Traditional healers and the managment of malaria in Kisumu District, Kenya

Article *in* East African medical journal · March 2007

DOI: 10.4314/eamj.v84i2.9504 · Source: PubMed

4 authors, including:

Jennifer Orwa
Kenya Medical Research Institute
37 PUBLICATIONS **613** CITATIONS
SEE PROFILE

Peter G Mwitari
Kenya Medical Research Institute
32 PUBLICATIONS **338** CITATIONS
SEE PROFILE

Esther Matu
Kenya Medical Research Institute
5 PUBLICATIONS **257** CITATIONS
SEE PROFILE

Journal of Complementary and Alternative Medical Research

5(3): 1-11, 2018; Article no.JOCAMR.41248
ISSN: 2456-6276

Phytochemical Screening and *in vitro* Antiproliferative Activity of the Fruit of *Annona muricata* and *Abelmoschus esculentus* Pods against Selected Cancer Cell Lines

Mercy Jepkorir[1], Tobias Ambundo[1], Chrispus Mutuku Ngule[2,3], Jecinta Wanjiru Ndungu'[4], Douglas Kahura Njuguna[5], Regina Wachuka Mbugua[2,6], Jean Chepng'etich[3] and Peter Githaiga Mwitari[2*]

[1]Department of Chemistry and Biochemistry, Laikipia University, P.O.Box 1100, Nyahururu, Kenya.
[2]Centre for Traditional Medicine and Drug Research, Kenya Medical Research Institute, P.O.Box 54840-00200, Nairobi, Kenya.
[3]Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 62000- 00200, Nairobi, Kenya.
[4]Department of Biochemistry and Molecular Biology, Egerton University, P.O.Box 536, Egerton, Kenya.
[5]Department of Biochemistry, Rongo University, P.O.Box 103-40404, Rongo, Kenya.
[6]Department of Biochemistry and Biotechnology, Kenyatta University, P.O.Box 43844-00100, Nairobi, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MJ, CMN, JC, JWN, RWM, TA and PGM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MJ and CMN managed the analysis of the study. Authors TA and PGM managed the data presentation and literature for this work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2018/41248
Editor(s):
(1) Sahdeo Prasad, Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Texas, USA.
Reviewers:
(1) M. V. N. L. Chaitanya, Guru Nanak School of Pharmacy, India.
(2) Sangeetha A/P Arullappan, Universiti Tunku Abdul Rahman (UTAR), Malaysia.
(3) Jolanta Flieger, Medical University, Poland.
Complete Peer review History: http://www.sciencedomain.org/review-history/24557

Original Research Article

Received 25th February 2018
Accepted 4th May 2018
Published 10th May 2018

**Corresponding author: E-mail: pmwitari@kemri.org;*

ABSTRACT

Incorporation of fruits and vegetables in diet can successfully be used in prevention and treatment of cancer. *Annona muricata* and *Abelmoschus esculentus* which belongs to the annonaceae and malvaceae family respectively have commonly been used in traditional medicine to treat various ailments. This study evaluated the phytochemical components of both *A. muricata* and *A. esculentus* and their antiproliferative activity on the breast, cervical and prostate cancer cell lines. Both *A. muricata* and *A. esculentus* were extracted using methanol and dichloromethane in a ratio of 1:1. Phytochemical screening was done using standard analytical procedures. The MTT assay was used to evaluate the antiproliferative activity *of A. muricata* and *A. esculentus* extracts against breast cancer, cervical cancer, prostate cancer and Vero cell lines. Phytochemical screening confirmed that the fruit of *A. muricata* and the pods of *A. esculentus* are rich in saponins, tannins, alkaloids, terpernoids, glycosides, flavonoids and phenols. *A. muricata* had an IC_{50} of 23.632±1.3465 µg/ml, 72.5860±1.9819 µg/ml and 93.6233±3.0570 µg/ml on Hela (cervical cancer cells), DU145 (Prostate cancer) and HCC 1395 (Breast cancer) cells respectively. *A. esculentus* demonstrated antiproliferative activity on Hela cells with an IC_{50} of 20.3840±1.2132 µg/ml on DU145 and HCC 1395 cells an IC_{50} of 50.013±0.2502 µg/ml and 171.6460±4.7642 µg/ml respectively. The standard drug used had an IC_{50} of 21.126 µg/ml on HCC and 24.850 µg/ml on Hela cells. Both plants selectively inhibited the growth of the cancerous cells tested (SI>3) with the highest selectivity observed in HCC 1395 cells. This study authenticates traditional use and suggests potential use of these plants in cancer management and treatment.

Keywords: Annona muricata; Abelmoschus esculentus; antiproliferative; phytochemical.

1. INTRODUCTION

Cancer is among the leading causes of morbidity and mortality in the world. Worldwide, 32.6 million people are living with cancer [1]. Approximately 14 million new cases and 8.2 million cancer-related deaths were reported in 2012. The burden of cancer is increasing in Africa. This can be attributed to lifestyle changes and increased industrialized population as well as increased prevalence of risk factors associated with economic transition including smoking, obesity, lack of physical exercise and generative behaviors [2]. According to the United Nation's population estimates, the population of Africa between 2010 and 2030 is projected to increase by 50% and by 90% for those aged 60 years, the age bracket that is mostly affected by cancer [3]. In developing countries, the mortality rate is increasing probably due to the adoption of westernized lifestyle, delayed cancer screening and inadequate access to medication [4]. Breast cancer is the most diagnosed cancer in women [5]. It is the leading cause of death among females in the world, having an estimate of 1.7 million cases and 521,900 deaths in 2012 [6]. Cervical cancer is the second most diagnosed cancer with an estimate of 527,600 new cases. Approximately 265,700 deaths worldwide in 2012 were due to cervical cancer [7]. It is also the third leading cause of deaths among females in developing countries [8]. In men, prostate cancer is the second most diagnosed cancer worldwide having an estimate of 1.1 million new cases in 2012. It is the fifth leading cause of cancer worldwide [6,9].

Currently, chemotherapy, radiotherapy and surgery are the main techniques used in cancer treatment and management. However, these techniques are expensive and have been associated with detrimental side effects. Drug resistance against the currently used anticancer drugs has been reported. Drawbacks being experienced with the current techniques necessitate the need for alternative leads in cancer treatment and management [10].

Plant-derived products are being used as alternative therapies because they are thought to be cheap, effective, safe and accessible. Regular consumption of fruits and vegetables has been associated with reduced risk of cancer and other chronic diseases [11]. High cost and side effects associated with the current techniques have compelled many people to shift from conventional medication to traditional medicine because they are not only readily available and affordable but are also perceived to have less harmful effects [12]. Research has revealed that plants possess secondary metabolites which can be used in the treatment of various diseases including cancer [13]. Many edible plants

possess therapeutic compounds and therefore are used as nutraceuticals [14,15]. Utilization of food such as fruits and vegetables has the potential to have a 7-31% reduction of all cancers worldwide [16]. Dietary phytochemicals are believed to induce apoptotic cell death in preneoplastic or neoplastic cells through different growth inhibitory mechanisms [17].

Abelmoschus esculentus also known as finger's lady in English speaking countries is a plant that belongs to the Malvaceae family. Commonly, it is referred as okra, gumbo and kingombo. In Kenya, okra is widely distributed in Kitui and Machakos Counties. Its geographical origin is believed to be West Africa, Ethiopia and South Africa. The plant grows well in tropical, subtropical and warm temperate regions [18]. Traditionally, *A. esculentus* has been used to treat various diseases. The decoction of the fruit is used to cure inflammation of mucous membrane especially respiratory tract [19]. The juice is used to treat a sore throat as a result of coughing. Its decoction is also used to treat fever, headache and arthritis [20]. The decoction is also used to ease the menstrual pain [21]. In 2010, a research conducted by French and Dutch researchers found that okra contains pectin which is effective in destroying highly metastatic mouse melanoma cells. The antioxidants present in okra are rich in beta-carotene which is important in cancer prevention [22]. Okra seeds are a good source of unsaturated oil and proteins. Seeds can also be used to obtain caffeine-free coffee [20]. It is rich in fiber, vitamin C and folate and because of this many people have incorporated it into their diet [23]. It is also known to be rich in antioxidants. Also, it contains appreciable amounts of calcium and potassium [24]. It is rich in phenolic compounds. Epidemiological studies suggest that consumption of foods rich in phenolic compounds reduce the risks associated with cardiovascular diseases, stroke and certain forms of cancer [25,26,27].

Annona muricata belongs to the family Annonaceae. The evergreen plant can grow to a height 4M tall. It is commonly found in tropical regions. The plant is commonly known as soursop in English, Mstafeli in Kenya, Ekitafeli in Uganda and Dukume in West Africa. Its medicinal use can be traced to many years ago and since then; there is a lot of research because of the bioactivity and toxicity it contains. The leaves, bark, fruit and seeds of *A. muricata* have been used for many traditional cultures for a variety of physical ailments. Traditionally leaves, roots, fruits and seeds have been used to treat various diseases such as, skin rashes, stomach pain, malaria and prostate cancer [28]. It has also been used in respiratory illness [29] and gastrointestinal problems [30]. *A. muricata* is rich in acetogenins which is the principal bioactive compound isolated [31,32]. Recent studies have suggested that *A. muricata* expresses analgesics effect, anti-inflammation effects, promotes apoptosis and cytotoxicity on cancer cells that may result from the presence of alkaloids, essential oils and acetogenins [33]. Acetogenins are active compounds capable of preventing abnormal or cancer type cells from proliferating. It is important to understand the existing relationship between phytochemical and the bioactivity of these plants [34]. Hence, this study aimed to determine the antiproliferative potential of *A. muricata* and *A. esculentus* on selected cancer and normal cell lines. Results provide preliminary affirmation that *A. muricata* and *A. esculentus* extracts could be used in the treatment and management of breast, prostate and cervical cancer.

2. MATERIALS AND METHODS

2.1 Plant Collection

The fruits of *A. muricata* and pods of *A. esculentus* were collected from Mwingi, Kitui County, 200km east of Nairobi with latitude 0° 56' 3.66" N and longitude 38° 03' 36.18" E in 2016 from a farmer's land after permission had been sought. This region was selected because the plants are abundant. The plant samples were picked, labelled and transported to Centre for Traditional Medicine and Drug Research (CTMDR) KEMRI, Nairobi. Identification of the collected samples was conducted by a specialist botanist and voucher specimens stored at the University of Nairobi Herbarium.

2.1.1 Sample preparation

The samples were washed under running tap water to remove soil particles and other particulate matter. The pods of *A. esculentus* were chopped into small pieces, and the fruits of *A. muricata* cut into quarters to ensure proper drying of the plant materials. The samples were then dried at room temperature under indirect sunlight exposure with good air ventilation. The dried fruits and pods were grounded into fine powder using a Gibbons electric miller (Wood-Rolfe Road, Tollesbury Essex, UK), packed,

labelled and stored in a well-aerated room until use.

2.1.2 Extraction

Briefly, 200 g of the fine powder was weighed using an electric top balance and put in a 500 ml flat-bottomed conical flask. Methanol and dichloromethane (DCM) in the ratio of 1:1 was added until the sample was completely submerged. The mixture was then agitated by shaking for thorough mixing then left to extract for 24 hours with frequent shaking. The mixture was then filtered using a Butchner funnel and Whatman No. 1 filter paper. The residue was then resoaked and left to extract for another 24 hours and filtered. The filtrate was concentrated using a rotary evaporator (Buchi water bath 8-480, Butch laboratechn IK AG, Switzerland) in a water bath at 40ºC [33]. The concentrated extracts were then weighed, labelled and stored at 4ºC until use.

2.1.3 Qualitative phytochemical screening

Qualitative phytochemical screening of *A. muricata* and *A. esculentus* was done using standard procedures [35]. These phytochemical components analysed included saponins, alkaloids, phenols, flavonoids, glycosides, terpernoids and tannins

2.2 Antiproliferative Assay

2.2.1 Cell culturing

DU145 (prostate cancer, HTB-81), HCC 1395 (breast cancer, CRL-2324), Hela (cervical cancer, CCL-2) and Vero Cells (Normal cell from monkey kidney) obtained from ATCC (Manassas, VA, USA) were used. The cells initially stored in liquid nitrogen were speedily thawed in a water bath at 37ºC and cultured in T-75 flasks with Eagle's Minimum Essential Media (MEM, SIGMA USA) supplemented with 100µl/ml streptomycin and 10% Fetal Bovine serum (FBS) then incubated at 5% CO_2 at 37ºC until 70% confluence was attained.

2.2.2 Anti-proliferation assay

Upon attainment of confluence, the cells were washed using Phosphate Buffer Saline (PBS) and harvested by trypsinization. The number of viable cells was determined by Trypan blue exclusion test (cell density counting) using a hemocytometer. An aliquot of 2.0×10^4 cells/ml suspension were seeded in a 96-well plate and incubated at 37ºC for 24h at 5% CO_2. After 24 hrs, 15 µl of the test sample extracts at seven different concentrations each serially diluted was added to the wells in rows H to B. Row A acted as the negative control. The plates were then incubated at 37ºC in 5% CO_2 for 48 hrs. The viability of the cells after extract addition and incubation was done using 3-(4-5-dimethyl-2-thiazoly)-2, 5-diphenyltetrazolium bromide (MTT) assay. The growth of the cells was then quantified by ability of the living cells to reduce the yellow 3- (4-5-dimethyl-2thiazoly)-2, 5-diphenyltetrazolium bromide (MTT) to a purple formazan product [36,37]. After 48 h, 10 µl MTT dye was added to the cells and incubated for 2hrs in 5% CO_2 at 37ºC. The formazan crystals formed were then solubilized using 50 µl of 100% DMSO. The absorbance was read using Multiskan EX Elisa reader (Bio Tek, US) at 576 nm and a reference wavelength at 620 nm. The percentage cell viability at different extracts concentration was calculated using the formula [38].

$$Percentage\ viability = \frac{At-Ab}{Ac-Ab} X\ 100$$

$$Percentage\ inhibition = 100 - \frac{At-Ab}{Ac-Ab} X100$$

Where,

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

The effect of *A. esculentus* and *A. muricata* on Vero cells was expressed as CC_{50} values (the concentration of extracts required to kill 50% of the treated cells). The effects of the extracts on cancer cells were represented in IC_{50} values. The CC_{50} and IC_{50} values were calculated using linear regression curves. Selectivity index (SI = CC50/IC50) of the three cancer cell lines was calculated from the CC_{50} ratio of the Vero cells and IC_{50} of the cancer cells.

2.3 Data Management and Analysis

All the activities were recorded in a laboratory notebook. Raw and processed data was entered in excel data sheets and analysis done using Statistical Package of Social Science (SPSS Version 20). The concentration required to inhibit 50% of the cells was calculated using Cruzi cytotoxicity software. The differences between the control and the treatments were tested for statistical significance using One-way Analysis of

Variance (ANOVA). A value of $p \leq 0.05$ was considered to indicate statistical significance. The IC_{50} and CC_{50} values were expressed as Mean ±Standard Error of Mean (S.E.M). Tables and graphs were used for clear presentation of the results.

3. RESULTS AND DISCUSSION

Generally, the potential medicinal value of any plant is usually attributed to the phytochemicals present which have a certain biological importance defined by the biotic activities. These important phytochemicals are alkaloids, tannins, saponins, flavonoids, terpernoids, phenols and glycosides compounds. Flavonoids, terpernoids, glycosides, saponins and phenols were found in both plants. Alkaloids were present in *A. muricata* but absent in *A. esculentus*. Similarly, tannins were present in *A. esculentus* and absent in *A. muricata* as illustrated in Table 1 below.

Saponins are secondary metabolites that are natural glycosides possessing a wide range of pharmacological activities including cytotoxic and chemo preventive properties [39]. Flavonoids in recent research have been shown to inhibit initiation, promotion and progression of cancerous cells [40]. Flavonoids are reported to have a potent dietary chemo preventive activity [41]. Tannins are water soluble polyphenols usually found in various edible plants. A number of anti-mutagenic activities have been shown to be reduced by tannin molecules [42].

Terpenoids, also referred to as isoprenoid, are phytochemical components found in most plants. Several studies experimentally carried out have shown that monoterpenes, a class of terpernoids may be advantageous in prevention and management of cancer such as prostate, breast and colon [43]. Phenols also play a role in anticancer activity through processes such as apoptosis and inhibition of DNA binding [44].

This study focused to determine antiproliferative activity of organic extract of *A. muricata* and *A. esculentus* on cervical (Hela), prostate (DU145) and Breast (HCC) cancer cell lines. Both plant extracts exhibited a growth inhibitory effect on Hela, HCC and DU145 cancer cell lines (Fig. 3 below). A plant extract with an $IC_{50} \leq 20\mu g/ml$ is considered active, 20 µg/ml-100 µg/ml moderately active, weakly active when it ranges between 100 µg/ml→1000 µg/ml and inactive at above 1000 µg/ml [45]. From the extracts tested, *A. esculentus* had the highest antiproliferative activity on Hela with an IC_{50} of 20.384±1.2132 as shown in Fig. 1 below. Research done on flavones from *A. esculentus* exhibited pro-angiogenic activity by activating the VEGF-A/VEGFR2-P13/Akt Signaling axis [46]. Therefore, these results can be attributed to the same pathway of activity (Table 1). DU 145 had an IC_{50} of 50.013 ± 0.2502, and IC_{50}171.646±4.7642 on HCC 1395 respectively. On the other hand, *A. muricata* inhibited the growth of the cancer cell lines with highest activity on Hela which had an IC_{50} of 23.632±1.3465. Crude leave extract of *A. muricata* inhibited proliferation of 4T1 breast cancer cell line [47]. This was similar to HCC1395 breast cancer cell line as shown in Figure 1 and 2 below. Similarly, the fruit extract reduced the size of breast tumor in a 5week treatment in rats [48]. The cytotoxic mechanism suggests the inhibition of multiple signaling pathways which regulate metastasis, metabolism, induction of necrosis and cell cycle [29]. Crude leaf extract of *A. muricata* demonstrated the *in vitro* inhibition of prostate cancer proliferation and a tremendous effect on growth inhibition of the tumor compared to isolated flavonoid compound [32]. This illustrates interaction of the different phytochemicals present and the bioactive acetogenins.

Table 1. Phytochemical composition of *A. esculentus* and *A. muricata*

Compounds	*Abelmoschus esculentus*	*Annona muricata*
Alkaloids	–	+
Flavonoids	+	+
Terpernoids	+	+
Glycosides	+	+
Tannins	+	–
Saponins	+	+
Phenols	+	+

Key: + (Present), (-) Absent

Both *A. muricata* and *A. esculentus* were non-toxic to normal cells. They both showed selective toxicity to cancer cell lines (SI>3) with the highest selectivity index on Hela 1395 (SI of 23.575 and 28.939 respectively) as illustrated in Table 2 below. These results are consistent with the study carried out on lectin isolated from A. esculentus promoted selective antitumor effects on MCF-7 breast cancer cells [49]. As illustrated in Table 3, all the extracts except *A. muricata* on Hela cells (P=0.05) showed a significant difference from 5-Fluoro uracil (P=0.05).

This study reports the anti-proliferative potential of *A. muricata* and *A. esculentus*

on prostate, cervical and breast cancer cells. This activity could be attributed to the phytochemical components present. The study reveals that these plants could act as potent agents in treatment and management of breast, cervical and prostate cancer.

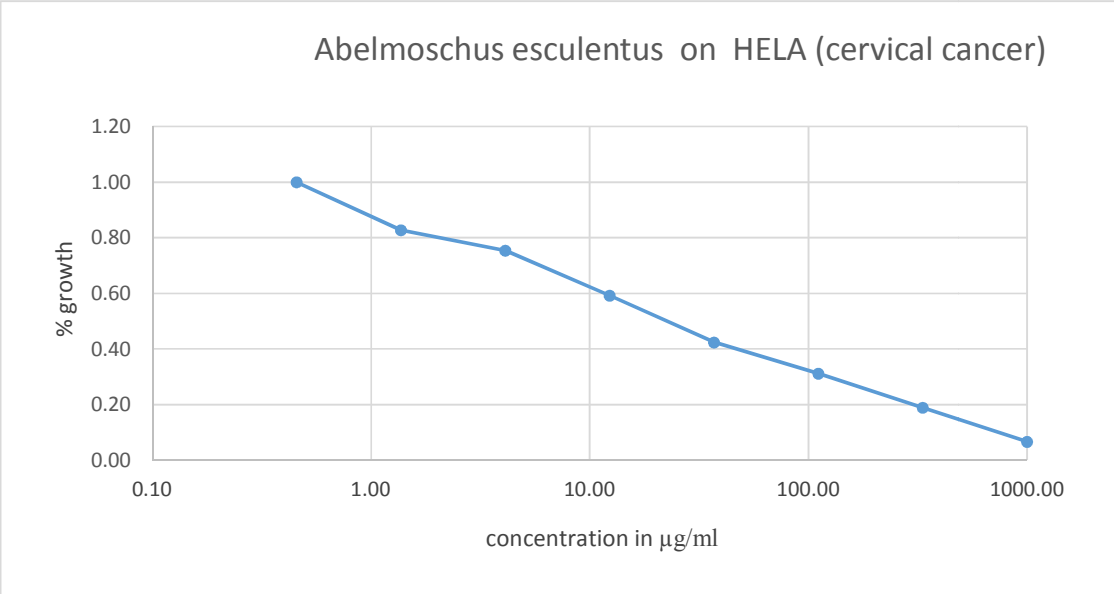

Fig. 1

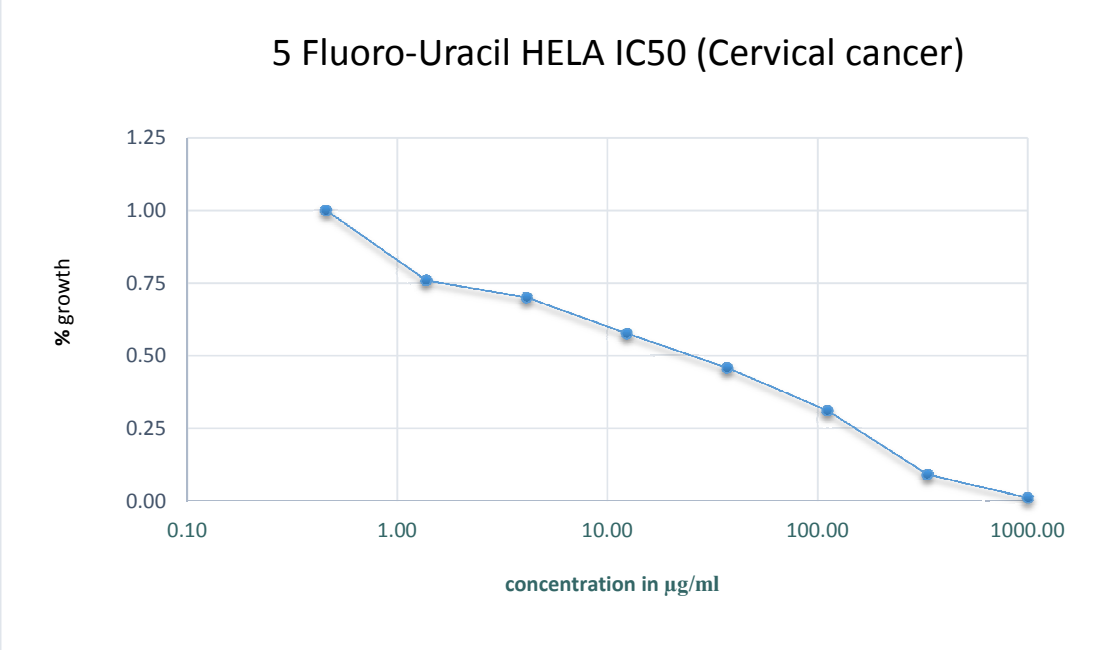

Fig. 2

Fig. 1 and 2. Show the growth inhibition percentage of cancer cells against concentration in µg/ml of the extracts and standard drug. The plant extracts of *A. esculentus* and 5 Fluoro-Uracil inhibited the growth of the selected cancerous cells in a concentration dependent manner

Table 2. Antiproliferative activity (Mean ±SEM) and selectivity indices of *A. muricata* and *A. esculentus* extracts on prostate, cervical, breast and Vero cells used

Extracts	Vero	DU145	Selectivity index	Hela	Selectivity index	HCC	Selectivity index
A. muricata	615.4587±3.9957	73.3390±1.8651	8.245	26.4340±1.9317	23.575	93.6233±3.0570	6.4422
A. esculentus	663.2197±7.864	48.2210±2210	12.920	22.7257±1.1834	28.939	171.646±4.7642	3.652

Table 3. Tukey's multiple comparisons of the IC_{50} values of selected cell lines treated with the plant extracts and the conventional drug (5-fluorouracil)

Comparison	P value
HCC 1395 *A. muricata* vs. HCC1395 -5-fluoro uracil	0.001
HCC 1395 *A. esculentus* vs HCC1395 -5-fluoro uracil	0.001
DU145 *A. muricata* vs DU145 -5-fluoro uracil	0.001
DU145 *A. esculentus* vs DU145 5-fluoro uracil	0.001
Hela *A. muricata* vs Hela 5-fluoro uracil	0.460
Hela *A. esculentus* vs Hela 5-fluoro uracil	0.01
Vero *A. muricata* vs Vero 5-fluoro uracil	0.01

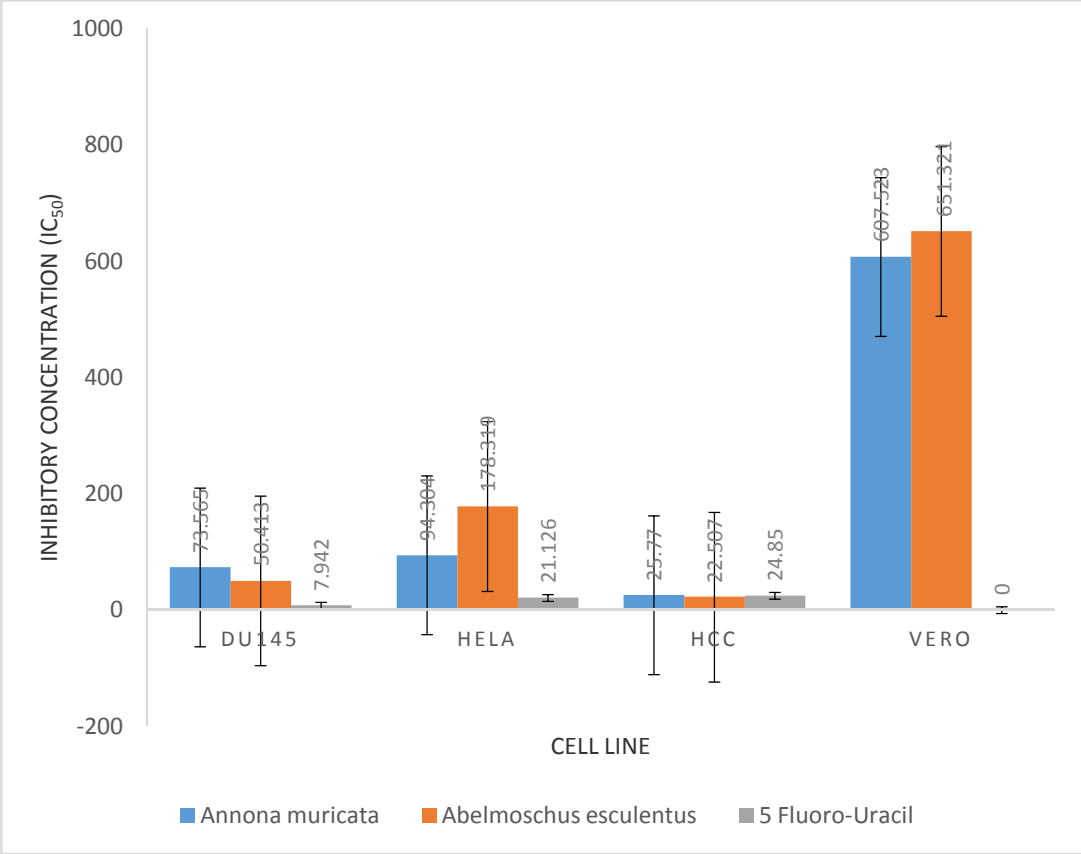

Fig. 3. Effects of *A. muricata* and *A. esculentus* extracts on selected cancer cell lines

4. CONCLUSIONS

The antiproliferative activity of *A. muricata* and *A. esculentus* has been well established in this study. The antiproliferative potential could be attributed to the essential phytochemicals exhibited by both plant extracts. This can be attributed to the synergistic interaction of the various phytochemicals enriched in the crude extracts. The extracts also selectively inhibited the cancerous cells. In vivo studies are envisaged to make a milestone in determining these extracts efficacy and safety. Moreover, isolation and identification of pure compounds and determination of different growth inhibitory mechanisms of action is needed to shed more light on these findings.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Authority to conduct this study was sought and granted from Kenya Medical Research Institute (KEMRI), CTMDR Centre Scientific Committee (CSC) and Scientific and Ethics Review Unit (SERU) through study approval number KEMRI/SERU/CTMDR/035/3346.

ACKNOWLEDGEMENT

The authors acknowledge the Director, KEMRI and the Center for Traditional Medicine and Drug Research (CTMDR), KEMRI for provision of space, equipment and consumables to undertake the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Siegel RL, Miller K, Jemal A. Cancer statistics, 2018. CA: A Cancer Journal for Clinicians. 2018;68(1):7-30.
2. World Health Organization. World Cancer Report; 2014.

3. International Agency for Research on Cancer; 2014.
4. Kanavos P. The rising burden of cancer in the developing world. Annals of oncology. 2006;17(8):15-23.
5. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics. CA: A Cancer Journal for Clinicians. 2007;57(1): 43-66.
6. Torre, Lindsey A, Freddie Bray, Rebecca L. Siegel, Lortet-Tieulent J, Jemal A. Global cancer statistics 2012. CA: A Cancer Journal for Clinicians. 2015;87-108.
7. Peng J, Qi S, Wang P, Li W, Liu C, Li F. Diagnosis and prognostic significance of c-Met in cervical cancer: A meta-analysis. Disease markers; 2016.
8. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International Journal of Cancer. 2010;127(12):2893-2917.
9. Bashir MN. Epidemiology of prostate cancer. Asian Pac J Cancer Prev. 2015; 16(13):5137-41.
10. Cancer Statistics. American Cancer Society. CA Cancer J. Clin. 2015;65:5-29.
11. Hung HC, Joshipura KJ, Jiang R, Hu FB, Hunter D, Smith-Warner SA, Willett WC. Fruit and vegetable intake and risk of major chronic disease. Journal of the National Cancer Institute. 2004;96(21): 1577-1584.
12. Molassiotis A, Fernadez Ortega P, Pud D, Ozden G, Scott JA, Panteli V, Madsen E. Use of complementary and alternative medicine in cancer patients: A European survey. Annals of Oncology. 2005;16(4): 655-663.
13. Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. Current Medicinal Chemistry. 2004; 11(11):1451-1460.
14. Zhao J. Nutraceuticals, nutritional therapy, phytonutrients and phytotherapy for improvement of human health: A perspective on plant biotechnology application. Recent Patents on Biotechnology. 2007; 1(1):75-97.
15. Ravindranath MH, Muthugounder S, Presser N, Viswanathan S. Anticancer therapeutic potential of soy isoflavone, genistein. In Complementary and Alternative Approaches to Biomedicine. 2004; 564:121-165.
16. Shukla Yogeshwer, Pal S. Complementary and alternative cancer therapies: Past, present and future scenario." Asian Pacific Journal of Cancer Prevention. 2004;5(1):3-14.
17. Tan AC, Konczak I. Sze DMY, Ramzan I. Molecular pathways for cancer chemo-prevention by dietary phytochemicals. Nutrition and cancer. 2011;63(4):495-505.
18. National Research Council. Okra lost crops of Africa: Volume ii: Vegetables. Lost crops of Africa. National Academies Press; 2008. (Retrieved 2008 -07-15)
19. Pandita Deepu, Anu Pandita, Shanu Pandita. Herbaceous medicinal & therapeutic plants of district samba of Jammu province, Jammu & Kashmir (India). Int J Indig Med Plants. ISSN 46. 2013;2051-4263.
20. Gemede HF. Ratta N, Haki GD, Woldegiorgis AZ, Beyene F. Nutritional quality and health benefits of okra (*Abelmoschus esculentus*): A review. Global Journal of Medical Research; 2014.
21. Naim Zannatul, Ibrahim Md. Debnath, Dipti Rana S, Billah Md. Mukul, Md. Emdadul. Current drug therap anti-inflammatory, analgesic andanti-nociceptive efficacy of peel of *Abelmoschus esculentus* Fruits in Laboratory Animal; 2015.
22. Krinsky Norman I, Elizabeth J. Johnson. Carotenoid actions and their relation to health and disease. Molecular Aspects of Medicine. 2005;26(6):459-516.
23. American Dietetic Association. Position of the American dietetic association and dietitians of Canada: Vegetarian diets. Journal of the Academy of Nutrition and Dietetics. 2003;103(6):748.
24. Gemede HF, Ratta N, Haki GD, Woldegiorgis AZ, Beyene F. Nutritional quality and health benefits of okra (*Abelmoschus esculentus*): A review. J Food Process Technol. 2015;6(458):2.
25. Hollman PCH, Katan MB. Dietary flavonoids: Intake, health effects and bioavailability. Food and Chem Toxicol. 1999;37:937-942.
26. Klimczak I, Małecka M, Szlachta M, Gliszczyńska Świgło A. Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. Journal of Food Composition and Analysis. 2007;20(3):313-322.
27. Kubola J, Siriamornpun S. Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem

and fruit fraction extracts *in vitro*. Food Chemistry. 2008;110(4):881-890.

28. Coria-Téllez Ana V, Efigenia Montalvo-Gónzalez, Elhadi M. Yahia, Eva N. Obledo-Vázquez. Annona muricata: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arabian Journal of Chemistry; 2016.
29. Torres MP, Rachogani S, Purohit V, Ganti AK, Batra SK. Graviola: A novel promising natural derived drug that inhibits tumoregeni city and metastatis pf pancreatic cancer cell in In vitro and *in vivo* through altering cell metabolism. Cancer Lett. 2012;322:1-11.
30. Hamid RA, Foong CP, Ahmad Z, Hussain MK. Antinociceptive and anti-ulcerogenic activities of the ethanolic extract of *Annona muricata* leaf. Rev. Bras. Farmacogn. 2012;22:630–641.
DOI: 10.1590/S0102-695X2012005000001
31. Pieme CA, Kumar SG, Dongmo MS, Moukette BM, Boyoum FF, Ngogang JY, Saxena AK. Anti proliferative activity and induction of apoptosis by *Annona muricata* (*Annonaceae*) extract on human cancer cells. BMC Complementary and Alternative Medicine. 2014;14(1):516.
32. Yang C, Gundala SR, Mukkavili R, Vangala S, Reid MD, Aneja R. Synergistic interactions among flavonoids and acetogenins in Graviola (*Annona muricata)* leaves confer protection against prostate cancer. Carcinogens. 2015;36(6) 656-665.
33. De Sousa OV, Vieira GDV, De Pinho JDJR, Yamamoto CH, Alves MS. Anti-nociceptive and anti-inflammatory activities of the ethanol extract of *Annona muricata* L. leaves in animal models. International Journal of Molecular Sciences. 2010;11(5): 2067-2078.
34. Gavamukulya Yahaya, Faten Abou-Elella, Fred Wamunyokoli, Hany AEl-Shemy. Phytochemical screening, anti-oxidant activity and in vitro anticancer potential of ethanolic and water leaves extracts of *Annona muricata* (*Graviola*). Asian Pacific Journal of Tropical Medicine. 2014;7:S355-S363.
35. Maobe MA, Gatebe E, Gitu L, Rotich H. Preliminary phytochemical screening of eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii region, southwest Kenya. European Journal of Applied Sciences. 2013;5(10):01-06.
36. Patel S, Gheewala N, Suthar A, Shah A. *In-vitro* cytotoxicity activity of *Solanum nigrum* extract against Hela cell line and vero cell line. Int J Pharm Pharm Sci. 2009;1:38-46.
37. Lau CS, Ho CY, Kim CF, Leung KN, Fung KP, et al. Cytotoxic activities of *Coriolus versicolor* (Yunzhi) extract on human leukemia and lymphoma cells by induction of apoptosis. Life Sci. 2004;75:797-808.
38. Awasare S, Bhujbal S, Nanda R. *In vitro* cytotoxic activity of novel oleanane type of *Triterpenoid saponin* from stem bark of Manilkara zapota Linn. Asian J Pharm Clin Res. 2012;5:183-188.
39. Podolak I, Galanty A, Sobolewska D. Saponins as cytotoxic agents: A review. Phytochemistry Reviews. 2010;9(3):425-474.
40. Ramos S. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. The Journal of Nutritional Biochemistry. 2007;18(7):427-442.
41. Walle T, Ta N, Kawamori T, Wen X, Tsuji, PA, et al. Cancer chemopreventive properties of orally bio available flavonoids methylated versus unmethylated flavones. Biochemical Pharmacology. 2007;73(9): 1288-1296.
42. Koleckar V, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L, et al. Condensed and hydrolysable tannins as antioxidants influencing the health. Mini Reviews in Medicinal Chemistry. 2008; 8(5):436-447.
43. Gupta C, Prakash D. Phytonutrients as therapeutic agents. Journal of Complementary and Integrative Medicine. 2014;11(3):151-169.
44. Sa G, Das T. Anticancer effects of curcumin: Cycle of life and death. Cell Division. 2008;3(1):14.
45. Atjanasuppat K, Wongkham W, Meepowpan P, Kittakoop P, Sobhon P, Bartlett A, Whitfield PJ. *In vitro* screening for anthelmintic and antitumor activity of ethnomedicinal plants from Thailand. Journal of Ethnopharmacology. 2009;123: 475-482.
46. Zhu Gui-Song, Ling-Yi Tang, Dong-Ling LV, Meng Jiang. Total flavones of *Abelmoschus esculentus* manihot exhibits pro-Angiogenic activity by activating the VEGF-A/VEGFR2-P13/Akt signaling axis.

The American Journal of Chinese Medicine. 2018;1-17.

47. Syed Najmuddin SU, Romli MF, Hamid M, Alitheen NB, Nik Abd Rahman NM. Anticancer effect of *Annona muricca* Linn leaves crude extract (AMCE) on breast cancer cell line. BMC Complement Altern Med. 2016;16(1):311.
48. Dai Y, Hogan S, Schmelz EM, Ja YH, Canning C, Zhou K. Selective growth inhibition of human breast cancer cells by graviola fruit extract in In vitro and *in vivo* involving down regulation of EGFR espression. Nurt Cancer. 2011;63:795-801
49. Monte LG, Tatiane S, Reis BL, Braganhol E, Rafael FP, Odir AD, Rodrigo RL, Carlos AAG, Fabricio RC, Luciano SP. Lectin *Albelmoschus esculentus* (okra) promotes selective antitumor effects in human breast cancer cells. Biotechnology Letters. 2014: 36(3):461-469.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24557