Journal of Complementary and Alternative Medical Research

6(2): 1-9, 2018; Article no.JOCAMR.44413
ISSN: 2456-6276

Phytochemical Composition and *In vitro* Anti-Proliferative Activity of *Oxygonum sinuatum* (Meisn.) Dammer on Selected Cancerous Cells

Douglas Kahura Njuguna[1], Karori Mbuthia[1], Chrispus Mutuku[2], Mercy Jepkorir[3], Jecinta Wanjiru Ndung'u[4], Reginah Mwangangi[5], Jean Chepngetich[6] and Peter Mwitari[2*]

[1]Department of Biochemistry and Molecular Biology, Egerton University, P.O.Box 536, Egerton, Kenya.
[2]Center for Traditional Medicine and Drug Research, KEMRI, P.O.Box 54840-00200, Nairobi, Kenya.
[3]Department of Chemistry and Biochemistry, Lakipia University, P.O.Box 1100, Nyahururu, Kenya.
[4]Department of Biochemistry, Rongo University, P.O.Box 103-40404, Rongo, Kenya.
[5]Department of Biochemistry and Biotechnology, Kenyatta University, P.O.Box 43844-00100, Nairobi, Kenya.
[6]Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 6200-00200, Nairobi, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Authors DKN, CM and JC designed the study and wrote the protocol. Authors DKN, PM and JC performed the statistical analysis and wrote the first draft of the manuscript. Authors KM and PM managed the analyses of the study. Authors MJ, JWN and RM managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2018/44413
Editor(s):
(1) Dr. Aditi Singh, Amity Institute of Biotechnology, Amity Univesity, Lucknow, India.
Reviewers:
(1) Ahmed Mohammed Abu-Dief Mohammed, Sohag University, Egypt.
(2) Daohong Chen, China.
(3) Ramaiah Maddi, Hindu College of Pharmacy, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/26695

Original Research Article

Received 07 July 2018
Accepted 30 September 2018
Published 19 October 2018

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

ABSTRACT

Cancer is a leading cause of deaths worldwide. The current search for alternative drugs is imperative. Medicinal plants are the most promising source of new anticancer agents. This study evaluated the phytochemical composition, and anti-proliferative activity of Methanol-Dichloromethane (1:1) extracts from the leaves, stem and fruits of *Oxygonum sinuatum*. Phytochemicals screening was done using standard procedures which were based on color change and/or precipitation. The MTT assay was used to test for the anti-proliferative activity of the plant extracts on selected cancer cell lines. In addition, normal Vero cells were used. 5-flourouracil was used as the standard drug. Results from this study revealed that stem, leaves and fruits extracts contained alkaloids, glycosides, saponins, tannins, flavonoids, terpenoids, steroids and phenols. The leaves, stem and fruits extracts showed moderate anti-proliferative activity on *4T1,* Hcc 1395, 22Rv1 and DU145 with the best activity been registered by fruits extract on *4T1* cell line*;* 35.841±3.549µg/ml. The fruit extract showed no activity on DU145 cell line. The leaves and fruit extract showed no toxicity to Vero normal cells (CC_{50} >1000µg/ml) however, the stem extract was toxic to Vero cell, CC_{50} 413.733±21.022 µg/ml. The highest selectivity index was registered by fruit extract on *4T1;* SI=27.90. The leaves and stem extracts also showed high selectivity (SI >3) on 22Rv1 and DU145 respectively. *The anti-proliferative activity of Oxygonum sinuatum on the selected cancer cell lines can be attributed to the phytochemical compounds present in the plant extracts.*

Keywords: Oxygonum sinuatum; anti-proliferation; phytochemicals; selectivity index (SI).

1. INTRODUCTION

Cancer is one of the leading causes of deaths worldwide accounting for 8.2 million deaths annually, which is approximately, 13% of the total deaths [1]. New cancer cases per year are estimated to be 14 million a figure predicted to rise to 22 million within the next two decades [1]. Regrettably, 70% of the total cancer deaths occur in low and middle income countries [2]. The major cancer predisposing factors include; tobacco use, excessive alcoholism, some infectious agents, obesity, physical inactivity and westernized diet [3]. In Africa, cancer mortality rate is greater than that of HIV, TB, and malaria combined [4]. Cancer causes 7% of the total national mortality annually in Kenya ranking third after infectious and cardiovascular diseases [5]. The poor prognosis of cancer is due to lack of enough facilities for diagnosis and treatment, limited access to health facilities and high cost of conventional treatment [6]. The major conventional treatment methods include chemotherapy, radiotherapy, immunotherapy and surgery. Despite being expensive and beyond reach by majority of people in poor countries, these methods are also laden with various side effects [1]. This has necessitated for the search of alternative cancer management and treatment agents.

In Africa, up to 80% of the population relies on the use of herbal medicine for their primary healthcare [7,8]. The conventional medicine in Kenya provides for only 30% of the population, this means that more than two-thirds of Kenyans rely on traditional medicine for their healthcare needs [9]. Many people perceive medicinal plants to be safer than their synthetic alternatives. Wide range of plant extracts have been used as raw or processed drugs in the treatment of various diseases. The different parts used include root, stem, flower, twigs, exudates as well as whole plant [10]. Various phytochemical compounds found in medicinal plants have been a source of innumerable therapeutic agents and a tool for development of conventional medicine [11].

Oxygonum sinuatum (Meisn.) Dammer (Polygonaceae) is an annual weed growing in the drier areas and widely distributed in Kenya [12]. *O. sinuatum* is a decumbent plant, its stem grows up to 90 cm long [13]. The plant is polygamous with prickly fruits. Traditionally the plant is used in treatment of various diseases including cancer [14-17]. However, its anti-proliferative potential has not been fully investigated. Previous studies on methanolic extract of the whole plant demonstrated antioxidant and anti-inflammatory activity as well as broad spectrum antibacterial effect [18]. A different study by Uma and Bharti [19] correlated the antioxidant activity of methanolic extract from the whole plant to phenolic compounds in the plant. Kamuhabwa et al. [20] showed the anti-proliferative potency of the whole plant methanolic extract on skin and colon carcinomas.

2. MATERIALS AND METHODS

2.1 Plant Collection

The plant was collected from Ngo'ng Hills (1.3618°S, 36.6566°E) Kajiado County and transported to the Center for Traditional Medicine and Drug Research CTMDR, KEMRI for taxonomic identification and processing. A botanical sample was deposited at CTMDR, KEMRI and given the voucher number KEMRI/CTMDR/DKN/1/2016. The plant parts were sorted into leaves, stems, and fruits, dried at room temperature and ground into fine powder using a laboratory mill.

2.2 Extraction Procedures

The grounded samples were weighed using a top balance and put in a 500 ml flat bottomed flask. Methanol and dichloromethane (DCM) (1:1) was added until the plant material was completely submerged. The mixture was then agitated for thorough mixing then left to extract for 24 hours with frequent shaking to ensure effective extraction. After the 24 hours the mixture was filtered using Butchner funnel; Whatman no. 1 filter paper. The extracts were then concentrated using a rotary vacuum evaporator with a water bath at 40°C. The concentrated extracts were stored at 4°C until use.

2.3 Qualitative Phytochemical Analysis

The extracts' phytochemicals composition was tested using standard procedures with slight modifications of the procedures [21,22].

Alkaloids; Mayer's Test: About 2 ml of the plant extract was put in a clean test tube. Few drops of Mayer's reagent were added and observations made. Appearance of white precipitate indicates presence of alkaloids.

Cardiac glycosides; Keller Killiani Test: Approximately 2 ml of the plant extract was put in a test tube, 1 ml of glacial acetic acid containing 1 drop of ferric chloride was added. This mixture was added to 1 ml of concentrated sulphuric acid and observations made. Formation of a brown ring indicates presence of cardiac glycosides.

Saponins: The crude extract was mixed with 5 ml of water and vigorously shaken. The mixture was left to stand for about 15 seconds. Formation of a stable form indicates presence of Saponins.

Tannins: To about 0.5 g of the plant extract in a test tube, 20 ml of double distilled water was added and heated to boiling. The mixture was then filtered and 1% $FeCl_3$ was added to the filtrate and observation made. A brownish green coloration indicates the presence of tannins.

Flavonoids: To a portion of the plant extract in a test tube, 5 ml of dilute ammonia was added followed by addition of 2 ml of concentrated sulphuric acid. The appearance of a yellow color indicates the presence of flavonoids.

Terpenoids: About 2 ml of the plant extract was put in a test tube. To this, 2 ml of chloroform was added followed by 1ml of concentrated sulphuric acid. Observations were then made. A brown ring at the junction of two layers indicates the presence of terpenoids.

Steroids; Liebermann Rurchard Reaction: To a sample of the plant extract in a test tube, 10 ml of chloroform was added and filtered. To 2 ml of the filtrate, 2 ml of acetic acid was added followed by addition of concentrated sulphuric acid. The formation of blue-green ring at the junction of two layers indicates presence of steroids.

Phenols: Portion of the plant extract was put in a test tube and treated with few drops of 2% ferric chloride. Observations were then made. The appearance of blue green coloration indicates presence of phenols.

2.4 Anti-proliferative Assay

2.4.1 Sample preparation

Briefly, 10 mg of the plant extracts (leaves, stem, and fruits extracts) were weighed in a 1.5 ml eppendorf tube using analytical balance. To each sample 100 µl dimethyl sulfoxide (DMSO) solution was added and the mixture vortexed, 900 µl of PBS was added to the mixture to make 1 ml of the solution.

2.4.2 Cell line culturing

Vero cell line (normal) and 4T1 (breast), 22Rv1 (prostate), Hcc 1395 (prostate), DU 145 (breast) cancer cell lines (ATCC) were obtained from Center for Virology Research (CVR), KEMRI. The cells were revived in a water bath at 37°C and cultured in T-75 flasks with Growth Essential Medium (GEM, SIGMA USA) supplemented with 10% Fetal Bovine Serum (FBS) and 100 µg/ml

streptomycin then incubated for 72 hours to attain confluence. All incubation was done at 5% CO_2 and 37°C [23].

2.4.3 MTT assay

Upon attainment of confluence, both Vero and cancer cells were washed using Phosphate Buffer Saline (PBS) and harvested by tripsinization. The number of viable cells was determined by Trypan blue exclusion test. Approximately 2.0 $\times 10^4$ cells/ml suspension for both the Vero and cancer cells were seeded in 96-well plates and incubated for 24 hours for the cells to attach to the plates. Briefly, 15 µl of the test sample extracts at seven different concentrations each serially diluted was added from rows H to B and the plates incubated for 48 hours. Row A acted as the negative control. The viability of the cells after extracts addition and incubation was determined using MTT cell proliferation assay which is based on the ability of the living cells to reduce the yellow 3- (4-5-dimethyl-2-thiazoly)-2, 5-diphenyltetrazolium bromide (MTT) dye to a purple formazan product [24,25,26]. After 48 hrs 10 µl MTT dye was added to the cells and incubated for 4 hrs. All the media was then removed from the plates and 50 µl of DMSO was added to solubilize the formazan product. Absorbance was read on a scanning multi well spectrophotometer at 562 nm [27]. The inhibitory concentration (IC_{50}) on cancer cells and cytotoxic concentration (CC_{50}) on normal Vero cells was determined [28]. The Selectivity index ($SI=CC_{50}/IC_{50}$) was also calculated.

2.5 Data Analysis and Presentation

Analysis of statistical data obtained from this study was done using Cruzi 7 Drug Cytotoxicity Software and Statistical Package of Social Science (SPSS Version 20, IBM, USA). The difference between the treatment and the control in this study was tested for statistical significance using one-way analysis of variance. A value of $p \leq 0.05$ was considered significant. The IC50 and CC50 values were expressed as Mean ± Standard deviation (SD). Tables were used to enhance the presentation of the results.

3. RESULTS

3.1 The Yield of the Extracts

The plant extracts yield was calculated and recorded as a percentage. The fruit extract had the highest yield of 11%, the leaves extract had 10% while the stem extract had the lowest yield of 5%.

3.2 The Phytochemistry Results

Phytochemical analysis revealed the presence of alkaloids, glycosides, saponins, tannins, flavonoids, terpenoids, steroids and phenols in all the extracts. The tests were based on precipitation and color change. Table 1 illustrates these results.

3.3 The Anti-proliferative Activity

The three plant parts extracts registered significantly higher anti-proliferative activity ($P \leq 0.05$) on 4T1, 22Rv1, Hcc 1395 and DU145. The highest activity was registered by fruit extract on 4T1 with an IC_{50} of 35.841±3.549 µg/ml which was statistically comparable to that of the reference drug on the same cell line. The fruits extract had no activity on DU145 cell. The stem extract was toxic to Vero cells while the leaves and fruit extract showed no toxicity to the Vero cells (CC50 >1000 µg/ml). These results are illustrated in Table 2.

4. DISCUSSION

The leaves, stem and fruits extracts of O. sinuatum showed anti-proliferative effects against 4T1 (mouse breast cancer cell), Hcc 1395 (human breast cancer cell), 22Rv1 (human prostate cancer cell) and DU 145 (human metastatic prostate cancer cell) as depicted by the IC50 values. An IC50 value is the amount of the concentrated extract required to inhibit 50% of the cell growth. The stem extract showed toxicity to Vero cell line, this could be attributed to the presence of other compound(s) in the stem extract which were not investigated in this study. According to the American National Cancer Institute (NCI) drug screening program, a crude extract is considered to have a better *in vitro* anti-proliferative activity if the IC_{50} value is less than or equal to 30µg/ml following incubation for 48 hours to 72 hours. A crude extract with an IC_{50} of between30µg/ml to 1000 µg/ml is considered to have moderate activity [29,30,31]. Therefore, the three extracts (leaves, stem and fruit extracts) had moderate activity on the four cancer cell lines used in this study as illustrated in Table 1. The extracts IC_{50} mean values were statistically different to the IC_{50} mean values of the 5-flourouracil - a standard anticancer drug except for the fruit extract on 4T1 (breast cancer cell line) whose IC_{50} was statistically comparable to that of 5-flourouracil on the same cell line.

Table 1. Phytochemical screening results and observations

Phytochemical tested	Plant part			Observations
	Leaves	Stem	Fruits	
Alkaloids	+	+	+	White precipitate
Glycosides	+	+	+	Formation of a brown ring
Saponins	+	+	+	A stable foam
Tannins	+	+	+	Brown-green colouration
Flavonoids	+	+	+	Yellow colouration
Terpenoids	+	+	+	Brown ring at junction of two layers
Steroids	+	+	+	Blue-green ring at junction of two layers
Phenols	+	+	+	Blue-green colouration

Key: + indicates the presence of the phytochemical tested

Table 2. Anti-proliferative assay results; CC50s, IC50s, and selectivity indices (SI) of the methanol-dichloromethane leaves, stem and fruits extracts from O. sinuatum on Vero (normal), 4T1, 22Rv1, Hcc 1395 and DU 145 cancer cells

Cell line	Plant part			Reference drug
	Leaves	Stem	Fruits	5-Flourouracil
Vero (Normal)				
CC_{50} (µg/ml)	>1000	413.733±21.022[a]	>1000	>1000
4T1 (breast)				
IC_{50} (µg/ml)	745.460±17.126[a]	222.792±2.220[a]	35.841±3.549[b]	21.063±2.368
SI (CC_{50}/ IC_{50})	1.341±0.023[a]	1.857±0.045[a]	27.901±1.714[b]	48.084±5.406
22Rv1 (prostate)				
IC_{50} (µg/ml)	181.480±13.972[a]	956.966±10.996[a]	759.836±4.985[a]	15.738±1.609
SI (CC_{50}/ IC_{50})	5.543±0.0.427[a]	1.045±0.012[a]	1.316±0.066[a]	64.212±6.565
Hcc 1395 (breast)			.	
IC_{50} (µg/ml)	867.058±7.354[a]	529.199±12.707[a]	367.485±16.108[a]	24.531±3.368
SI (CC_{50}/ IC_{50})	1.153±0.007[a]	1.891±0.046[a]	2.727±0.119[a]	41.548±5.704
DU 145 (prostate)				
IC_{50} (µg/ml)	559.292±16.532[a]	114.870±15.825[a]	>1000	10.292±2.350
SI (CC_{50}/ IC_{50})	1.798±0.036[a]	3.602±0.632[a]	*	102.507±23.405

Key: [a] (superscript) indicates that the IC_{50} mean values are statistically different compared to those of the reference drug: [b] (superscript) indicates that the IC_{50} mean values are statistically comparable to that of the reference drug

The selectivity index (SI) of an extract shows its ability to inhibit the growth of cancer cells more than it does to the normal (Vero) cell line. The (SI) of an extract is calculated as a ratio of the CC_{50} on the Vero cell line to the IC_{50} of the cancer cell line. An extract with the SI greater than three is considered to be highly selective [32] and may indicate the potential use of the plant extract in cancer management. The highest selectivity index was registered by fruit extract on *4T1* (SI=27.90). The leaves and stem extracts also showed high selectivity (SI >3) on 22Rv1 and DU145. The extracts selectivity indices ranged from 1.04 to 27.90.

This study is consistent with previous studies by Kamuhabwa et al. [20] which showed that the whole plant methanolic extract of O. sinuatum had anti-proliferative activity against HT29 (colon adenocarcinoma) and A431 (skin carcinoma) but registered no activity on HeLa (Cervical cancer cells). Another study by Crowford et al. [33] showed that the crude methanolic extract from O. sinuatum inhibited mammalian endothelial cell proliferation and migration. The anti-proliferative activity of the three plant extracts against the four cancer cell lines was not directly correlated to the phytochemical profile of the plant. However, this activity can be attributed to the presence of

various secondary metabolites that were found to be present in the three plant extracts as shown in Table 1. In addition to this, other studies have shown that *O. sinuatum* contains anthraquinones, diterpenoids and phenols [19,33]. Various phytochemical compounds have been isolated and shown to have anti-proliferative activity against various cancer cell lines [34,35].

Alkaloids are a group of cyclic compounds that have a nitrogen atom in their chemical ring structure. Alkaloids have been isolated from various plants and their therapeutic activity tested. Vinblastine and vincristine are alkaloids isolated from the Madagascar periwinkle *Cantharanthus roseus*. They were the first alkaloids to be used as anticancer agents [34]. Cardiac glycosides are effective therapies for congestive heart failure and arrhythmia [36]. Recently, cardiac glycosides have been documented to have anti-proliferative activity on human carcinoma and leukemia cells [37]. Saponins have also been documented to have anti-proliferative effect on various cancer cells [38].

Phenolic compounds form a large group of molecules including the flavonoids, tannins and coumarins [39]. Phenolic compounds are known for their antioxidant and anti-proliferative activity on various cancers including leukemia, colon adenocarcinoma, cervical carcinoma prostate and bladder carcinomas [39]. Phenols from whole plant methanolic and aqueous extracts of *O. sinuatum* showed a high antioxidant activity [19]. Flavonoids are a group of water soluble compounds containing 15 carbon atoms. Takemura [40] provided a comprehensive review of chemoprevention by flavonoids on breast cancer. Flavonoids isolated from flowers of Tecoma stains demonstrated significant antitumor activity on Hep-2 (Human larynx) carcinoma cell line [41]. Tannins a subclass of hydroxybenzoic acids also manifests anti-proliferative activity against human hepatocellular carcinoma, human prostate cancer, human cervical cancer and mouse sarcoma cell lines [42]. Terpenoids and steroids are unsaturated cyclic or linear hydrocarbons with a varying number of isoprene units. Corticosteroids have been in use in management of cancer [43]. A Study by Joao [44] showed that oxysterols were active against LAMA-84 (leukemia) cells.

Although this study did not directly depict the exact mechanisms of action of the plant extracts, Crawford et al. [33] showed that crude methanolic extract of *O. sinuatum* have anti-angiogenic activity both in vitro and in vivo. Emodin, an anthraquinone and coleon A lactone, a diterpenoid from O. sinuatum [33] has been shown to be an inhibitor of protein tyrosine kinase [45], casein kinase II [46] and human epidermal growth factor receptor 2 (HER-2) [47] all of which are involved in cell division. O. sinuatum could have imparted its anti-proliferative activity through the same mechanisms.

5. CONCLUSION

This study provides the phytochemical profile of methanol-dichloromethane stem, leaves and fruits extracts of *O. sinuatum*, and further gives the anti-proliferative activity of the three plant extracts against breast and prostate cancer cell lines. The anti-proliferative activity of these extracts can be attributed to the phytochemicals that are present in the extracts. This study provides important basis for further investigation on the development of herbal medicine from *O. sinuatum* as alternative therapy for cancer treatment and management. This study recommends further studies to isolate the bioactive components from the three extracts and elucidate their mechanisms of action.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was sought from Kenya Medical Research Institute (KEMRI) Centre for Traditional Medicine and Drug Research (CTMDR) Centre Scientific Committee (CSC) and Scientific and Ethics Review Unit (SERU) (KEMRI/SERU/CTMDR/030/3331) before conducting the study.

ACKNOWLEDGEMENT

The authors are very grateful to the Center for Traditional Medicine and Drug Research (CTMDR), KEMRI for their support during the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization; WHO. World cancer report. International Agency for Research on Cancer; 2014.
2. International Agency for Research on Cancer; 2013.
3. Anand P, Kunnumakkara B, Sundaram C, Harikumar B, Tharakan T, Lai S, Sung B, Aggarwal B. Cancer is a preventable disease that requires major lifestyle changes. Pharm. Res. 2008;25(9):2097–116.
   DOI: 10.1007/s11095-008-9661-9
   PMC: 2515569
   PMID: 18626751
4. Bray F, Ren JS, Masuyer E, Ferlay J. Global estimation of cancer prevention for 27 sites in the adult population. Int J Cancer. 2008;132(5):1133-1145.
   Available:http://dx.doi.org/10.1002/ijc.27711
   PMID: 22752881
   (Accessed 20th May 2016)
5. The Kenya National Cancer Control Strategy 2011 – 2016. Ministry of Health; 2016.
6. Korir A, Okerosi N, Ronoh V, Mutuma G, Parkin M. Incidence of cancer in Nairobi, Kenya (2004-2008). International Journal of Cancer. 2015;137:2053-2059.
7. Bandaranayake WM. Turning medicinal plants into drugs. Modern Phytomedicine. 2006;23:25–57.
8. Huosain K, Majeed TM, Ismail Z, Sadikaum A, Ibram P. Traditional and complementary medicines. Quality assessment strategies and safe usage. Southern Medicine Review. 2009;2(1):19–23.
9. National Coordinating Agency for Population Development (NCAPD). Draft Policy on Traditional Medicine and Medicinal Plants, Nairobi; 2007.
10. Srivastava J, Lambert J, Vietmeyer N. Expanding role in development. Medical Plants Word Bank Technical. 1996;320.
11. Kroschwitz JI, Howe GM, Kirk O. Encyclopedia of chemical technology. 1992;19(5):535–542.
12. Hamill FA, Apio NK, Mumbira Bukenya Z, Mosango M, Magonyi OW, Soejarto D. Traditional herbal drugs of Southern Uganda. II. Literature Analysis and Antimicrobial Assays. 2010;84(299):54–78.
13. Kokwaro JO. Medicinal plants of East Africa. 3rd Ed. East Afsrica Literature Bureau; Kampala, Nairobi, Dar es Salaam. 2009;368.
14. Kareru P, Kanji PG, Gachanja AN, Keriko JM, Mungai G. Traditional medicine among the Mbeere people. Africa Journal of Traditional and Complementary Medicines. 2007;4(1):75–86.
15. Mainen J, Donald F, Pamela K, Anke W. The ethnomedicine of the Haya people of Bugabo Ward. Kagera Region, North West Tanzania. Journal of Ethnobiology and Ethnomedicine. 2009;5:24.
16. Amadi E. Evaluation of herbal drugs used to treat fungal and bacterial diseases in Mbeere Eastern Kenya. IJHM. 2009;1(4): 85–87.
17. Dominic O, Charles N, Jemimah A, Peter K, James M, Stephen G. Medicinal plants used in treatment and management of cancer in Kakamega County Kenya. Journal of Ethnopharmacology. 2014;151: 1040–1055.
18. Olila D, Bukenya Ziraba R, Kamoga D. Bio-prospective studies in medicinal plants used to manage poultry diseases in Mt. Elgon Region of Uganda. Research Journal of Pharmacology. 2007;1:56–60.
19. Uma S, Bharti O. *In vitro* 5-Lipoxygenase inhibition of polyphenol antioxidant from undomesticated plants of South Africa. Journal of Medicinal Plants Research. 2008;2:207–212.
20. Kamuhabwa A, Nshimo C, Witte P. Cytotoxicity of some medicinal plant extracts used in Tanzanian traditional medicine. Journal of Ethnopharmacology. 2000;70:143–149.
21. Harbone JB. Phytochemical methods. 3rd Ed. A guide to modern techniques of plants analysis. Chapman Hall Ltd, London; 1976.
22. Trease GE, Evans WCA. Textbook on pharmacognosy. 15th Ed. Bailierie Tindall. London. 2008;386–480.
23. Masters JR. Animal cell culture. A practical approach. 3rd Ed. Oxford University Press. London; 2008.
24. McCauley J, Zivanovic A, Skropeta D. Bioassays for anticancer activity. Methods in Molecular Biology. 2013;1055:191–205.

25. Abdel-Rahman LH, Abu-Dief AM, Newair EF, Hamdan SK. Some new nano-sized Cr(III), Fe(II), Co(II), and Ni(II) complexes incorporating 2-((E)-(pyridine-2-ylimino)-methyl)-napthalen-1-ol ligand: Structural characterization, electrochemical, anti-oxidant, antimicrobial, antiviral assessment and DNA interaction. J. Photochem. Photobiol. 2016;B160:18–31.
26. Abdel-Rahman LH, Abu-Dief AM, Ismael Mohammed, Mohamed Mounir AA, Hashem Nahla A. Synthesis, structure elucidation, biological screening, molecular modeling and DNA binding of some Cu (II) chelates incorporating imines derived from amino acids. Journal of Molecular Structure. 2016;1103:232-244.
27. Tim M. Rapid colorimetric assay for cellular growth and survival. Application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983;16(2):55-56.
28. Nemati F, Dehpouri AA, Eslami BJ, Mahdavi V, Mirzanejad S. Cytotoxicity properties of some medicinal plant extract. Iranian Red Crescent Medicinal Journal. Mazandran, Iran. 2013;47(1):e8871.
29. Boik J. Natural products in cancer-promising Nontoxic antitumor agents from plants and other natural sources. The American Journal of Clinical Nutrition. 2001;105(3):529.
30. Praying P, Barusrux S, Weerapreeyakul N. Cytotoxic activity screening of some indigenous Thai plants. Fitoterapia. 2008;79:598-601. [Pubmed] [Cross Ref]
DOI: 10.1016/j.fitote.
31. Siti MM, Nurhanan MY, Haffiz JM, Ilham AM, Getha K, Asiah O, Norhayati I, Sahira HL, Suryani SA. Potential anticancer compound from *Cerbera odollam*. Journal of Tropical Science. 2011;23(1): 89–96.
32. Nabende PN, Karanja SM, Mwatha JK, Wachira SW. Anti-proliferative activity of *Prunas africana*, *Warburgia stuhlmannii* and *Mayenus senegalensis* extracts in breast and colon cancer cell line. European Journal of Medicinal Plants. 2015;5(4):366–376.
33. Crawford AD, Liekens S, Kamuhabwa AR, Maes J, Munok S, Busson R, Rozenski J, Esguerra CV, De Witte PAM. Zebrafish bioassay-guided natural product discovery. Isolation of angiogenesis inhibitors from East African medicinal plants. PLoS ONE. 2011;6(2).
PMCID: e14694
34. Cragg GM, Newman DJ. Plant as source of anticancer agents. J Ethno-pharmacology. 2005;100:72–79.
35. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian plants. Africa J. Biotechnolgy. 2006;5:357–361.
36. Ehle M, Patel C, Giugliano RP. Dixogin. Clinical highlights: A review of digoxin and its use in contemporary medicine. Crit Pathw Cardiol. 2011;10:93-98.
37. Newman RA, Yang P, Pawlus AD, Block KI. Cardiac glycosides as novel cancer therapeutic agents. Mol Interv. 2008;8:36–49.
38. Sun L, Lin S, Zhao R, Yu B, Yuan S, Zhang L. The Saponins monomer of dwarf lily turf tuber, Dt-13, reduced human breast cell cancer cell adhesion and migration during hypoxia via regulation of tissue factor. Biological and Pharmaceutical Bulletin. 2010;33(7):1192–1198.
39. Carocho M, Ferreira I. The role of phenolic compounds in the fight against cancer. Anticancer Agents in Medicinal Chemistry. 2013;13:1236–1258.
40. Takemura H, Sakakibara H, Yamazaki S, Shimoi K. Breast cancer and flavonoids-a role in prevention. Curr Pharm Des. 2013;19(34):6125-32.
41. Kameshwaran S, Suresh V, Arunachalam G, Kanthlal SK, Mohanraj M. *In vitro* and *in vivo* activity of methanolic flower extract of Tecoma stains flower. Int Rev J Pharm. 2012;3(3):246–252.
42. Wang C, Chen L, Yang L. Antitumor activity of four macro-cyclic ellagitannins from *Cuphea hyssopifolia*. Cancer Lett. 1999;140:195–200.
43. James EW, Clay MA, Michael CP. Corticosteroids in advanced cancer. Oncology. 2001;15(20):225–236.
44. Joao FS, Manuel CS, Joao NM, Sergio S, Luisa SM. Sterols as anticancer agents. Synthesis of Ring B oxygenated steroids cytotoxic profile and comprehensive SAR analysis. J Med Chem. 2010;53(21):7632–7638.
45. Jayasuriya H, Koonchanok NM, Geahlen RL, McLaughlin JL, Chang CJ. Emodin,

a protein kinase inhibitor from *Polygonum cuspidatum*. J Nat Prod. 1992;55:696.

46. Yim H, Lee YH, Lee CH, Lee SK. Emodin, an anthraquinone delivative isolated from the rhizomes of *Rheum palmatum* selectively inhibits the activity of casein kinase II as a competitive inhibitor. Planta Med. 1999;65:9.

47. Zhang L, Chang CJ, Bacus SS, Hung MC. Suppressed transformation and induced differentiation of HER-2/ neu-over-expressing breast cancer cells by emodin. Cancer Res. 1995;55:3890.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26695