

The Nelson Mandela African Institution of Science and Technology

<http://dspace.nm-aist.ac.tz>

Life sciences and Bio-engineering

Research Articles [LISBE]

2019-07-10

In vitro antiproliferative potential of Annona senegalensis Pers. and Allophylus africanus P Beauv. plant extracts against selected cancer cell lines

Biseko, Emiliana Zacharia

Journal of Medicinal Plants Research

<https://doi.org/10.5897/JMPR2019.6785>

Downloaded from Nelson Mandela-AIST's institutional repository

Full Length Research Paper

***In vitro* antiproliferative potential of *Annona senegalensis* Pers. and *Allophylus africanus* P Beauv. plant extracts against selected cancer cell lines**

Emiliana Zacharia Biseko^{1*}, Hulda Shaidi Swai¹, Regina Wachuka Mbugua², Jecinta Wanjiru Ndung'u², Jean Chepng'etich² and Jeremiah Waweru Gathirwa²

¹Department of Global Health and Biomedical Sciences, School of Life Science and Bioengineering, Nelson Mandela African Institution of Science and Technology, P. O. Box 447, Arusha, Tanzania.

²Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute (KEMRI), P.O. Box 54840 00200, Nairobi, Kenya.

Received 14 May, 2019; Accepted 20 June, 2019

The medicinal plants *Annona senegalensis* Pers. and *Allophylus africanus* P Beauv. are used in Tanzania traditional medicine for the treatment of cancer. However, there is no scientific documentation on their therapeutic effectiveness. The present study evaluated antiproliferative potential as an indicator of anticancer activity of *A. senegalensis* and *A. africanus* plant species from Tanzania. *A. senegalensis* and *A. africanus* were collected from Ugweno village at Kilimanjaro, Tanzania. Different types of extracts were prepared in dichloromethane/methanol (DCM:MeOH), petroleum ether, DCM, ethyl acetate (EtOAc), MeOH and water respectively. Antiproliferative activity against HCC 1396 (breast), HEP-2 (throat) and CT 26 (colon) cancer cell lines was assessed by the MTT cell viability assay. The results of the present study showed that the antiproliferative activity varied between plant extracts and the cancer cell lines. The highest antiproliferative activity was achieved with petroleum ether extract of *A. senegalensis* against HEP-2 with an IC₅₀ value of 0.42 ± 0.09 µg/ml. This also depicted the highest selectivity to cancerous cells (SI value 94.19) compared to the other extracts. *A. africanus* also depicted good antiproliferative activity against HEP-2 with IC₅₀ values of 1.00 ± 0.41 and 2.37 ± 1.45 µg/ml for DCM:MeOH and petroleum ether extracts, respectively. The findings validate the traditional use of *A. senegalensis* and *A. africanus* in the treatment of cancer. Results also support previous studies which demonstrated the effect of extraction solvent used in extraction of bioactive agents from medicinal plants. Further studies involving the isolation of pure antiproliferative compounds against cancer cells from the two plants are recommended to elucidate bioactive molecules.

Key words: *Annona senegalensis*, *Allophylus africanus*, antiproliferative, cancer cells, plant extracts.

INTRODUCTION

Cancer is a leading cause of death worldwide (Max Parkin et al., 2005). Globally, the number of people dying of cancer is expected to increase from 8.2 million in 2012 to 14.6 million by 2035 (WHO, 2014; Siegel et al., 2018).

Cancer is a threat to the economy as a lot of money has been invested in cancer treatment but survival rate remains unchanged (Kaur et al., 2009). Cancer is associated with several factors such as environmental,

lifestyle, social, cultural, hormonal and gender factors (Aslam et al., 2014). Among these factors, tobacco use, lack of physical exercise, unhealthy diet, alcohol consumption, automobile exhaust pollutant, Solar UV radiation and bacterial or viral infections are included (Prakash et al., 2013).

Cancer treatments approach involve chemotherapy, surgery, radiation therapy, immunotherapy, targeted therapy, and hormonal therapy, of which have side effects (Baskar et al., 2012; WHO, 2014). Of all treatments, chemotherapy is the most effective but due to high dose requirements, it kills normal cells, hence causing side effects such as fatigue, nausea, hair loss, vomiting, loss of appetite, constipation, anaemia and diarrhoea (Conklin, 2004; Prakash et al., 2013). Herbal medicines have been used as alternative sources of treatment since the early ages (Kooti et al., 2017). It has been used by our ancestors to treat various diseases (Elansary et al., 2018). According to the World Health Organization (WHO), almost 75% of the world's population use herbal medicines (Yasser, 2016; Napagoda et al., 2018). Plants produce secondary metabolites which have been reported to possess therapeutic effects which are non-toxic to normal cells, hence not harmful to the body (Greenwell and Rahman, 2015; Priya et al., 2015). The metabolites are tannins, carbohydrates, alkaloids, saponins, terpenoids, phenolic compounds, steroids, glycosides and flavonoids (Khalid et al., 2018). Study of Al-asady et al. (2014) indicated that the glycoside Fraction I from *Convolvulus arvensis* had more cytotoxic inhibition at 10 mg/ml against rhabdomyosarcoma (RD) tumour cell line *in vitro* after 72 h, compared with other extracts (aqueous and methanol) crude extracts of the leaves, stems and roots. Glycoside FI had cytotoxicity concentration 50% (CC 50%) 1.775, 0.870 and 0.706 mg/ml after 24, 48, and 72 h, respectively.

In Tanzania, medicinal plants play an important role in providing primary health care to rural and urban communities (Kisangau et al., 2007). Traditional health practices also provide a source of income to traditional healers within the country (Kitula, 2007). However, there is lack of scientific documentation on the therapeutic effect of most of the medicinal plants. In an attempt to fill the gap, we selected *Annona senegalensis* and *Allophylus africanus* to validate traditional use for cancer treatment. *A. senegalensis* popularly known as the African custard apple or wild custard apple is a shrub or small tree of about 2-7 m or more belonging to the family Annonaceae (Okoli et al., 2010; Mustapha, 2013). It is native and widely distributed in Africa (Okoye et al., 2012). Ethnomedicinally, it has been reported to exhibit

antimicrobial, antioxidant, antiparasitic, anti-inflammatory, anticonvulsant, antimalarial, trypanocidal, anti-snake venom, anti-nociceptive and anthelmintic activities (Ajaiyeoba et al., 2006; Awa et al., 2012; Mustapha, 2013). It has been reported to be effective against cervical, skin and pancreatic cancers (Graham et al., 2000; Okoye et al., 2014). *A. africanus* belongs to the family Sapindaceae (Balogun et al., 2016). It is widely spread in tropical and subtropical regions of the America, Africa, Asia and Indian archipelago (Chavan and Gaikwad, 2016). Scientifically, it is reported to have strong antimalarial, antibacterial and antioxidant activities (Sofidiya et al., 2012; Balogun et al., 2016). One of the species from the same genus, *Allophylus cobbe*, was confirmed to have anticancer activity against human prostate cancer cell lines (Ghagane et al., 2017).

This study evaluate the *in vitro* antiproliferative potential of *A. senegalensis* and *A. africanus* that are used in Tanzania traditional medicine as anticancer remedies by using three cancer cell lines namely; HCC 1396 (breast), HEP- 2 (throat) and CT 26 (colon).

MATERIALS AND METHODS

Plants collection

The fresh stem bark of each plant was sustainably collected from Ugweno village in Kilimanjaro region of Tanzania during the dry season in the month of December, 2017. Traditional healers guided plants collection which were then identified by a taxonomist at the Tropical Pesticides Research Institute (TPRI) Herbarium located in Tanzania. Voucher specimens assigned numbers EB.01 and EB.02 for *A. senegalensis* and *A. africanus* respectively were then deposited at the herbarium

Plant processing and extraction

Plant materials (stem bark) were chopped into small pieces, air dried and ground to a fine powder using an electric blender then stored at room temperature until used. Extraction was done using six solvents for each plant making a total of 12 extracts. The solvent used was dichloromethane/methanol (DCM:MeOH) at a ratio of 1:1 (Fouche et al., 2008). Briefly, 500 g of each plant powder was soaked completely into a mixture of 1 L of DCM and 1 L of MeOH for 72 h. The extract solutions were filtered and concentrated using a rotary evaporator. Extraction was also done sequentially with petroleum ether, DCM, ethyl acetate (EtOAc) and MeOH starting from least polar to most polar solvent respectively. For sequential extraction, 500 g of each plant powder was soaked in 1 L of petroleum ether, and then the filtrate re-soaked in the rest of solvents sequentially. All solvents were filtered after every 48 h and extracts concentrated by rotary evaporator (Bandar et al., 2013). The remaining powder material was further extracted in aqueous medium by soaking 500 g of fine powder of each plant material in 1 L of water at 60°C for 1 h. The filtrate was then freeze-dried to free

*Corresponding author. E-mail: bisekoe@nm-aist.ac.tz. Tel: +255 769 440 982.

powder (Rukunga et al., 2009).

Phytochemical analysis

The 12 plant extracts were screened for secondary metabolites using standard methods (Sowmya and Lakshmidevi, 2013; Ajuru et al., 2017). Secondary metabolites tested were alkaloids, tannins, glycosides, flavonoids, saponins, and terpenoids.

In vitro antiproliferative screening

Cell lines culturing

CT 26: Colon carcinoma (colon cancer), HEp- 2: Human larynx carcinoma (throat cancer), and HCC 1396: Human breast carcinoma (breast cancer) were used as the cancer cell lines. VERO P23 (African green monkey kidney) was used as the normal cells for reference purpose. The cell lines were originally obtained from the American Type Culture Collection (ATCC) and sub-cultured at the Center for Traditional Medicine and Drug Research (CTMDR), Kenya Medical Research Institute (KEMRI). The cell lines were cultured in Dulbecco Modified Essential Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 1% L-glutamine and 100 µg/ml of streptomycin at 37°C in a 5% CO₂ and 95% humidity.

Antiproliferative assay

Upon attainment of confluence, cells were washed with saline phosphate buffer and harvested by trypsinization. The number of viable cells was determined using the trypan blue exclusion method (cell density count) using a hemocytometer. They were then seeded in 96 well plates at a concentration of 2×10^5 cell/ml in 100 µl per well and incubated for 24 h at 37°C in a 5% CO₂ and 95% humidity to let cells adhere onto the surface of the wells. Three columns of each plate were left without cells. These were filled with 100 µl per well of media only to serve as a blank. Each extract was then added onto row H of the plate at a concentration of 100 µg/ml. This was followed by three folds serial dilution to get different concentrations from 100, 33.33, 11.11, 4.0, 1.33, 0.44 and 0.146 µg/ml from row H to B respectively. Row A was left as a negative control. Doxorubicin, a standard drug for cancer treatment was used as positive control (Wang et al., 2004). All concentrations were replicated three times for each plant extract and then incubated for 48 h at 37°C in a 5% CO₂ and 95% humidity. The extracts were also added to the columns filled with media only so as to evaluate the effect of extract concentrations without the cells and to allow evaluating whether extracts alone would cause MTT quenching. After 48 h incubation, 10 µl of MTT dye (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) was added to each well and incubated for 2 h to allow interaction with the living cells to form formazan (Twentyman and Luscombe, 1987). The blue insoluble formazan product which is directly proportional to the number of living cells present during MTT exposure was then dissolved by 50 µl DMSO. Absorbance was then read at a wavelength of 540 nm and a reference wavelength of 720 nm using ELISA Reader (MULTSKAN GO Thermo scientific, USA). The effect of the plant extracts on the cells was expressed as IC₅₀ values, (drug concentration inhibiting cell growth by 50% compared to untreated cells). Antiproliferative activity was classified according to the standards of the National Cancer Institute (NCI) as follows; high antiproliferative when an IC₅₀ <20 µg/ml, antiproliferative for an IC₅₀ between 20 µg/ml to 30 µg/ml, moderately for IC₅₀ between 30 µg/ml to 100 µg/ml and inactive with IC₅₀ >100 µg/ml (Boik, 2001). The percentage growth inhibition was calculated using the following

formula below (Bézivin et al., 2003).

$$\text{Percentage inhibition} = 100 - (At - Ab) / (Ac - Ab) \times 100$$

Where; At=Absorbance value of test compound (cells plus extracts)
Ab=Absorbance value of blank (media only)
Ac=Absorbance value of negative control (cells plus media)

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA, MiniTab Version 18) to determine differences (p<0.05) among plant extracts IC₅₀. Multiple comparisons of IC₅₀ were done by Tukey test. Experimental results are expressed as mean ± SEM and all measurements were in triplicate. Selectivity index (SI) was determined by the equation below;

$$SI = CC_{50} (\text{VERO cells}) / IC_{50} (\text{cancer cell})$$

RESULTS AND DISCUSSION

The use of herbal medicines in the treatment of various diseases has received increasing attention due to their varied phytochemical contents with multiple biological activities. *A. senegalensis* and *A. africanus* were collected from Ugweno village of Kilimanjaro region in Tanzania and evaluated for antiproliferative activity. A total of 12 extracts were made by using six solvents for each plant species. The percentage yield of each extract is shown in Table 1. Extraction yields varied from 1 to 6.8% depending on the type of solvent used for extraction. Highest yields were obtained with aqueous extractions. This may be due to the high solubility of different plant compounds in this solvent (Senguttuvan et al., 2014). The stem bark of *A. senegalensis* and *A. africanus* was screened for secondary metabolites and results presented in Tables 2 and 3, respectively. Presence of flavonoids was observed in all extracts. Alkaloids, saponins, glycosides and tannins were absent in petroleum ether extracts of both plants. All tested metabolites were detected in ethyl acetate extract of *A. senegalensis*. The presence or absence of phytochemicals in extracts used can be explained by the different polarities of compounds which were selectively more soluble in different solvents (Ngo et al., 2017; Thouri et al., 2017; Snehlata et al., 2018).

The concentration of plant extracts that inhibited cell growth by 50% (IC₅₀) for the twelve plant extracts was calculated and the results displayed in Table 4. Tabulated results show that antiproliferative activity varied between plant extracts and the cancer cell lines. This variation may be due to the type of bioactive compounds present in the different extraction solvents used (Gberikon et al., 2015). The highest antiproliferative activity was achieved with petroleum ether extract of *A. senegalensis* against HEp-2 with an IC₅₀ value of 0.42 ± 0.09 µg/ml. This demonstrated the efficiency of petroleum ether over the other extraction solvents for extracting antiproliferative

Table 1. Extraction yield (%) of *A. senegalensis* and *A. africanus*.

| Plant sample (stem bark) | Sample weight (g) | Extracted weight (g) | Yield (%W/W) |
|--------------------------|-------------------|----------------------|--------------|
| AS Pet ether | 500 | 5 | 1.0 |
| AA Pet ether | 500 | 6 | 1.2 |
| AS DCM:MeOH | 500 | 25 | 5.0 |
| AA DCM:MeOH | 500 | 10 | 2.0 |
| AS DCM | 500 | 6 | 1.2 |
| AA DCM | 500 | 8 | 1.6 |
| AS Ethyl acetate | 500 | 9 | 1.8 |
| AA Ethyl acetate | 500 | 11 | 2.2 |
| AS MeOH | 500 | 15 | 3.0 |
| AA MeOH | 500 | 26 | 5.2 |
| AS Aqueous | 500 | 34 | 6.8 |
| AA Aqueous | 500 | 32 | 6.4 |

AS: *Annona senegalensis*, AA: *Allophylus africanus*

Table 2. Phytochemical analysis of different solvent extracts of *A. senegalensis*.

| Solvent | Alkaloids | Saponins | Flavonoids | Glycosides | Terpenoids | Tannins |
|---------------|-----------|----------|------------|------------|------------|---------|
| Pet ether | - | - | + | - | + | - |
| DCM:MeOH | + | + | + | + | + | + |
| DCM | + | - | + | - | + | + |
| Ethyl acetate | + | + | + | + | + | + |
| MeOH | + | + | + | + | - | + |
| Aqueous | + | + | + | + | - | + |

Present phytochemicals denoted by (+) sign; absent phytochemicals denoted by (-) sign.

Table 3. Phytochemical analysis of different solvent extracts of *A. africanus*.

| Solvent | Alkaloids | Saponins | Flavonoids | Glycosides | Terpenoids | Tannins |
|---------------|-----------|----------|------------|------------|------------|---------|
| Pet ether | - | - | + | - | + | - |
| DCM:MeOH | + | - | + | + | + | + |
| DCM | - | - | + | - | + | - |
| Ethyl acetate | + | + | + | + | - | + |
| MeOH | + | + | + | + | - | + |
| Aqueous | + | + | + | + | - | - |

Presence of phytochemicals denoted by (+) sign; absence of phytochemicals denoted by (-) sign.

compounds against HEP-2 from *A. senegalensis* stem bark. Among all plant extracts, the following exhibited high activity; DCM extract of *A. senegalensis*: IC_{50} 10.41 ± 2.07 $\mu\text{g/ml}$ and MeOH extract of *A. africanus*: IC_{50} 7.33 ± 0.43 $\mu\text{g/ml}$ against HCC 1396; petroleum ether extract of *A. senegalensis*: IC_{50} 0.42 ± 0.09 $\mu\text{g/ml}$ and DCM:MeOH extract of *A. africanus*: IC_{50} 1.00 ± 0.4 $\mu\text{g/ml}$ against HEP-2 cancer cells; petroleum ether extract of *A. senegalensis*: IC_{50} 9.19 ± 0.81 $\mu\text{g/ml}$ and MeOH extracts of *A. africanus*: IC_{50} 9.04 ± 1.05 $\mu\text{g/ml}$ against CT 26

cancer cells. Water is the common solvent used by traditional healers for extraction of medicinal plants due to its availability (Mekonnen and Abebe, 2017). In our study, the aqueous extracts exhibited antiproliferative activity ranging from moderate to none. This signified the inefficiency of an aqueous medium as an extraction solvent for antiproliferative compounds from these plant species. Four extracts were observed to be inactive ($IC_{50} > 100$ $\mu\text{g/ml}$) while the rest were moderately active with IC_{50} ranging between 30 and 100 $\mu\text{g/ml}$.

Table 4. Mean IC₅₀ of the plant extracts on HCC 1396, HEp-2, CT 26 and mean CC₅₀ on VERO cell line.

| Plant | Solvent | IC ₅₀ (µg/ml) HCC 1396 | IC ₅₀ (µg/ml) HEp- 2 | IC ₅₀ (µg/ml) CT 26 | CC ₅₀ (µg/ml) VERO |
|------------------------|---------------|-----------------------------------|---------------------------------|--------------------------------|-------------------------------|
| <i>A. senegalensis</i> | Pet Ether | 21.88±2.18 ^{cd} | 0.42±0.09 ^a | 11.59±2.58 ^b | 39.56±1.73 ^b |
| <i>A. africanus</i> | Pet Ether | 41.10±1.42 ^e | 2.37±1.45 ^a | 9.19±0.81 ^b | 62.70±2.04 ^{bc} |
| <i>A. senegalensis</i> | DCM:MeOH | 27.41±2.28 ^d | 4.50±0.72 ^a | 54.02±4.13 ^f | >100 |
| <i>A. africanus</i> | DCM:MeOH | 12.61±1.67 ^{bc} | 1.00±0.41 ^a | 21.52±0.06 ^c | 78.20±1.47 ^{cd} |
| <i>A. senegalensis</i> | DCM | 10.41±2.07 ^b | 12.36±3.20 ^b | 12.19±2.70 ^b | 52.21±1.95 ^b |
| <i>A. africanus</i> | DCM | 8.76±0.43 ^b | 5.02±0.71 ^a | 19.04±0.78 ^c | 57.73±1.05 ^b |
| <i>A. senegalensis</i> | Ethyl acetate | 17.19±0.19 ^c | 12.00±1.11 ^b | 26.08±0.04 ^d | 93.33±0.67 ^d |
| <i>A. africanus</i> | Ethyl acetate | 18.60±0.28 ^c | 9.48±0.42 ^b | 27.61±4.57 ^d | 68.33±3.79 ^c |
| <i>A. senegalensis</i> | MeOH | 47.98±4.52 ^f | 97.12±2.88 ^f | 36.52±3.23 ^e | >100 |
| <i>A. africanus</i> | MeOH | 7.33±0.43 ^b | 25.38±2.57 ^c | 9.04±1.05 ^b | 55.72±1.00 ^b |
| <i>A. senegalensis</i> | Aqueous | 76.31±1.22 ^g | 76.20±2.38 ^e | 65.03±0.04 ^g | >100 |
| <i>A. africanus</i> | Aqueous | 28.58±0.71 ^d | 65.10±3.49 ^d | >100 | >100 |
| Doxorubicin | - | 1.14±0.01 ^a | 0.21±0.04 ^a | 2.94±0.05 ^a | 10.94±0.06 ^a |
| LSD _(0.05) | - | 6.07 | 3.73 | 4.95 | 25.43 |

Values are expressed as Mean±SEM. Doxorubicin was used as a positive control. The IC₅₀ values of the plant extracts were compared with the doxorubicin for each cell line. Values that do not share a letter are significantly different (p<0.05). LSD is least significance difference between two mean.

Table 5. Selectivity index of *A. senegalensis* and *A. africanus* plant extracts.

| Plant | Solvent | HCC 1396 | HEp-2 | CT 26 |
|------------------------|---------------|----------|-------|-------|
| <i>A. senegalensis</i> | Pet Ether | 1.81 | 94.19 | 3.41 |
| <i>A. africanus</i> | Pet Ether | 1.53 | 26.46 | 6.82 |
| <i>A. senegalensis</i> | DCM:MeOH | 3.65 | 22.22 | 1.85 |
| <i>A. africanus</i> | DCM:MeOH | 6.2 | 78.2 | 3.63 |
| <i>A. senegalensis</i> | DCM | 5.02 | 4.22 | 6.42 |
| <i>A. africanus</i> | DCM | 6.6 | 9.92 | 2.74 |
| <i>A. senegalensis</i> | Ethyl acetate | 5.43 | 7.78 | 3.58 |
| <i>A. africanus</i> | Ethyl acetate | 3.67 | 7.21 | 2.47 |
| <i>A. senegalensis</i> | MeOH | 2.08 | 1.03 | 2.74 |
| <i>A. africanus</i> | MeOH | 7.6 | 2.2 | 6.16 |
| <i>A. senegalensis</i> | Aqueous | 1.31 | 1.31 | 1.54 |
| <i>A. africanus</i> | Aqueous | 3.49 | 1.54 | N/A* |
| Doxorubicin | - | 9.6 | 52.1 | 3.8 |

N/A*: Not applicable because the test extract did not inhibit growth of the cells.

The previous study by Okoye et al. (2014) showed root bark of *A. senegalensis* has anticancer activity against pancreatic and cervical cancer cells. This study, therefore, revealed that the stem bark of the same plant species has antiproliferative activity against colon, breast and throat cancer cells. Likewise, the study support a previous study conducted by Sofidiya et al. (2012) which showed that *A. africanus* had the best antioxidant activity which could be related to anticancer activity.

All the plant extracts were not cytotoxic to VERO (normal) cells (CC₅₀ >39 µg/ml). The plant extracts were found to have higher CC₅₀ compared to the positive

control drug, doxorubicin whose CC₅₀ was 10.94 µg/ml. There was a variation of selectivity among plant extracts and cancer cell lines tested as indicated in Table 5. SI value >3 were considered selective for cancer cell line while SI values <3 were considered non-selective to specific cancer cell line (Bézivin et al., 2003). Some extracts showed selectivity against one cancer cell line but not against the others. However, at least one extract for each plant species showed selectivity to all cancer cell lines. These are DCM:MeOH for *A. africanus* and DCM for *A. senegalensis*. Generally, aqueous extract of *A. senegalensis* was found not to be selective (SI<3) to any

cancer cell line. This could be due to the presence of large quantities of polar based compounds that dilutes concentration of the active compounds (Bézivin et al., 2003). Petroleum ether extract of *A. senegalensis* depicted the highest selectivity on HEP-2 cancer cell lines with SI value of 94.19.

Doxorubicin, a standard drug for cancer treatment was used as positive control. The results showed that doxorubicin was more potent than all the plant extracts with an IC₅₀ value of 1.14 ± 0.01 µg/ml for HCC 1396, 0.21 ± 0.04 µg/ml for HEP-2, and 2.94 ± 0.05 µg/ml for CT 26. This was expected as the drug is purified as opposed to the extracts which were in crude form. This result agrees with that obtained by Al-asady et al. (2014) who found that the glycoside Fraction I from *Convolvulus arvensis* had more cytotoxic inhibition at 10 mg/ml against rhabdomyosarcoma (RD) tumour cell line *in vitro* after 72 h, compared with other extracts (aqueous and methanol) crude extracts of the leaves, stems and roots. Glycoside FI had cytotoxicity concentration 50% (CC 50%) 1.775, 0.870 and 0.706 mg/ml after 24, 48, and 72 h, respectively. The CC₅₀ of doxorubicin on VERO cells was also low (10.94 ± 0.06 µg/ml), which gave more evidences that doxorubicin provides side effect against normal tissue (Wang et al., 2004). Of particular interest, petroleum ether extract of *A. senegalensis* depicted high activity against HEP-2 at an IC₅₀ value of 0.42 ± 0.09 µg/ml comparing well to the reference standard doxorubicin. Selectivity index for the same was also high (SI = 94.19). This implied its high potential for the development of a safe anticancer agent. The potency of plant extracts varied with plant species and the screened cancer cell lines. High potency (IC₅₀ <20 µg/ml) coupled with high selectivity (SI>3) was observed on extracts of *A. senegalensis* extracted using DCM against HCC 1396, petroleum ether on HEP-2 and CT 26. For *A. africanus*, this was observed on DCM:MeOH against HEP-2, MeOH against HCC 1396 and CT 26 as depicted in Tables 4 and 5. This indicated that the aforementioned are suitable extraction solvents for antiproliferative compounds from these plants respectively. Extracts from both polar and non-polar solvents showed varied levels of activity (Bandar et al., 2013). This signified the possibility of *A. senegalensis* and *A. africanus* to possess both polar and non-polar compounds with antiproliferative activity as indicated in Tables 4 and 5. Regarding the variation on the performance of plant extracts shown by solvent used for extraction, the results supported study conducted by Koffi et al. (2010) and Dhawan and Gupta (2016), which showed that the solvent type used in extraction has an effect to the potency of medicinal plants.

Conclusion

This study indicated that, *A. senegalensis* Pers. and *A. africanus* P Beauv. have potential antiproliferative activity

on throat, breast and colon cancer cells. The different solvent used for extraction showed varied activity and selectivity against the cancer cells. Petroleum ether extract of *A. senegalensis* was in particular found to have a high potential for the development of an anticancer agent against throat cancer. These findings validate the traditional use of *A. senegalensis* and *A. africanus* in the treatment of cancer. Current findings also support previous studies which indicated the effect of extraction solvents used on the extraction of bioactive molecules from medicinal plants. Further studies involving the isolation of pure antiproliferative compounds against cancer cells from the two plants are recommended to elucidate bioactive molecules.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors wish to thank the African Development Bank (AfDB) for financial support. The authors are grateful to the Kenya Medical Research Institute (KEMRI) for the provision of infrastructure to conduct the study. The authors acknowledge traditional healers for assistance in the collection of the medicinal plants and all the other people who participated in this work.

FUNDING

This study was supported by the African Development Bank (AfDB).

CONSENT FOR PUBLICATION

Publication of the result finding was granted by the Nelson Mandela African Institute of Science and Technology

REFERENCES

- Ajaiyeoba E, Falade M, Ogbale O, Okpako L, Akinboye D (2006). In vivo antimalarial and cytotoxic properties of *Annona senegalensis* extract. *African Journal of Traditional, Complementary and Alternative Medicines* 3(1):137-141.
- Ajuru MG, Williams LF, Ajuru G (2017). Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger delta region of Nigeria. *Journal of Food and Nutrition Sciences* 5(5):198-205. <https://doi.org/10.11648/j.jfns.20170505.16>
- Al-asady AAB, Suker DK, Hassan KK (2014). Cytotoxic and cytogenetic effects of *Convolvulus arvensis* extracts on rhabdomyosarcoma (RD) tumor cell line *in vitro*. *Journal of Medicinal Plant Research* 8(15):588-598. <https://doi.org/10.5897/JMPR2013.5272> effects of chemotherapy in cancer patients and evaluation of patients
- Aslam MS, Naveed S, Ahmed A, Abbas Z, Gull I, Athar MA (2014).

- Side opinion about starvation based differential chemotherapy. *Journal of Cancer Therapy* 5:817-822. <https://doi.org/10.4236/jct.2014.58089>
- Awa EP, Ibrahim S, Ameh DA (2012). GC/MS Analysis and antimicrobial activity of diethyl ether fraction of methanolic extract from the stem bark of *Annona senegalensis* Pers. *International Journal of Pharmaceutical Sciences and Research* 3(11):4213-4218.
- Balogun OS, Oladosu IA, Liu Z (2016). Fatty acid profile and in vitro biological activities of *Allophylus africanus* (P. Beauv). *Journal of Herbs, Spices and Medicinal Plants* 22(3):238-246. <https://doi.org/10.1080/10496475.2016.1193587>
- Bandar H, Hijazi A, Rammal H, Hachem A, Saad Z, Badran B (2013). Techniques for the extraction of bioactive compounds from Lebanese *Urtica dioica*. *American Journal of Phytomedicine and Clinical Therapeutics* 1(6):507-513. <http://www.jpsonline.com/admin/php/uploads/221>
- Baskar R, Lee KA, Yeov R, Yeoh K (2012). Cancer and radiation therapy: Current advances and future directions. *International Journal of Medical Science* 9(3):193-199. <https://doi.org/10.7150/ijms.3635>
- Bézivin C, Tomasi S, Lohézic-Le Dévéhat F, Boustie J (2003). Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. *Phytomedicine* 10:499-503. <https://doi.org/10.1078/094471103322331458>
- Boik J (2001). Natural Compounds in Cancer Therapy (1 St). United State of America. <https://doi.org/10.1002/ptr.1054>
- Chavan RB, Gaikwad DK (2016). The Ethnobotany, phytochemistry and biological properties of *Allophylus* species used in traditional medicine: a review. *World Journal of Pharmacy and Pharmaceutical Sciences* 5(11):664-682. <https://doi.org/10.20959/wjpps201611-8039>
- Conklin KA (2004). Chemotherapy-associated oxidative stress: Impact on chemotherapeutic effectiveness. *Integrative Cancer Therapies*, 3(4):294-300. <https://doi.org/10.1177/1534735404270335>
- Dhawan D Gupta J (2016). Comparison of different solvents for phytochemical extraction potential from *Datura metel* plant leaves. *International Journal of Biological Chemistry* 11:17-22. <https://doi.org/10.3923/ijbc.2017.17.22>
- Elansary HO, Szopa A, Kubica PB, Ekiert H, Ali HM, Elshikh MS, Elansary DO (2018). Bioactivities of traditional medicinal plants in Alexandria. *Evidence-Based Complementary and Alternative Medicine*. Volume 2018, Article ID 1463579, pp 1-13.
- Fouche G, Cragg GM, Pillay P, Kolesnikova N, Maharaj VJ, Senabe J (2008). In vitro anticancer screening of South African plants. *Journal of Ethnopharmacology* 119:455-461. <https://doi.org/10.1016/j.jep.2008.07.005>
- Gberikon G, Adeoti I, Aondoackaa A (2015). Effect of ethanol and aqueous solutions as extraction solvents on phytochemical screening and antibacterial activity of fruit and stem bark extracts of *Tetrapleura tetrapleura*, *Streptococcus salivarius* and *Streptococcus mutans*. *International Journal of Current Microbiology and Applied Sciences* 4(5):404-410.
- Ghagane SC, Puranik SI, Nerli RB, Hiremath MB (2017). Evaluation of in vitro antioxidant and anticancer activity of *Allophylus cobbe* leaf extracts on DU-145 and PC-3 human prostate cancer cell lines. *Cytotechnology* 69:167-177 <https://doi.org/10.1016/j.imr.2017.01.004>
- Graham JG, Quinn ML, Fabricant DS, Farnsworth NR (2000). Plants used against cancer – an extension of the work of Jonathan Hartwell. *Journal of Ethnopharmacology* 73:347-377.
- Greenwell M, Rahman PKSM (2015). Medicinal Plants: Their use in anticancer treatment. *International Journal of Pharma Sciences Research* 6(10):4103-4112. [https://doi.org/10.13040/IJPSR.0975-8232.6\(10\).4103-12](https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12)
- Kaur M, Agarwal C, Agarwal R (2009). Anticancer and cancer chemopreventive potential of grape seed extract and other. *The Journal of Nutrition* 139:1806S-1812S. <https://doi.org/10.3945/jn.109.106864.1806S>
- Khalid S, Shahzad A, Basharat N, Abubakar M, Anwar P (2018). Phytochemical screening and analysis of selected medicinal plants in phytochemistry and biochemistry. *Journal of Phytochemistry and Biochemistry* 2(1):2-4.
- Kisangau DP, Lyaruu HVM, Hosea KM, Joseph CC (2007). Use of traditional medicines in the management of HIV / AIDS opportunistic infections in Tanzania: a case in the Bukoba rural district. *Journal of Ethnobiology and Ethnomedicine* 29(3):1-8. <https://doi.org/10.1186/1746-4269-3-29>
- Kitula AR (2007). Use of medicinal plants for human health in Udzungwa Mountains Forests: a case study of New Dabaga Ulongambi Forest Reserve. *Journal of Ethnobiology and Ethnomedicine* 7(3):1-4. <https://doi.org/10.1186/1746-4269-3-7>
- Koffi E, Sea T, Dodehe Y, Soro S (2010). Effect of solvent type on extraction of polyphenols from twenty three ivorian plants. *Journal of Animal and Plant Sciences* 5(3):550-558.
- Kooti W, Servataryi K, Behzadifar M, Asadi-samani M, Sadeghi F, Nouri B, Marzouni HZ (2017). Effective medicinal plant in cancer treatment, Part 2: Review study. *Journal of Evidence-Based Complementary and Alternative Medicine* 22(4):982-995. <https://doi.org/10.1177/2156587217696927>
- Max PD, Bray F, Ferlay J, Pisani P (2005). Global cancer statistics, 2002. *A Cancer Journal for Clinicians* 55:74-108. <https://doi.org/10.3322/canjclin.55.2.74>
- Mekonnen N, Abebe E (2017). Ethnobotanical knowledge and practices of traditional healers in Harar, Haramaya, Bati and Garamuleta, Eastern Ethiopia. *Ethiopian Veterinary Journal* 21(2):40. <https://doi.org/10.4314/evj.v21i2.4>
- Mustapha AA (2013). *Annona senegalensis* Persoon: A multipurpose shrub, its phytotherapeutic, phytopharmacological and phytomedicinal uses. *International Journal of Science and Technology* 2(12):862-865.
- Napagoda MT, Sundarapperuma T, Fonseka D, Amarasiri S, Gunaratna P (2018). An Ethnobotanical study of the medicinal plants used as anti-inflammatory remedies in Gampaha district, Western province, Sri Lanka. *Hindawi Scientifica* 2:1-9.
- Ngo T, Scarlett CJ, Bowyer MC, Ngo PD, Vuong Q (2017). Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis* L. *Journal of Food Quality*, Vol. 2017, Article ID 9305047, pp. 1-8.
- Okoli CO, Onyeto CA, Akpa BP, Ezike AC, Akah PA, Okoye TC (2010). Neuropharmacological evaluation of *Annona senegalensis* leaves. *African Journal of Biotechnology* 9(49):8435-8444. <https://doi.org/10.5897/AJB10.033>
- Okoye TC, Akah PA, Ezike AC, Okoye MO, Onyeto CA, Ndukwu F, Ohaegbulam E, Ikele L (2012). Evaluation of the acute and sub-acute toxicity of *Annona senegalensis* root bark extracts. *Asian Pacific Journal of Tropical Medicine* 5(4):277-282. [https://doi.org/10.1016/S1995-7645\(12\)60039-X](https://doi.org/10.1016/S1995-7645(12)60039-X)
- Okoye TC, Akah PA, Nworu CS, Ezike AC (2014). Kaurenolic acid isolated from the root bark of *Annona senegalensis* induces cytotoxic and antiproliferative effects against PANC-1 and HeLa cells. *European Journal of Medicinal Plants* 4(5):579-589. <https://doi.org/10.9734/EJMP/2014/6926>
- Prakash O, Kumar A, Kumar PA (2013). Anticancer potential of plants and natural products: a review. *American Journal of Pharmaceutical Sciences* 1(6):104-115. <https://doi.org/10.12691/ajps-1-6-1>
- Priya LM, Priya BK, Kotakadi VS, Josthna P (2015). Herbal and medicinal plants molecules towards treatment of cancer: a mini review. *American Journal of Ethnomedicine* 2(2). <http://www.ajethno.com>
- Rukunga GM, Gathirwa JW, Omar SA, Muregi FW, Muthaura CN, Kirira PG, Mungai GM, Kofi-Tsekpo WM (2009). Anti-plasmodial activity of the extracts of some Kenyan medicinal plants. *Journal of Ethnopharmacology* 121(2):282-285. <https://doi.org/10.1016/j.jep.2008.10.033>
- Senguttuvan J, Paulsamy S, Karthika K (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochoeris radicata* L. for in vitro antioxidant activities. *Asian Pacific Journal of Tropical Biomedicine* 4:S359-S367. <https://doi.org/10.12980/APJTB.4.2014C1030>
- Siegel RL, Miller KD, Jemal A (2018). Cancer statistics, 2018. *Cancer Journal of Clinicians* 68:7-30. <https://doi.org/10.3322/caac.21442>
- Snehlata K, Sheel R, Kumar B (2018). Evaluation of phytochemicals in polar and non-polar solvent extracts of leaves of *Aegle marmelos* (L). *Journal of Biotechnology and Biochemistry* 4(5):31-38. <https://doi.org/10.9790/264X-0405013138>
- Sofidiya MO, Jimoh FO, Aliero AA, Afolayan AJ, Odukoya OA, Familoni

- OB (2012). Evaluation of antioxidant and antibacterial properties of six Sapindaceae members. *Journal of Medicinal Plants Research* 6(1):154-160. <https://doi.org/10.5897/JMPR11.1364>
- Sowmya S, Lakshmidivi N (2013). Qualitative phytochemical analysis of nonpolar to polar solvent extracts of selected medicinal plants. *International Journal of Current Research* 5(12):3618-3621.
- Thouri A, Chahdoura H, Arem A, El Hichri AO, Hassin R, Achour L (2017). Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var . Korkobbi and Arechti). *BMC Complementary and Alternative Medicine* 17(248):1-10. <https://doi.org/10.1186/s12906-017-1751-y>
- Twentyman PR, Luscombe M (1987). A study of some variables in a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. *British Journal of Cancer* 56:279-285. <https://doi.org/10.1038/bjc.1987.190>
- Wang S, Konorev EA, Kotamraju S, Joseph J, Kalivendi S, Kalyanaraman B (2004). Doxorubicin induces apoptosis in normal and tumor cells via. *The Journal of Biological Chemistry* 279(11):25535-25543. <https://doi.org/10.1074/jbc.M400944200>
- WHO (World Cancer Report) (2014). The International Agency for Research on Cancer.
- Yasser HEM (2016). Biochemistry and Analytical biochemistry in-vitro anti-cancer activity of extracts Dracaen Cinnabari Balf . F Resin from Socotra Island in Yemen Republic. *Biochemistry and Analytical Biochemistry* 5(3):1-17. <https://doi.org/10.4172/2161-1009.1000296>