeds are de-hulled by boiling, rubbing by hand, and then sun drying the kernels before grinding Fermentation of powdered de-hulled seeds is known to increase protein digestibility. It also reduces the trypsin inhibition activity but increases tannin content. Baobab seeds are ground with peanuts and water and sugar added to make a sauce used with porridge. Seed pulp is sometimes known as monkey bread and [19] is eaten and traded in the different regions. Traditionally it used in scurvy related diseases, laxative purpose, anti-diabetic, anti-diarrheal,

Corresponding Author:
**Huiam Salah Eldeen Izz Eldeen
Abd Alaziz**
Department of Pharmacognosy,
Faculty of Pharmacy,
Omdurman Islamic University,
Omdurman, Sudan

anti- Trypanosoma [3, 7, 8]. The powder of raw seeds is used as Hiccoughs in infants & children [8].

Materials and Methods

Reagents

All solvents, chemicals and reagent used are 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 modification.

Test for Alkaloids: 0.5g of extract was diluted to 10ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and 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, Dragendort'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 Dragendort'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 extract diluted with 3ml of distilled water and filtered. Dilute ammonia (5ml) was added to the filtrate of the extract. 1ml of concentrated sulphuric acid was added. A yellow coloration that disappear on standing indicates the presence of flavonoids.

Test for Saponins: 0.5g of extract was added 5 ml of distilled water in a test tube. 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 0.5 g 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 was 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 (H₂SO₄) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette 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 2ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish-brown

coloration of the interface indicates the presence of terpenoids.

Test for Cardiac glycosides: (Keller-Killiani Test): 0.5g of extract diluted to 5ml in water was added 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 deoxysugar 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: two reagents were used first one **Salkowski's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

The second was Libermann 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 conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

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

Antibacterial Activity

Preparation of test micro-organisms

The micro-organisms used in this study were *Salmonella typhimurium* (ATCC 14028), *Streptococcus pyogenes* (ATCC 21059), *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (NCTC8236), *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922), *Pseudomonas eruginosa* (ATCC27853), and *Klebsiella pneumonia* (ATCC 13883) which were grown at 37°C for 24 h in nutrient broth.

Broth Microdilution assays

The microdilution technique, using 96-well microplates [11], was used to obtain the inhibitory effect of the *Adansonia digitata* seeds 80% ethanol extract against the selected standard bacterial strains, MBC, and MIC. The extract at 50, 100, 150, and 200 mg/ml was added in the 96-well plate with 48-hour-old micro-organisms culture which had been serially diluted to 5 x 10⁶ CFU/ml following incubation at 37 °C. The final concentration of extracts and positive controls (CHX) 25.0 mg/ml was prepared. Microbial growth was indicated by adding 40 µl (0.2 mg/ml) p-iodonitrotetrazolium violet (INT) (Sigma-Aldrich, South Africa) to micro-plate wells and incubated at 37 °C for 48 h. MIC values were determined as the lowest concentration of plant extracts that inhibited the growth of micro-organism and for which there was no change in the colour change of INT. The MBC of the seed extract were determined by adding 50 µl of the suspensions from the wells, which did not show any growth after incubation during

MIC assays, to 150 µl fresh broth. These suspensions were re-incubated at 37 °C for 48 h and 40 µl (0.2 mg/ml) INT added. The MBC values were determined as the lowest concentration of plant extract that inhibited 100% growth of microorganisms and for which there was no change in the color change of INT [12].

Antioxidant Assay

The free radical scavenging activities were measured using 1,1 diphenyl-2-picryl-hydroxyl (DPPH) assay [13]. With slight 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 mM) methanolic solution to obtain final concentrations of the extracts (which ranged from 100 to 0.8 µg/ml). The plates were incubated at 37 °C for 30 minutes and the absorbance was measured at 517 nm, using the enzyme-linked immunosorbent 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 = $(AC-AS)/AC \times 100$, 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)), by *means of the Sulfo-Rhodamine-B stain method [14]. 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% CO₂ at 37 °C. The plant

extract was serially diluted, making up various concentrations with 100, 250, 500 and 1000 µg/ml added to the microtiter 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 as: actinomycin-D *(at various concentrations, ranging from 0.05 to 5.00 µg/ml) for Fibroblast cell (BKH), breast cancer cell (MCF7), Lung cancer cell A549. Cisplatin (at different concentration ranging 1 to 10 µg /ml) for breast cancer cells (MCF7) and lung cancer cells (A549), Paclitaxel (at concentration ranging from 1 to 5 µg /ml) for (HEPG2), hydroquinone and kojic acid at concentrations ranging from (1 to 100 µg /ml) and (0.5 to 10 µg/ml) respectively for melanocytes. After incubation, the absorbance of the colour was spectrophotometrically quantified using an ELISA plate reader, which measured the optical density at 490 nm with a reference wavelength of 690 nm.

Results

Adansonia digitata L. seeds 80% ethanol extract was viscous, brown, 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 phenols as tannins and flavonoids. Saponin and diterpens are not detected (Table 1).

The *Adansonia digitata* seeds 80% ethanol extract revealed no antibacterial activity against all standard bacterial strains tested.

The antioxidant activity of *Adansonia digitata* L. seeds 80% ethanol extract was exhibited 50% RSA radical scavenging activity with IC₅₀ 0.093 µg/ml (Table 2).

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 IC₅₀ value revealed no cytotoxic effect against the cancer cell tested except the breast cancer cell with IC₅₀ 11.5 µg/ml. the normal cell line was affected by the ethanol extract with IC₅₀ 11.5 µg/ml and 24.5µg/ml against normal fibroblasts cell and normal melanocytes cell respectively (Table 3)

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

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

Key: + = detected - = not detected

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

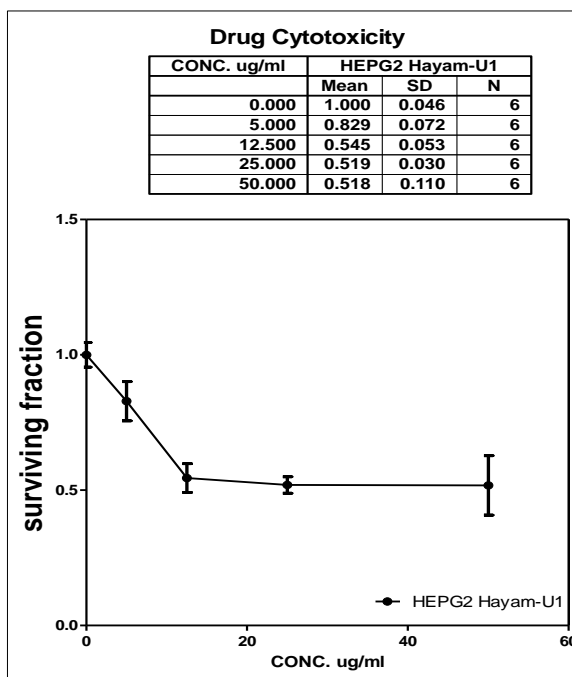
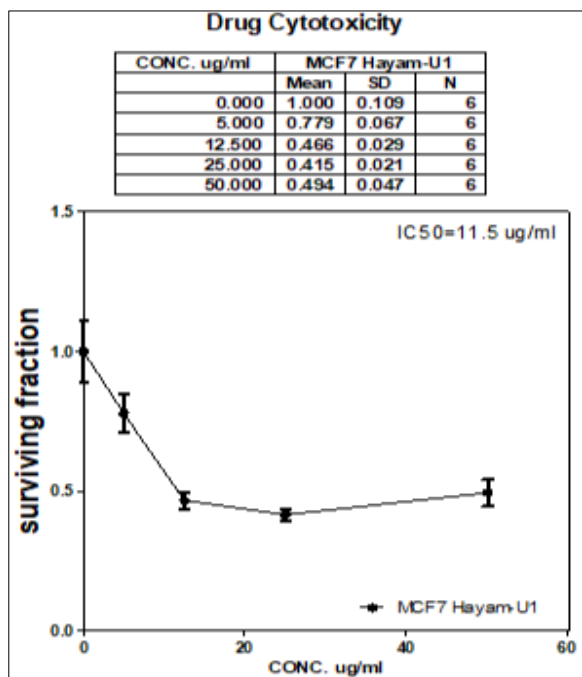
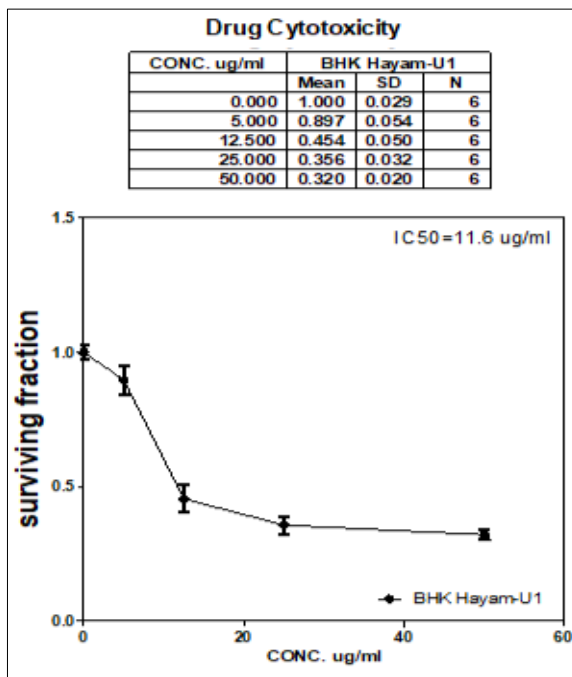
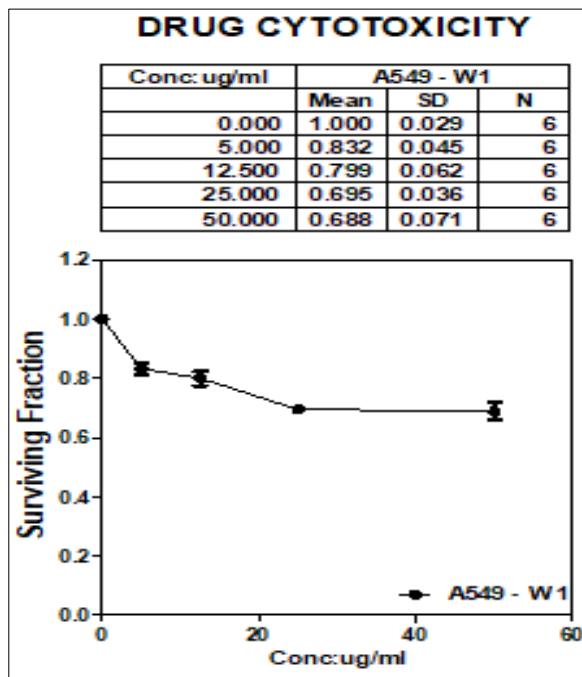
| Sample | RSA% ± SD | IC ₅₀ (µg/ml) ± SD |
|--------------------------|--------------|-------------------------------|
| Seed 80% ethanol extract | 50.66±0.034 | 0.093±0.004 |
| Vitamin C | 98.94 ± .051 | 0.0167 ±0.195 |

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

Table 3: *In vitro* Cytotoxic effect of *Adansonia digitata* seeds 80% ethanol extract against two normal cell line and three carcinoma cell line

| Sample | IC ₅₀ (µg/ml) | | | | |
|---------------------------------|---------------------------|------|---------------------|------------------|-----------|
| | Cell line | | | | |
| | Normal cell line | | Carcinoma cell line | | |
| | BKH | HFB4 | MCF7 | HEPG2 | A549 |
| Seeds 80% ethanol extract | 11.6 | 24.5 | 11.5 | < 50.5 | < 50.5 |
| Doxorubicin positive control | 1.00 | NT | 3.8 | NT | 0.6+ 0.08 |
| Cisplastin positive control | NT | NT | 1.4+ 0.54 | NT | 7.6+ 1.1 |
| Paclitaxel positive control for | NT | NT | NT | Aproximately 3.5 | NT |
| Hydroquinone positive control | NT | 72 | NT | NT | NT |
| Kojec acid positive control | NT | 6 | 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



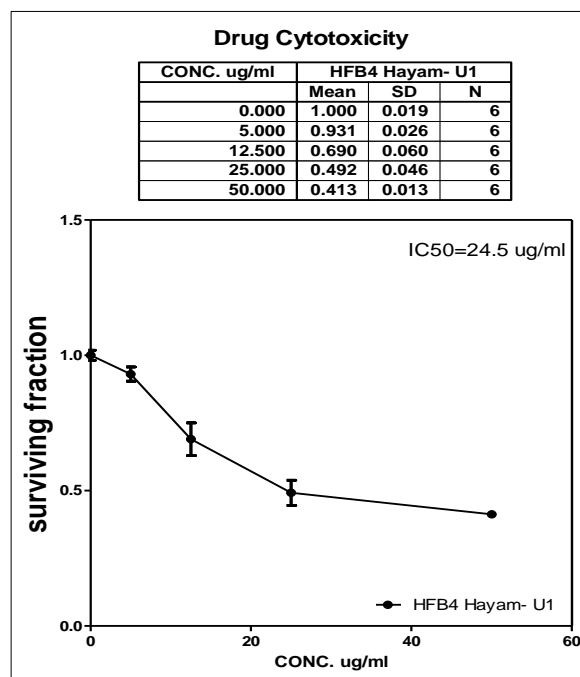


Fig 1: The cytotoxicity effects of *Adansonia digitata* seeds 80% ethanol extract on the growth of human cell (two normal cell line and three carcinoma cell line)

Discussion

Several Phytochemical groups were detected in the seed aqueous alcohol extract of *Adansonia digitata* as anthraquinone glycoside, alkaloids, phenol compounds and proteins, fats, carbohydrates this was agree with [15, 16]. The presence of these constituents was supported the purposes of its traditional use and agree with Murray *et al.* (2001) whose reported that baobab seed flour is an important source of energy and protein, also this finding was agree with [16]. The review reported that all parts of the tabaldy tree revealed a significant antibacterial activity specially the seed oil but the compounds extracted by the aqueous ethanol revealed no inhibition effect against all Gram positive and Gram negative standard bacterial strain tested although the phenols as tannins and flavonoids were detected. The extract revealed the presence of the flavonoids and this result support the significant antioxidant activity of the extract tested. The cytotoxicity of the aqueous ethanol extract revealed significant effects against the normal cell that mean the soluble compounds in aqueous ethanol are toxic to the dermal cell IC_{50} for extract on melanocytes and fibroblast was 24.5 $\mu\text{g/ml}$ and 11.6 $\mu\text{g/ml}$ respectively hydroquinone and kojic acid were used as stander in test of melanocytes cell line their result were 72&6 $\mu\text{g/ml}$ respectively hydroquinone has highly cytotoxic although kojic acid has less IC_{50} not affected the cell [18], IC_{50} for extract was less than hydroquinone and more than kojic acid. For fibroblast stander used was doxorubicin IC_{50} value was $\approx 1\mu\text{g/ml}$ this value as less than that of extract. On the other hand these polar compounds revealed a cytotoxic effect against the breast carcinoma cell with $IC_{50} = 11.5 \mu\text{g/ml}$. this agree with) [17]. While standers used in test doxorubicin, cisplatin as result in 3.8 and 1.4+₋0.54 $\mu\text{g/ml}$ respectively these value were less than that of extract that means stander had cytotoxic effect more than them.

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 require.

Acknowledgement

I want to thank Department of pharmacognosy at Omdurman Islamic University for facilitating and providing the required material and talented staff to do the necessary research work its facilities to complete required task. For. Ciuro University faculty of pharmacy, National Cancer Institute (Eygpt), National Centre for Research department of Medicinal and Aromatic Plant and Traditional Medicine Research Institute in Sudan they were help me to do all experiment I was need. For all assistant and technician in pharmacognosy department.

References

1. Ajayi, Ajayi IA, Dawodi FA, Oderinde RA. Fatty acid composition and metal content of *Adansonia digitata* seeds and seed oil. La Rivista Italiana delle Sostanze Grasse. 2003; 80:41-43.
2. Ezeagu IE. Baobab (*Adansonia digitata* L.) seed protein utilization in young albino rats: biochemical ingredients and performance characteristics. Animal Research International. 2005; 2(2):301-305.
3. Sundarambal M, Muthusamy P, Radha R. A review on *Adansonia digitata* Linn. Journal of Pharmacognosy and Phytochemistry. 2015; 4(4):12.
4. Ernest Small. Top 100 Exotic Food Plants. CRC Press Taylor & Francis group Boca Raton London, New York, 2012, 67-72.
5. Asolkar IV, Kakkar KK, Chakre OJ. Glossary of Indian Medicinal Plants with Active Principles. Edn 3, CSIR, New Delhi. 1965; 10(1):22.
6. Chopra RN, Nagar S, Chopra IC. Glossary of Indian Medicinal Plants. Edn 7, CSIR New Delhi, 2006, 6.

7. De Caluwé E, Halamová K, Van Damme P. *Adansonia digitata* L. a review of traditional uses, phytochemistry and pharmacology. *Afrika focus*. 2010; 23:11-51.
8. Kaboré D, Sawadogo-Lingani H, Diawara B, Compaoré C, Mamoudou H, Jacobsen M. A review of baobab (*Adansonia digitata*) products: effect of processing techniques, medicinal properties and uses. *African Journal of Food Science*. 2011; 5(16):833-844.
9. Evan William Charles. Trease and evan's Pharmacognosy Ebook, Elsevier Health Science, 16th Edition, 2009.
10. Harborne A. *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media, 1998.
11. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med*. 1998; 64(8):711-713.
12. Cohen MA, Husband MD, Yoder SL, Gage JW, Roland GE. Bacterial eradication by clinafloxacin, CI-990, and ciprofloxacin employing MBC test, in-vitro timekill and in-vivo time-kill studies. *J Antimicrob. Chemother*. 1998; 41(6):605-614.
13. Gurava SS, Deshkar NS, Tiloo SK, Duragkar NJ, Burade K. Antimicrobial and Antioxidant Evaluation of *Flacourtia ramontchi* L. *Herit. J Herbs Spices Med. Plants*. 2013; 19(1):76-95
14. Zheng YT, Chan WL, Chan P, Huang H, Tam SC. Enhancement of the antiherpetic effect of trichosanthin by acyclovir and interferon. *FEBS Lett*. 2001; 496:139-142.
15. Murthy HA, Karungamye PN. Highly Efficient Baobab (*Adansonia digitata*) Fruit Seeds and Pulp Extracts as Potential Green Corrosion Inhibitors for Mild Steel in 0.5 M HCl Solution, 2017.
16. Abdulkarim SM. Baobab Tree (*Adansonia digitata* L) Parts: Nutrition, Applications in Food and Uses in Ethno-medicine – A Review. *Annals of Nutritional Disorders & Therapy*. 2014; 1(3):1-9.
17. Elsaid FG. The Effect of Seeds and Fruit Pulp of *Adansonia digitata* L (Baobab) on Ehrlich Ascites Carcinoma. *Food and nutrition science*. 2013; 4(3):38-46.
18. Eenest Curto V, Cecil Kwong, Heino Hermersdorfer T, Hansruedi Glatt T. Chie Santis++, Thomas p Dooley. Inhibitors of mammalian melanocyte tyrosinase: *in vitro* comparisons of alkyl esters of gentisic acid with other putative inhibitors *Science Direct, Biochemical Pharmacology*. 1999; 57(6):663-672.
19. Zahra'u B, Mohammed AS, Ghazali HM, Karim R. Baobab Tree (*Adansonia digitata* L) Parts: Nutrition, Applications in Food and Uses in Ethno-medicine – A Review. *Ann Nutr Disord & Ther*. 2014; 1(3):1011.