Evaluation of antioxidant and anticancer activities of aqueous extract of the fruit pulp of *Adansonia digitata* Linn and its fractions

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Abstract

Introduction: Adansonia digitata L (baobab) is a forest tree, primarily in Africa and Asia. Fruit pulp extract of baobab possesses anti-inflammatory, hepatoprotective, anticlastogenic and other medicinal properties.This study evaluates the *in vitro* antioxidant and anticancer potentials of the aqueous extract of the fruit pulp of baobab and its fractions.

Methods: Cold extraction was done on the pulp for 72 h. The extract was filtered and concentrated; and then resuspended in water and sonicated. The mixture was sequentially fractionated to obtain n-hexane fraction (NHF), chloroform fraction (CRF), ethyl acetate fraction (EAF), n-butanol fraction (NBF) and residual aqueous fraction (AQF).The DPPH, ABTS, nitrite scavenging capacity and reducing antioxidant power assays were determined spectrophotometrically. Anticancer activity on A-549, KB, T-24 and A-498 cell lines was evaluated using sulphurhodamin B assay.

Results: The DPPH radical scavenging activity indicates that NBF has IC_{50} (43.44µg/mL) closest to that of the standard (Ascorbic acid) (27.22 µg/mL). For ABTS scavenging activity, NBF with IC_{50} value of 38.28µg/mL is closest to the standard (7.41µg/mL). For nitrite scavenging capacity, EAF has IC_{50} value (66.05µg/mL) closest to the standard (35.52µg/mL). Overall antioxidant results showed that NBF fraction has the highest antioxidant capacity of all the fractions. The various fractions were not significantly cytotoxic. However, morphological observation with phase contrast microscope showed that CRF, EAF and NBF induced cytotoxic effects on human oral cancer cells (KB).

Conclusion: Aqueous extract of the fruit pulp of *baobab* and its fractions could be applied as natural sources of antioxidant and the fractions are cytotoxic on KB cells.

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Keywords: *Adansonia digitata,* Antioxidant activity, Cytotoxicity, anticancer activity, aqueous extract, sulphurhodamin B assay.

Résumé

Contexte: Adansonia digitata L (baobab) est un arbre forestier, principalement en Afrique et en Asie. L'extrait de pulpe de fruit de baobab possède des propriétés anti-inflammatoires, hépatoprotectrices, anticlastogéniques et autres. Cette étude évalue les potentiels antioxydants et anticancéreux in vitro de l'extrait aqueux de pulpe de fruit de baobab et de ses fractions.

Méthodes: Une extraction à froid a été effectuée sur la pulpe pendant 72 h. L'extrait a été filtré et concentré; puis remis en suspension dans l'eau et soniqué. Le mélange a été fractionné séquentiellement pour obtenir une fraction de n-hexane (NHF), une fraction de chloroforme (CRF), une fraction d'acétate d'éthyle (EAF), une fraction de n-butanol (NBF) et une fraction aqueuse résiduelle (AQF). Le DPPH, l'ABTS, la capacité de piégeage des nitrites et les dosages de pouvoir antioxydant réducteur ont été déterminés par spectrophotométrie. L'activité anticancéreuse sur la ligne des cellules A-549, KB, T-24 et A-498 a été évaluée en utilisant un dosage de sulfurhodamine B.

Résultats: L'activité de piégeage des radicaux DPPH indique que le NBF a une CI50 (43,44 µg / mL) plus proche de celle du standard (acide ascorbique) (27,22 µg / mL). Pour l'activité de piégeage ABTS, le NBF avec une valeur IC50 de 38,28 µg / mL est le plus proche de la norme (7,41 µg / mL). Pour la capacité de piégeage des nitrites, l'EAF a la valeur IC50 (66,05 µg / mL) la plus proche de la norme (35,52 µg / mL). Les résultats globaux des antioxydants ont montré que la fraction NBF a la capacité antioxydante la plus élevée de toutes les fractions. Les différentes fractions n'étaient pas significativement cytotoxiques. Cependant, l'observation morphologique au microscope à contraste de phase a montré que le CRF, l'EAF et le NBF induisaient des effets cytotoxiques sur les cellules cancéreuses orales humaines (KB).

Conclusion: L'extrait aqueux de pulpe de fruit de baobab et ses fractions pourrait être appliqué comme source naturelle d'antioxydant et les fractions sont cytotoxiques sur les cellules KB.

Mots clés: *Adansonia digitata, activité antioxydante, cytotoxicité, activité anticancéreuse, extrait aqueux, dosage de sulfurhodamine* B.

Introduction

Cancer epidemic has continually posed a great challenge globally and the consequential mortality is on the increase. An estimate of 1,685,210 cases was projected in the US for the year 2016, with an average death of around 1,600 per day [1].Despite considerable epidemiological, immunological, genomic, surgical and pharmacological efforts, deaths from cancer continue to increase. The current efficacy of cancer prevention and treatment is still very low [2], necessitating an urgent search for novel anticancer agents.

Cancer cells are made up of billions of cells, originating from an initial cell which multiplies clonally, bypassing apoptosis and accumulating genetic (and/or epigenetic) alterations which results into the formation of neoplastic cells [3]. Blocking of apoptosis can bring about the accumulation of aberrant cells which can lead to critical point in the development of malignancies [4,5]. Many plants have shown great medicinal properties; most of which have been used safely for the management of various diseases. Africa and Asia continents possess numerous medicinal plants, an example of which is *Adansonia digitata L* (baobab). In Nigeria, it is called Ose or Igi-ose by the Yoruba tribe and kukaa by the Hausas. It belongs to the family bombacaceae [6], and the genus *Adansonia* [7]. It is also found in some parts of India, some examples of local names of the tree in India are: Hindi: Gorakhimli, Marathi: Gorakh chinch, Gujarati: Bukha.

Adansonia digitata L (Baobab) is known in many African countries as the "tree of life" due to its many traditional, medicinal and nutritional uses [8]. All parts of the tree including the leaves, bark, fruits and roots are employed traditionally in several African countries as food stuffs and for therapeutic purposes [9]. The fruit pulp of *Adansonia digitata* is employed as analgesic, anti-diarrhoea and in the treatment of smallpox and measles [10], and to stimulate or counteract immune responses [11,12]. Fruit pulp extract have been found to possess anti-inflammatory activities according to Vimalanathan and Hudson, [13]. The aqueous extract was found to possess significant hepatoprotective activity [14,15,16], and anticlastogenic activity [16]. Vertuani *et al.* [17] also reported the antioxidant capacity of derived products from baobab using photochemiluminescence (PCL) assay. Studies showing the anticancer activity of plants in the bombacaceae family are very rare, but research suggests that baobab may have anti-tumor properties [18]. Reports from Guinea also show that baobab fruit extract possess anti-tumor activities [10,18]. The interest in drugs of plant origin is due to the fact that conventional medicine can be inefficient and very expensive, coupled with the numerous side effects. Although, some medicinal uses of *Adansonia digitata* have been documented, there is little information on the anticancer and antioxidant properties of the aqueous extract of the fruit pulp of *Adansoniadigitata*. This study therefore, was to evaluate the *in vitro* antioxidant and inhibitory effects of the aqueous extract and fractions of *Adansonia digitata* on the growth of some cancer cells.

Materials and methods

Reagents

The reagents 1, 1-diphenyl-1-picrylhydrazyl (DPPH), 2', 2',2 -azinibis 3-ethyl benzothiazoline-6-sulfonic acid (ABTS), ascorbic acid, iron (III) chloride hexahydrate, Folin and Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM), RPMI 1640 medium, Eagle's Minimum Essential Medium (EMEM), fetal bovine serum (FBS) and penicillin–streptomycin were obtained from Invitrogen (Carlsbad, CA, USA).

Plant preparation, extraction and fractionation

The leaves and fruits of the tree were used for its identification/authentication and the voucher number stored at the herbarium of Forestry Research Institute, Jericho, Ibadan Nigeria, with FHI NO. 109859. The fruits were broken open and pulp separated from the seeds with the use of a sieve. Cold extraction was carried out on the pulp by soaking in distilled water for 72 h at room temperature. The extract was filtered and the filtrate concentrated using a rotary evaporator at temperature of 40ºC. The crude extract obtained was concentrated and dried. The dry sample was resuspended in distilled water (200 mL) and sonicated for 5 min. The mixture was then transferred to a 1-L separating funnel and sequentially fractionated with n-hexane (3 x 200 mL), chloroform (3 x 200 mL), ethyl acetate (3 x 200 mL) and n-butanol (3 x 200 mL). The resulting fractions and residual aqueous fraction (AQF) were then concentrated to dryness to get n-hexane fraction (NHF), chloroform fraction (CRF), ethyl acetate fraction (EAF), n-butanol fraction (NBF) and aqueous fraction (AQF). The dry residues were stored at -20°C until they were required for further analysis.

Antioxidant assays

DPPH radical scavenging activity

Analysis of DPPH radical-scavenging activity was carried out according to the Blois method [19]. DPPH (0.3 mM) was added to each sample (i.e the crude extract and different fractions). After incubation for 30 min in the dark at room temperature, the absorbance was measured at 518 nm using a microplate reader. Ascorbic acid was used as a positive control. Percent reduction of the DPPH radical was calculated in the following way:

Inhibition concentration (%) = 100 - (A_{sample}/ $A_{control}$)×100.

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test sample) and A_{sample} is the absorbance of the test sample. Tests were carried out in triplicate.

ABTS radical cation scavenging activity

The ABTS assay is based on the ability of different fractions to scavenge the ABTS radical cation in comparison to a standard (Ascorbic acid) [20].The radical cation was prepared by mixing 7mM ABTS with 2.45mM potassium persulfate (1:1 v/v) and leaving the mixture for 24h until the reaction was completed and the absorbance was stable. The ABTS radical solution was diluted with PBS to an absorbance of 0.7± 0.02 at 732nm. The photometric assay was conducted with 180µL of ABTS radical solution and 20µL of each sample; measurements were taken at 732nm after 1min. The antioxidant activity of the crude extract and fractions were calculated by determining the decrease in absorbance.

Nitrite-scavenging capacity

Nitrite-scavenging capacity was evaluated by the method of hydrochloric acid naphthalene ethylenediamine coloration. One milliliter of each sample or 1mL of 50% ethanol (blank) was mixed with 1mL of 5mg/L nitrite solution and 1mL of citric acid buffer (pH 3). After reacting for 30 min at 37°C, 1mL of 4g/L amino benzene sulfonic acid sodium (in 20% hydrochloric) was added in the mixture and then 0.5mL of 2g/L hydrochloric acid naphthalene ethylenediamine (in water) was also added after 3 min. The mixture was reacted for 15min and measured at 538nm. Ascorbic acid was used as positive control. Nitrite-scavenging ratio (%)=(1 - A sample/A blank)×100

Measurement of Reducing Power

The reducing power was determined using the method described by Yen and Duh, [21]. A serial dilution of the extract and fractions were performed in 0.2M phosphate buffer pH 6.6 containing 1% ferrocyanate. The mixture was incubated at 50ºC for 20 mins. 10% trichloroacetic acid (TCA, 2.5mL) was added to a portion of this mixture (5mL) and centrifuged at 3,000g for 10 mins. The supernatant was separated and mixed with distilled water (2.5mL) containing 1% ferric chloride (0.5mL). The absorbance of this mixture was measured at 700nm. The intensity in absorbance was used to measure the antioxidant activity of the extract and its fractions.

Cell cultures

The cell lines were maintained according to the protocols described by Celis [22] and Freshney [23]. Human lung carcinoma cell line (A-549), human oral cancer cell line (KB), human bladder cancer cell line (T-24) and human renal cancer cell line (A-498) were used.They were thawed and mixed with culture medium followed by centrifugation and determination of cell concentration and viability.The cell lines were grown in RPMI 1640 medium, DMEM or EMEM modified with 10% FBS and 1% penicillin-streptomycin and incubated at 37°C in 5% CO_2.

Cell Viability

The cells viability was assessed by trypan blue dye exclusion method using hemocytometer [24].Cells per mL were calculated as follows: Cells per mL = N x 5 x D.F. / volume, where, N is the total number of cells counted in 5 squares, D.F. is the dilution factor and volume is the depth of the counting chamber (0.1 $mm^3 = 1x10^{-4}$). Therefore, cells/mL = N x 5 x D.F. x 10^4. Dilution factor was calculated by dividing the total volume used (20 µL) with the cell suspension volume (10 µL).

The percent cell viability was determined as follows: Viable cells x 100 / Total number of cells

Treatment of cell lines

The cells were passaged twice weekly. Fractions of the aqueous extract of the fruit pulp of *Adansonia digitata* were initially dissolved in DMSO and then with culture medium at a final concentration of 10 % ($^v/_v$) and the mixture was filter-sterilized using 0.22µm syringe filter unit (Millipore, USA). The fractions were added to cells in complete medium after 24 h of growth and were diluted at a final concentration of 10, 20, 40, 80µg/mL. The experiments were in triplicates and repeated at least three times.

Cell cytotoxicity assay [Sulforhodamine– B (SRB)Assay]

In order to evaluate the cytotoxic effects of the fractions, we performed cytotoxicity studies with A-549, KB, T-24 and A-498. The cell lines were exposed

to various sample concentrations (10, 20, 40, or 80 µg/mL).The SRB assay was carried out as previously described by [25, 26]. Sulforhodamine B [70*i*L, 0.4% (w/v)] in 1% acetic acid solution were added to each well and left at room temperature for 20 min. SRB was removed and the plates washed 5 times with 1% acetic acid before air drying. Bound SRB was solubilized with 200*i*L 10m MunbufferedTris-base solution and plates were left on a plate shaker for at least 10 min. Absorbance was read in a 96-well plate reader at 492nm subtracting the background measurement at 620 nm. The test optical density (OD) value was defined as the absorbance of each individual well, minus the blank value ('blank' is the mean optical density of the background control wells). Mean values and CV from 3 replicate wells were calculated automatically.

Gas chromatography-mass spectrum analysis (GC - MS)

The GC-MS analysis of the crude aqueous extract of the fruit pulp of *Adansoniadigitata* was carried out on a Shimadzu GCMS-QP2010 PLUS Gas Chromatography/ Mass Spectrometry (GC-MS) Japan, using helium gas and 5% diphenyl/95% dimethyl polysiloxane as mobile and stationary phases, respectively. The oven and injection temperature were 100°C and 250°C respectively. The injection mode was split and helium at 1.75 mL/min was used as carrier gas. Mass spectra were recorded in electronic impact (EI) mode at 70eV, scanning the 50–700 *m/z* range. The source and interphase temperature were 200°C and 250°C respectively. Different constituents were identified based on comparison of the retention times with those of known samples.

Statistical analysis

The results were expressed as mean ± Standard Error of Mean. Differences between the groups were analyzed by one-way analysis of variance (ANOVA) with Sidak's*post hoc* test using Prism version 6 (GraphPad Software Inc.) USA. P-values less than 0.05 were considered statistically significant for differences in mean. Curves and statistical analysis for cytotoxicities were performed using Excel 7.0 software for Windows.

Results

Extraction yield of the fruit pulp of Adansoniadigitata and its fractions

The yield of the extract and each fraction obtained from the dry plant material were measured. Table 1 shows the extraction yield, the highest solid residue yield was obtained as the residual aqueous fraction. The starting material was 105.6g of the dried aqueous extract of the fruit pulp of *Adansonia digitata* yielding the following:

Table 1: Total residue yields of various solvent fractions of the aqueous extract of the fruit pulp of *Adansonia digitata*

Solvent Fractions	Residue yield (grams)
n-hexane fraction (NHF)	0.28
Chloroform fraction (CRF)	0.44
Ethyl acetate fraction (EAF)	0.35
n-butanol fraction (NBF)	2.69
Aqueous fraction (AQF)	24.58

Antioxidant capacities of the fruit pulp of Adansonia digitata and its fractions

Table 2: Total antioxidant capacities of various solvent fractions of the aqueous extract of the fruit pulp of *Adansonia digitata.*

Solvent	DPPH IC_{50} Values (µg/mL)	ABTS IC_{50} Values (µg /mL)	Nitrite-scavenging capacity IC_{50} Values (µg /mL)
CAQ	61.05578	83.08417	85.82
NHF	140.7155	151.0153	170.61
CRF	132.6549	106.6434	136.94
EAF	55.61728	61.67109	66.05
NBF	43.43602	38.28261	105.56
AQF	70.52023	123.0868	91.95
STD	27.21875	7.414966	35.52

IC_{50} = Concentration of test sample required to produce 50% inhibition of the DPPH radical, ABTS radical or Nitrite scavenging capacity. Values are mean of three different determinations.

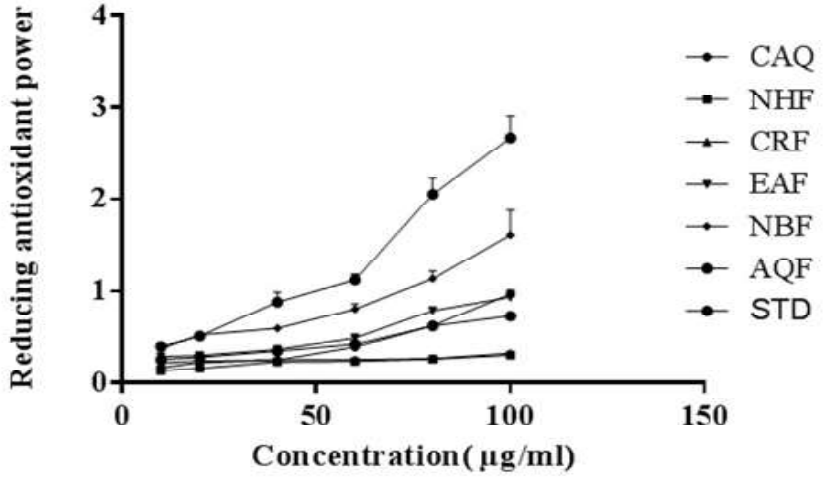

Fig. 1: *Reducing antioxidant power of the crude and various solvent fractions of the aqueous extract of the fruit pulp of Adansonia digitata (AEFAD). Each value represents mean ± SEM of 3 determinations. CAQ= Crude aqueous extract, NHF= n-hexane fraction, CRF= Chloroform fraction, EAF= Ethyl acetate fraction, NBF= n-butanol fraction, AQF= Aqueous fraction, STD= Standard (ascorbic acid).*

Results of the radical scavenging capacities determined by DPPH, ABTS and nitrite scavenging assays are shown in Table 2, while the result for the reducing power assay is shown in Figure 1.In the DPPH assay, the DPPH radical scavenging activity of all fractions from *Adansonia digitata* extract increased as shown in Table 2; the IC_{50} values of radical scavenging activity for DPPH were found to be 61.06, 140.72, 132.65, 55.62, 43.44, 70.52 and 27.22 for the Crude Aqueous, n-hexane, chloroform, ethyl acetate, n-butanol, aqueous fractions and standard (ascorbic acid), respectively. The n-butanol fraction showed the highest DPPH radical-scavenging activity. Considering the ABTS assay, the NBF demonstrated the highest scavenging activity, followed by the EAF, CAQ, CRF, AQF and NHFand this trend was almost similar to that of the DPPH except for CRF that showed more activity than aqueous fraction in the ABTS assay. In the reducing power assay, the ferric complex reducing abilities of different fractions showed that NBF exhibited very strong ferric ion reducing activity,

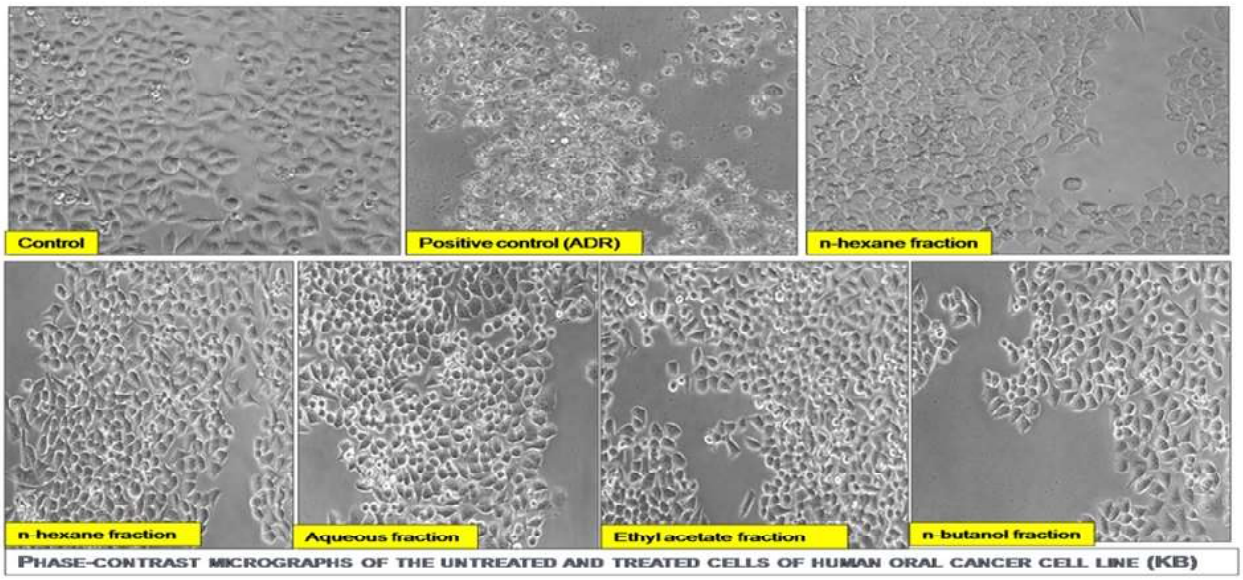

Figure 2: *Phase-contrast micrographs showing the effect of the fractions of aqueous extract of Adansonia digitata on the viability of human oral cancer cell line KB. KB cells were treated with Adriamicin (ADR) or with 80 µg/mL of fractions for 48 hours, the cell viability was assessed by SRB assay.*

Table 3: The GC/MS Analysis of the bioactive compounds in Aqueous Extract of *the* fruit pulp of *Adansonia digitata* (AEFAD)

S/N	Retention time	Compound name	Molecular formular	Molecular weight	Peak area (%)
1	17.30	Pentadecanoic acid	C H O	270	96.17
2	18.71	Hexadecanoic Acid	$C^{17}H^{34}O^{2}$	256	39.45
3	20.30	11-Octadecenoic acid	$C^{16}H^{32}O^{2}$	296	51.45
4	20.60	Octadecanoicacid	$C^{19}H^{36}O^{2}$	298	92.56
5	21.50	Oleic acid	$C^{19}H^{38}O^{2}$	282	35.98
6	21.7	Nonadecanoic acid	$C^{18}H^{34}O^{2}$	298	34.40
7	24.70	3,11-Tetradecadiene-1-ol	$C^{19}H^{38}O^{2}$ 14 26	210	39.60

(Figure 1) and the five fractions in descending order of strength of ferric ion reducing activity were CRF > NHF > CAQ > EAF > AQF. The fruit pulp of *Adansonia digitata* extract and its fractions exhibited good antioxidant activities against various oxidative systems *in vitro.*

Results of gas chromatography-mass spectrum analysis (GC - MS)

The GC/MS analysis of the crude aqueous extract of the fruit pulp of *Adansonia digitata* clearly revealed that it contains seven bioactive chemical compounds namely: Pentadecanoic-acid, Hexadecanoic-Acid, 11-Octadecenoic-acid, Octadecanoic-acid, Oleic-acid, Nonadecanoic-acid and 3,11-Tetradecadiene-1-ol. Table 3 shows the retention time, molecular formula, molecular weight and peak area of the compounds listed. While figure 3 shows the chromatogram.

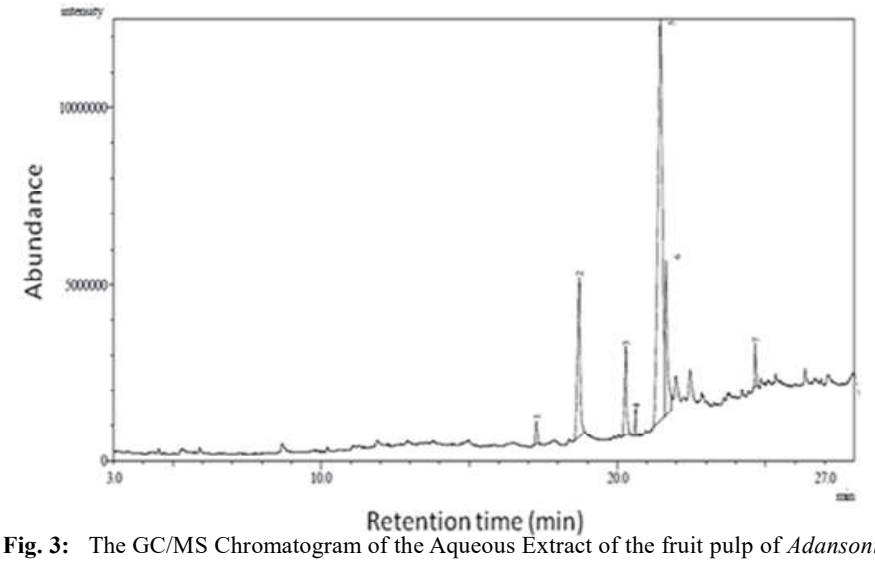

Fig. 3: The GC/MS Chromatogram of the Aqueous Extract of the fruit pulp of *Adansonia digitata* (AEFAD)

Cytotoxicity studies of the aqueous extract of the fruit pulp of Adansonia digitata and its fractions on various cancer cell lines.

Cytotoxicity studies results using sulphurhodamin B (SRB) assay showed that the fractions from the fruit pulp of *Adansonia digitata*extract did not induce ($P > 0.05$) significant cytotoxic activities on the various cancer cell lines studied at the various concentrations tested. However, the results of the phase contrast micrographs showed that the AQF, CRF, EAF and NBF (at 80µg/mL) inhibited cell viability of the KB cells, (figure 2) as shown by some characteristic apoptotic morphological changes which are similar to the ones observed in the positive control treated with Adriamycin.

Discussion

The search for potent anticancer lead compounds from plant origin becomes necessary, owing to the high cost, side effects and toxicity of synthetic drugs [27]. Many scientists are working towards the understanding of the mechanism underlying

carcinogenesis and discovery of novel ways of carrying out treatments by employing the use of natural bioactive agents from medicinal plants which are biologically friendly [28]. Reactive oxygen species (ROS) have important roles in the different stages of carcinogenesis, such ROS include super oxide anion, hydrogen peroxide, hydroxyl radical etc. ROS can induce DNA damage, such as strand break, base modification and DNA protein cross-links [29]. Their presence in biosystem could lead to mutation, transformation, and ultimately cancer. Antioxidants can decrease oxidative stress induced carcinogenesis by a direct scavenging of ROS and/or by inhibiting cell proliferation secondary to the protein phosphorylation [29,30]. Generally, antioxidants prevent cellular damage by reacting with oxidizing free radicals, bringing about their elimination. However, in cancer treatment, the mode of action of certain chemotherapeutic agents involves the generation of free radicals to cause cellular damage and necrosis of malignant cells [31]. There is no evidence showing that natural antioxidants interfere with conventional cancer therapeutics *in vivo*. Reports have however revealed that patients treated with antioxidants, without chemotherapy or with chemotherapy and radiation have better quality of life and also have the tendency to live longer than patients receiving no supplements at all [32].

Sulphorhodamin B (SRB) cell cytotoxicity assay is an accurate and reproducible assay based upon the quantitative staining of cellular proteins by SRB. This assay has been used for high throughput drug screening at the National Cancer Institute (NCI) [25,26]. SRB is an anionic aminoxanthene dye that forms an electrostatic complex with the basic amino acid residues of proteins under moderately acidic conditions, which provides a sensitive linear response [25,26].

Although, there are some reports on the antioxidant properties of *Adansonia digitata*, little is known about the relationship between the antioxidant and anticancer potential of the plant. The aim of this experiment therefore, was to investigate *in vitro* antioxidant and cytotoxic effect of exposure of the various fractions of the fruit pulp of *Adansonia digitata* on some selected cancer cell lines using the reliable antioxidant assays and SRB assay, respectively.The DPPH, ABTS, nitrite scavenging capacity and reducing antioxidant power assay are widely used to determine the antioxidant capacity of plant extracts due to their simplicity, stability and reproducibility. In this study, the DPPH, ABTS, nitrite scavenging capacity and reducing antioxidant power assay provided comparable results for the antioxidant capacity measured in the fruit pulp of *Adansonia digitata* extract and its fractions. The DPPH radical scavenging activity of all the fractions from the aqueous extract of the fruit pulp of *Adansonia digitata* compare well with that of the standard, the IC_{50} value of NBF has the closest IC_{50} (43.44 µg/mL) to that of the standard (27.22 µg/mL), while the n-hexane fraction has the highest IC_{50} value (140.72 µg/mL). Therefore, NBF showed the highest DPPH radical scavenging activity. For the ABTS assay, the NBF fraction also demonstrated the highest scavenging activity of all the fractions tested with IC_{50} value of (38.28 µg/mL), as compared to the standard (7.41 µg/mL), followed by the EAF, CAQ and CRF. For the nitrite scavenging capacity, Ethyl acetate fraction gave the IC_{50} value (66.05 µg/mL) closest to the standard (35.52 µg/mL). In the reducing power assay the more antioxidant compounds convert the oxidation form of iron (Fe+3) in ferric chloride to ferrous (Fe+2) in the results of this research, the n-butanol fraction showed the highest antioxidant content when compared with other solvent fractions. Fruit pulp of *Adansonia digitata* extract and its fractions exhibited good antioxidant activities against various oxidative systems *in vitro.*Vertuani *et al.* 2002 had earlier reported the antioxidant capacity of derived products from *Adansonia digitata* by employing the photochemiluminescence (PCL) assay as the analytical method and the concept of integral antioxidant capacity (IAC), the study recommended *Adansonia digitata* fruit pulp as novel ingredient for food and/or nutriceutical application in health promotion [17].

In order to evaluate the cytotoxic effects of all samples, we performed cytotoxicity studies with human lung cancer cell line (A-549), human oral cancer cell line (KB), human bladder cancer cell line (T-24) and human renal cancer cell line A-498. The cell lines were exposed to various sample concentrations (10, 20, 40, or 80 ìg/mL). In the present study, cytotoxicity studies results demonstrate that the fractions from the fruit pulp of *Adansonia digitata* did not induce significant cytotoxic effects on the various cancer cell lines studied at the various concentrations tested. The results of the phase contrast micrographs showed that the AQF, CRF, EAF and NBF (at 80µg/mL) inhibited cell viability on the KB cells, these were exhibited by some characteristic apoptotic morphological changes which are similar to the ones observed in the positive control, treated with adriamycin.These results suggest that AQF, CRF, EAF and NBF of the fruit pulp of *Adansonia digitata* extract may possess stronger cytotoxic effects on KB at higher concentration and

may be useful in the treatment of cancer. This is corroborated by the previous report from Fahmy, 2013 which reported the antitumor effects of the extracts of seeds and fruit pulp of *Adansonia digitata* on ehrlich ascites carcinoma [33].

The spectrum profile of GC-MS confirmed the presence of 7 major components, which are: Pentadecanoic acid, Hexadecanoic Acid, 11-Octadecenoic acid, Octadecanoicacid, Oleicacid, Nonadecanoic acid, 3,11-Tetradecadiene-1-ol. Out of the seven compounds present, hexadecanoic acid is known to possess antitumor activity; this may be responsible for the cytotoxic activity observed. Lai *et al.* 2008 reported a notable antitumor effect of fatty acids such as hexadecanoic acid obtained from plant extracts [34]. Despite the phytochemical studies on the fruit pulp of *Adansonia digitata,* its biological activities and active constituents have not been examined to a large extent. The evidences presented here have shown that AQF, CRF, EAF and NBF (at 80µg/mL) exhibited some inhibitory effects on cell proliferation and could inhibit the growth of KB cell line at higher concentrations. The biological activities of the aqueous extract of the fruit pulp of *Adansonia digitata* and its fractions may be the result of the synergistic effects of the various compounds present in the extract, which suggests that the aqueous extract of the fruit pulp of *Adansonia digitata* can be used as an antioxidant supplement and also in the treatment of human oral cancer (KB) at higher concentration.

Conclusion

Taken together, the antioxidant and cytotoxic activities of the aqueous extract and fractions of the fruit pulp of *Adansonia digitata* may be the result of the synergistic effects of various compounds present in the extract. Also, these results suggest that AQF, CRF, EAF and NBF fractions possess stronger cytotoxic effects on KB especially at higher concentration and may be useful in the treatment of cancer.

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Summary

- The results show that all the fractions tested have varying antioxidant capacity.
- N-butanol fraction has the highest antioxidant activity, when compared with other fractions examined.
- Cytotoxicity studies results using Sulphurhodamin B (SRB) assay demonstrate that the fruit pulp of *Adansonia digitata* extract and its fractions did not induced significant cytotoxic effects on the various cancer cell lines studied.
- Phase contrast micrographs showed that the AQF, CRF, EAF and NBF (at 80µg/mL) inhibited cell proliferation on the KB cells.
- The spectrum profile of GC-MS confirmed the presence of 7 major components including hexadecanoic which has a notable antitumor effect.

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