Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(2): 52-57
Received: 19-01-2016
Accepted: 20-20-2016

Adeolu A Adedapo
Department of Veterinary Physiology, Biochemistry and Pharmacology
Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Ademola A Oyagbemi
Department of Veterinary Physiology, Biochemistry and Pharmacology
Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Olusegun A Fagbohun
Department of Veterinary Microbiology and Parasitology
Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Temidayo O Omobowale
Department of Veterinary Medicine
Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Momoh A Yakubu
Department of Environmental and Interdisciplinary Sciences, College of Science, Engineering and Technology, Texas Southern University, Houston, TX 77074, US

Correspondence
Adeolu A Adedapo
Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Evaluation of the anticancer properties of the methanol leaf extract of *Chromolaena odorata* on HT-29 cell line

Adeolu A Adedapo, Ademola A Oyagbemi, Olusegun A Fagbohun, Temidayo O Omobowale, Momoh A Yakubu

Abstract
Plant materials have been used for medicinal purposes since ancient time and scientific works are being carried out so as to discover/develop lead agents which can be used to meet the numerous health challenges of man. The plant used in this study is *Chromolaena odorata*.

The effect of methanol leaf extract of *Chromolaena odorata* (MLECO) on Human Colorectal Adenocarcinoma Cell lines HT-29 (ATCC® HTB-38™) proliferation was investigated using the Cell Titer 96 MTT Proliferation Assay where the viable cells were seeded at a density of 5×10^4 (100 μL/well). Varying log concentrations of extract (100-700 μg/mL) were added and incubated for 24, 48, and 72 h time points. Incubation of the extract in the presence of VEGF and ET-1 was also conducted at different times.

MTT assay showed that after 72 hours, the extract caused marked inhibitory effects on the cancer cell lines with lower concentration showing greater effect. When the plant extract was incubated alone with cancer cell lines at 200 and 800 μg/mL, the results showed that the latter concentrations was more potent at cell inhibition but when incubated with VEGF and ET-1, the 200 μg/mL +ET-1 was more potent at 24 hours.

The result showed that the methanol leaf extract of *Chromolaena odorata* alone caused marked inhibition of HT29 cell lines after 72 hours but in the presence of the mitogens (VEGF and ET-1), the effect on the cell line was that of proliferation showing that the mitogens interfered with the plant extract's ability to cause inhibition of cell line.

Keywords: *Chromolaena odorata*, anticancer properties, HT-29 cell line, MTT assay, VEGF, ET-1

1. Introduction
Plant materials have been used for medicinal purposes since ancient time because cost, availability, accessibility and effectiveness are some of the reasons attributable for its widespread use in modern times. Researches on plant based scientific works being are being carried out so as to discover/develop lead agents which can be used to meet the numerous health challenges of man (Ijioma *et al*., 2014) [26].

A lot of research efforts are directed over the years on the anticancer therapies; nevertheless, cancer mortality rates are still on the increase. For instance among the women folks, the highest number of cancer-related deaths are caused by cancers of the breast, lungs, stomach, colon and/or rectum, and cervix, while among men cancers of the lung, stomach, liver, colon and/or rectum, esophagus, and prostate result in the highest mortality (Thomasset *et al*., 2007, Jemal *et al*., 2011, Lewandoska *et al*., 2013) [54, 27].

Chemotherapy, despite its many side effects, is still the most popular way of treating cancer (Kordek *et al*., 2007) [31]. It is for this reason a lot of attention is being paid on the natural products especially from plant to ameliorate cancer treatment. Plants such as *Abrus precatorius, Azadirachta indica, Brassica oleracea*, *Cinnamomum zeylanicum, Curcuma longa*, *Piper longum*, *Plantago major*, *Ginkgo biloba, Podophyllum emodi*, *Saussurea lappa*, *Solanum nigrum*, *Caesalpinia bonducella* and *Terminalia catappa* are some well-known medicinal plants with anti-cancer property (Kathiresan *et al*., 2006) [28].

Chemoprevention, which consists in using synthetic, semi-synthetic or natural agents to inhibit or reverse the process of carcinogenesis, particularly in individuals with a high risk of developing cancer may be another alternative way of combating cancer. In fact epidemiological studies showed that incidence of some cancers is low among the Asians than their American and European counterparts may be due in part to the fact that their diet is

markedly richer in health beneficial plant-derived polyphenols (Mohammad *et al*., 2006; Khan and Mukhtar, 2008) [38, 30]. Phenolic compounds are secondary metabolites of plant origin which carry one (phenols) or several (polyphenols) hydroxyl moieties in their aromatic ring (Stalikas, 2010) [52]. Phenolic compounds consist of approximately 8,000 naturally occurring metabolites which are divided into the flavonoids and the non-flavonoids. Phenolic compounds undertake antioxidant, pro-oxidant, anti-inflammatory activities and also exert great influence on the bioavailability of nitric oxide in humans (Li, 2011) [35]. Plants rich in phenols could therefore be explored for their anticancer properties. *Chromolaena odorata* is being evaluated for their anticancer properties in this study.

Chromolaena odorata (L) King and Robinson) regarded as an invasive weed, has been used in Indonesia, Thailand, Malaysia, Vietnam and parts of Africa including Nigeria as a hemostatic (Schoonjans *et al*., 1996; Murphy *et al*., 2000, Rangwala *et al*., 2004) [50, 40, 48] wound healing (Bohlmann *et al*., 1982a, 1982b, Hwang *et al*., 2002) [7, 8, 25] anti-inflammatory drug (Agrawal, 1989; Braca *et al*., 1999) [1, 9] and for intestinal diseases and burns (Thang *et al*., 2001) [53]. Studies about its antioxidant, antibacterial, and anti-gonorrhea effects have been reported (Dat *et al*., 2009) [13]. Its phytochemical investigations revealed the presence of flavonoids, alkaloids, and terpenoids (Thang *et al*., 2001, Dat *et al*., 2009) [53, 13]. In this study, cell viability and other properties of this plant alone and in the presence of VEGF and ET-1 on HT-29 cell lines were examined.

2. Materials and Methods

2.1 Plant collection and extract preparation

Fresh leaves of *Chromolaena odorata* were collected from the campus of the University of Ibadan, Ibadan, Nigeria. The leaves were dried under shade for about 10 days after which they were ground to powder using an electric blender. 200 g of the powdered material was soaked in 1 litre of methanol and shaken vigorously. The sample was then filtered after 3 days using a Buckner funnel and Whatman No. 1 filtered paper. The extract was further concentrated using a water bath. The weight of the extract was 12.8 g.

2.2 *Cell Culture:* HT-29 colorectal cancer cell lines were supplied by Dr. Yakubu of the Vascular Biology Unit, Center for Cardiovascular Diseases, Texas Southern University. Cells were cultured in RPMI 1640 medium (GIBCO, Grand Island, NY, USA) containing 10% fetal bovine serum and antibiotics. Cells were incubated at 37ºC in a humidified atmosphere of 5% CO_2 in 95% air. HT-29 cells were treated with 100 nM of phorbol myristate acetate (PMA, Sigma-Aldrich Co., St. Louis, MO, USA) for 72 h to induce differentiation into macrophages. After differentiation, nonattached cells were removed by aspiration and adherent macrophages were washed with RPMI 1640 medium three times and then incubated in cell culture medium at 37ºC.

2.3 *Cell viability*: The effect of methanol leaf extract of *Chromolaena odorata* (MLECO) on cell proliferation in HT29 cells was investigated using the using the Cell Titer 96 MTT Proliferation Assay. The viable cells were seeded at a density of 5×10^4 (100 μL/well) in 96-well plates and incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37ºC for 24h to form a cell monolayer. After 24h, the supernatant on the monolayer was aspirated and 100 μL of medium and varying log concentrations of extract (100-700 μg/mL) were added and incubated for 24, 48, and 72 h time points. After the specific times of exposure to the extract, 20 μL of 5 mg/mL MTT in PBS was added to each well and incubated for 3 h at 37ºC in a 5% CO_2 atmosphere. Supernatants were removed and 150 μL of isopropanol was added and the plates were gently shaken for 15 min to solubilize the formazan crystals and absorbance was measured at 560 nm using Bio Tex ELX 800 plate reader. In another study, the effects of *Chromolaena odorata* alone and with mitogens (VEGF and ET-1) on colorectal cancer cell lines (HT29) viability after 24 and 48 hours were investigated.

3. Results

MTT assay showed that after 72 hours, the extract caused marked inhibitory effects on the cancer cell lines with lower concentration showing greater effect. When the plant extract was incubated alone with cancer cell lines at 200 and 800 μg/mL, the results showed that the latter concentrations was more potent at cell inhibition but when incubated with VEGF and ET-1, the 200 μg/mL +ET-1 was more potent at 24 hours. After 48 hours, 200 μg/mL alone was more potent than the 800 μg/mL but in the presence of the mitogens, the effects of the plant extract at both concentrations were significantly different from the control and in this case caused significant proliferation. There was no significant difference between all the groups (Figures 1-7).

4. Discussion

The result showed that the methanol leaf extract of *Chromolaena odorata* alone caused marked inhibition of HT-29 cells after 72 hours but in the presence of the mitogens (VEGF and ET-1), the effect on the cell line was that of proliferation showing that the mitogens interfered with the plant extract's ability to cause inhibition of cell line. The inhibition of cancer cell line in this study by the methanol leaf extract of *Chromolaena odorata* may have lend credence to the fact that natural product especially from plant hold the key to cancer therapy. It has been stated that plants rich in phenols are of great medicinal value because phenols are potential antioxidants because there is relation between antioxidant activity and presence of phenols in common vegetables and fruits (Cai *et al*., 2004; Fu *et al*., 2011) [10, 22]. In fact, a positive linear correlation between antioxidant capacities and total phenolic contents may indicate that phenolic compounds in some tested 50 medicinal plants could be the main components contributing to the observed activities and could therefore be rich potential sources of natural antioxidants (Gan *et al*., 2010) [23]. Most phenolic antioxidants are flavonoids, such as catechins, of different structures and antioxidant activities (Pokorný, 2000) [47].

Phytochemical analysis of *C. odorata* showed that phenolics, alkaloids, terpenoids and cardiac glycosides were compounds detected in the extracts of this plant and these compounds have been documented to possess medicinal properties as well as health-promoting effects (Salah *et al*., 1995; Del-Rio *et al*., 1997; Okwu, 2004; Liu, 2004) [49, 14, 43, 36]. It may thus be safe to say that the methanol extract of *C. odorata* has anti-oxidant potential (Akinmoladun *et al*., 2007; Vijayaraghavan *et al*., 2013; Bhargava *et al*., 2013; Archana *et al*., 2015) [2, 55, 6, 3].

Although a lot of attention has been focused on anticancer therapies for many years with much progress being made, nevertheless, cancer mortality rates are still on the increase. Cancers of the breast, lungs, stomach, colon and/or rectum, and cervix, are the highest number of cancer-related deaths among women while cancers of the lung, stomach, liver, colon

and/or rectum, esophagus, and prostate resulted in the highest mortality in men (Thomasset *et al*., 2007; Jemal *et al*., 2011) [54, 27]. It is thought that increase in industrialization, environmental pollution, together with life style (smoking, excessive consumption of highly processed food, long-term stress) are all looked upon as some of the causes of this phenomenon. Many chemical compounds present in air, water, food, synthetic materials, and other products act as carcinogens (Huff and LaDou 2007; Loeb and Harris 2008; Parzefall, 2008) [24, 37, 44].

In this study, the methanol leaf extract of *Chromolaena odorata* caused dose-dependent suppression of cell viability especially at 72 hours. The interesting thing is that the lowest concentration (200 µg/ml) caused the highest suppression of cell viability with the highest concentration causing the least. It thus showed that at higher concentration cell proliferation was encouraged and vice-versa. In the presence of VEGF and ET-1, the cell antiproliferative property of this extract was reversed.

VEGF is the primary stimulus for angiogenesis in tumours. Angiogenesis is the process whereby new blood vessels sprout in response to local stimuli is essential for the development, progression, and metastasis of malignant tumors (Folkman, 1995; Ferrara, 2002) [20, 17]. In the absence of angiogenesis, tumors cannot grow beyond 1–2 mm^3 in size (Bergers and Benjamin, 2003) [5]. Vascular endothelial growth factor (VEGF) as the primary stimulus of angiogenesis in tumors carry out this functions through binding to VEGF receptor-2 (VEGFR2; also known as flk/kdr) and VEGFR1 (also known as flt) expressed on endothelial cells (Ferrara *et al*., 2003) [18]. The stimuli primarily consist of the release of angiogenic factors, activation of metalloproteases to break down extracellular matrix, and subsequent remodeling. The switch to the angiogenic phenotype is crucial in both tumor progression and metastasis (Fidler and Ellis, 1994) [19]. In fact the key factor involved in nearly all human tumors is vascular endothelial growth factor (Senger *et al*., 1993; Dvorak *et al*., 1995) [51, 15]. As a matter of fact, overexpression of VEGFR in the endothelial cells of tumor vasculature further attests to the significance of VEGF in tumor angiogenesis (Leung *et al*., 1997; Chan *et al*., 1998) [33, 11]. VEGF also acts as a survival or an anti-apoptotic factor and has been shown to induce Bcl-2 in endothelial cells as well as in breast cancer cells (Pidgeon *et al*. 2001) [46]. The VEGF family of ligands includes VEGF-A, -B, -C, -D, -E, and placenta growth factor (PlGF). These ligands bind to three tyrosine kinase receptors: VEGFR-1 (Flt-1), VEGFR-2 (KDR, or the murine homolog Flk-1), and VEGFR-3(Flt -4), all of which have been well characterized on endothelial cells (Fan *et al*., 2005) [16].

VEGF-C has been implicated in the response to ROS in prostate cancer cell lines (Muders *et al*., 2009) [39]. Wang *et al* (2014) demonstrated that VEGF-C does protect breast cancer cells from ROS-induced cell death because an antioxidant factor—SOD3—served as a downstream effector of VEGF-C. In this case SOD3 is at least in part responsible for the ability of VEGF-C to protect against ROS-induced cell death and to mediate breast tumor progression. It thus means that any agent with anti-oxidant property may in a way be promoting the activity of VEGF in tumor angiogenesis. Could this have been responsible for the results obtained in this study in which antiproliferative effect of this extract was abolished in the presence of VEGF especially that this plant is said to have anti-oxidant property?

Endothelin-1 (ET-1), on the other hand is an endothelial cell–derived vasoconstrictor peptide, an important member of the endothelin family (Yanagisawa *et al*., 1988a, Yanagisawa *et al*., 1988b) [57, 58] with myriad developmental, physiological, and pathological functions (Kedzierski and Yanagisawa, 2001) [29]. ET-1 is a potent vasoconstrictor involved in the development of cardiovascular diseases and is an important regulator of heart development (Chen *et al*., 2010) [12]. The endothelin axis as it is so-called consists of three similar small peptides, ET-1, ET-2, and ET-3, two G protein–coupled receptors, ETAR and ETBR, and two membrane bound proteases, the ET-converting enzymes, ECE-1 and ECE-2 (Kedzierski and Yanagisawa, 2001) [29], that activate the secreted pro-forms of the peptide. ET-1 production is stimulated by a variety of cytokines and growth factors, hypoxia, and shear stress, while ETAR activation triggers signaling networks involved in cell proliferation, new vessel formation, invasion, inflammation, and metastatic spread (Kedzierski and Yanagisawa, 2001; Nelson *et al*., 2003; Bagnato *et al*., 2005) [29, 42, 4]. ET-1 is secreted by human carcinoma cell lines and detected in malignant tissue (Kuhihara *et al*., 1990; Nakagawa *et al*., 1990) [32, 41].

A study has shown that the fresh leaves and extract of *Chromolaena odorata* are used as a traditional herbal treatment of burns, soft tissue wounds and skin infections in developing countries (Phan *et al*., 2000) [45], where it has been established that the extract had an effect on the growth and proliferation of keratinocytes and fibroblasts in culture (Foster and Duk, 2006) [21]. It thus means that the plant in this study has mitogenic property which is similar to that of ET-1. It is therefore not surprising that the incubation of the extract of the plant along with ET-1 led to proliferative effect of the viable cell as shown in figures 5 and 7. It could therefore be concluded from this study that the use of the extract from this plant as anticancer agent should be treated with a lot of caution.

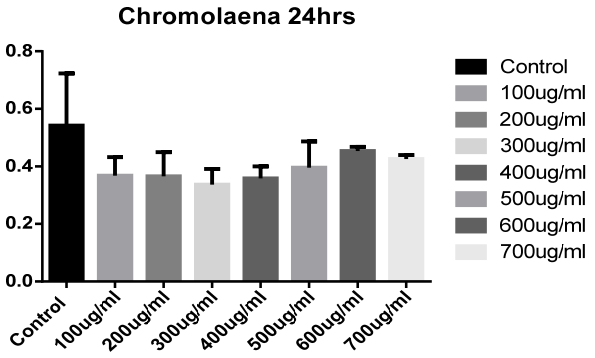

Fig 1: MTT assay on HT-29 cell line after 24 hours

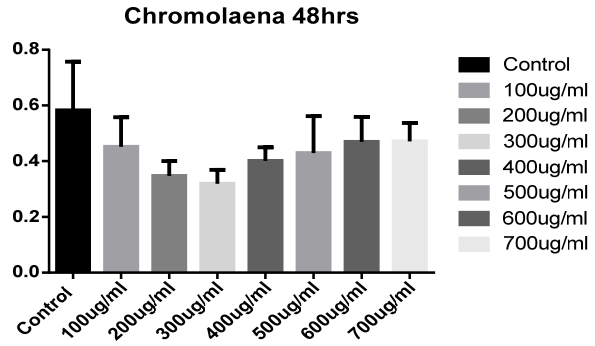

Fig 2: MTT assay on HT-29 cell line after 48 hours

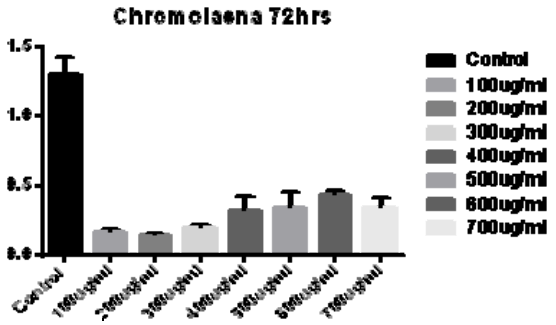

Fig 3: MTT assay on HT29 cell line after 72 hours

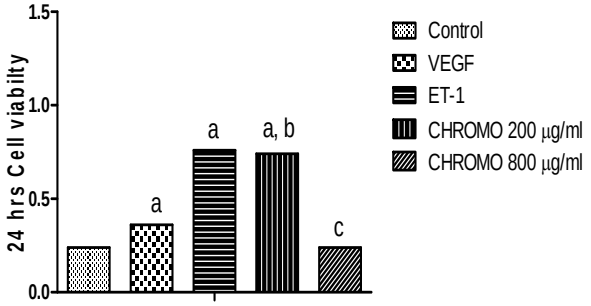

Fig 4: Effects of *Chromolaena odorata* alone and with mitogens (VEGF and ET-1) alone on colorectal cancer cell line (HT-29) viability after 24 hours. Superscripts "a" indicates significant difference compared with control; (b) indicates significant difference compared with VEGF and (c) compared with ET-1 respectively. Abbreviations: VEGF (vascular endothelial growth factor), ET-1 (endothelin-1).

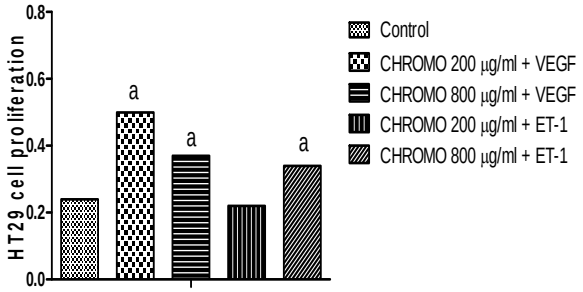

Fig 5: Effects of *Chromolaena odorata* on mitogens (VEGF and ET-1) induced-colorectal cancer cell lines (HT-29) proliferation after 24 hours. Superscripts "a" indicates significant difference compared with control; (b) indicates significant difference compared with VEGF and (c) compared with ET-1 respectively.

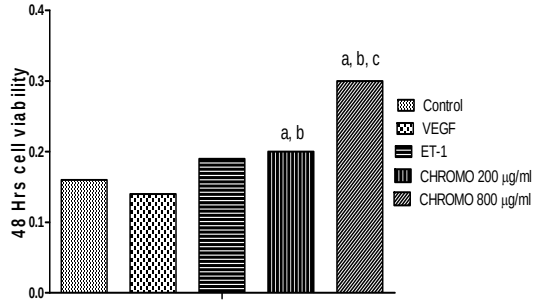

Fig 6: Effects of *Chromolaena odorata* alone and with mitogens (VEGF and ET-1) alone on colorectal cancer cell lines (HT-29) viability after 48 hours. Superscripts "a" indicates significant difference compared with control; (b) indicates significant difference compared with VEGF and (c) compared with ET-1 respectively.

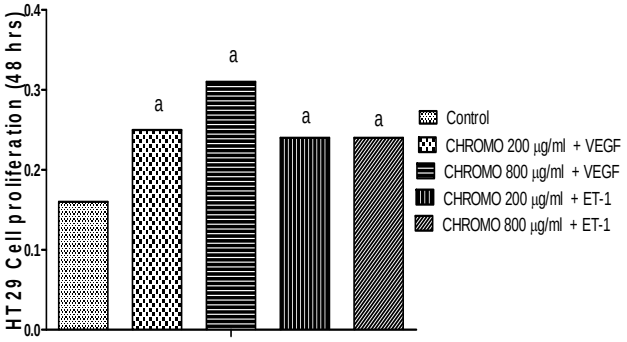

Fig 7: Effects of *Chromolaena odorata* on mitogens (VEGF and ET-1) induced-colorectal cancer cell lines (HT-29) on proliferation after 48 hours. Superscripts "a" indicates significant difference compared with control; (b) indicates significant difference compared with VEGF and (c) compared with ET-1 respectively.

Acknowledgement
The study was supported in part with a revitalization grant awarded to Drs. Adedapo and Omobowale by the University of Ibadan, Nigeria.

References

1. Agrawal PK. Carbon-13 NMR of flavonoids. Amsterdam: Elsevier, 1989, 96-157.
2. Akinmoladun AC, Ibukun EO, Dan-Ologe IA. Phytochemical constituents and antioxidant properties of extracts from the leaves of Chromolaena odorata. Sc. Res. Essay 2007; 2(6):191-194.
3. Archana CM, Jerlin SJ, Harini K, Pradeepa M, Geethan N. Radical scavenging and anti-oxidant activity of methanolic leaf extract of *Chromolaena odorata* (L) King and Robinson by in vitro assays. Int. J Pharm Sc Bus Mgt. 2015; 3(7):1-12.
4. Bagnato A, Spinella F, Rosano L. Emerging role of the endothelin axis in ovarian tumor progression. Endocr. Relat. Cancer. 2005; 12(4):761-772.
5. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat. Rev. Cancer. 2003; 3:401-410.
6. Bhargava D, Mondal CK, Shivapuri JN, Mondal S, Kar S. Antioxidant properties of the leaves of Chromolaena odorata Linn. J Inst Med. 2013; 35(1):53-56.
7. Bohlmann F, Borthakur N, King RM, Robinson H. Further prostaglandin-like fatty acids from Chromolaena morii. Phytochem. 1982a; 21:125-127.
8. Bohlmann F, Singh P, Jakupovic J, King RM, Robinson H. Three cadinene derivatives and a prostaglandin-like acid from Chromolaena species. Phytochem. 1982b; 21:371-374.
9. Braca A, Bilia AR, Mendez J, Morelli I. Three flavonoids from Licania densiflora. Phytochem. 1999; 51:1125-1128.
10. Cai YZ, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sc. 2004; 74(17):2157-2184.
11. Chan AS, Leung SY, Wong MP. Expression of vascular endothelial growth factor and its receptors in the anaplastic progression of astrocytoma, oligodendroglioma, and ependymoma. Am. J Surg Pathol. 1998; 22:816-826.
12. Chen M, Lin Y, Xie S, Wang J. Mitogen-activated protein kinase in endothelin-1-induced cardiac differentiation of mouse embryonic stem cells. J Cellular Biochem. 2010; 111(6):1619-1628.

13. Dat NT, Lee K, Hong Y, Kim YH, Minh CV, Lee JJ. A peroxisome proliferator-activated receptor-gamma agonist and other constituents from Chromolaena odorata. Planta Medica. 2009; 75:803-807.
14. Del-Rio A, Obdulio BG, Castillo J, Marin RR, Ortuno A. Uses and properties of citrus flavonoids. J Agric Food Chem. 1977; 45:4505-4515.
15. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am. J Pathol. 1995; 146:1029-1039.
16. Fan F, Wey JS1, McCarty MF, Belcheva A, Liu W, Bauer TW *et al.* Expression and function of vascular endothelial growth factor receptor-1 on human colorectal cancer cells. Oncogene. 2005; 24:2647-2653.
17. Ferrara N. Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. Seminars in Oncol. 2002; 29:10-14.
18. Ferrara N, Gerber HP, Le Couter J. The biology of VEGF and its receptors. Nature Med. 2003; 9:669-676.
19. Fidler IJ, Ellis LM. The implications of angiogenesis for the biology and therapy of cancer metastasis. Cell. 1994; 79:185-188.
20. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nature Med. 1995; 1:27-31.
21. Foster S, Duk JA. Watercress in Medical Plants, Houghton Miffin Co, New York: Academic Press, 2006.
22. Fu L, Xu BT, Xu XR, Gan RY, Zhang Y, Xia EQ *et al.* Antioxidant capacities and total phenolic contents of 62 fruits. Food Chem. 2011; 129(2):345-350.
23. Gan RY, Kuang L, Xu XR, Zhang YA, Xia EQ, Song FL *et al.* Screening of natural antioxidants from traditional Chinese medicinal plants associated with treatment of rheumatic disease. Molecules. 2010; 15(9):5988-5997.
24. Huff J, LaDou J. Aspartame bioassay findings portend human cancer hazards. Int. J Occup Environ Health. 2007; 13:446-448.
25. Hwang BY, Lee JH, Nam JB, Kim HS, Hong YS, Lee JJ. Two new furanoditerpenes from Saururus chinensis and their effects on the activation of peroxisome proliferator-activated receptor γ. J Nat Prod. 2002; 65:616-617.
26. Ijioma SN, Okafor AI, Ndukuba PI, Nwankwo AA, Akomas SC. Hypoglycemic, hematologic and lipid profile effects of Chromolaena odorata ethanol leaf extract in alloxan induced diabetic rats. Ann. Biol. Sc. 2014; 2(3):27-32.
27. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61:69-90.
28. Kathiresan K, Boopathy SN, Kavitha S. Coastal vegetation, an underexplored source of anticancer drugs. J. Nat. Prod. Radiance. 2006; 5(2):116-119.
29. Kedzierski RM, Yanagisawa M. Endothelin system: the double-edged sword in health and disease. Ann. Rev. Pharmacol. Toxicol. 2001; 41:851-876.
30. Khan N, Mukhtar H. Multitargeted therapy of cancer by green tea polyphenols. Cancer Lett. 2008; 269:269-280.
31. Kordek R, Jassem J, Jeziorski A, Kornafel J, Krzakowski M, Pawlęga J. Chemotherapy and hormone therapy for cancers: Oncology. A manual for students and doctors, red.: Ruzicka M., Siemaszko I., Via Medica, Gdańsk, Po-land. 2007, 93-100.
32. Kurihara M, Ochi A, Kawaguchi T, Niwa M, Kataoka Y, Mori K. Localization and characterization of endothelin receptors in human gliomas: a growth factor? Neurosurgery. 1990; 27(2):275-281.
33. Leung SY, Chan AS, Wong MP, Yuen ST, Cheung N, Chung LP. Expression of vascular endothelial growth factor and its receptors in pilocytic astrocytoma. Am. J Surg Pathol. 1997; 21:941-950.
34. Lewandoska U, Gorlach S, Owczarek K, Hrabec E, Szewczyk K. Synergistic Interactions between anticancer chemotherapeutics and phenolic compounds and anticancer synergy between polyphenols. Postepy. Hig. Med. Dosw (online). 2014; 68:528-540.
35. Li Y. Antioxidants in biology and medicine: Essentials, advances and clinical applications. New York: Nova Science Publishers. 2011, 266-335.
36. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J. Nutr. 2004; 134:3479S-3485S.
37. Loeb LA, Harris CC. Advances in chemical carcinogenesis: a historical review and prospective. Cancer Res. 2008; 68:6863-6872.
38. Mohammad RM, Banerjee S, Li Y, Aboukameel A, Kucuk O, Sarkar FH. Cisplatin-induced antitumor activity is potentiated by the soy isoflavone genistein in BxPC-3 pancreatic tumor xenografts. Cancer. 2006; 106:1260-1268.
39. Muders MH, Zhang H, Wang E, Tindall DJ, Datta K. Vascular endothelial growth factor-C protects prostate cancer cells from oxidative stress by the activation of mammalian target of rapamycin complex-2 and AKT-1. Cancer Res. 2009; 69:6042-6048.
40. Murphy GJ, Holder JC. PPAR-γ agonists: therapeutic role in diabetes, inflammation and cancer. Trends Pharm. Sc. 2000; 21:469-474.
41. Nakagawa K, Nishimura T, Shindo K, Kobayashi H, Hamada T, Yokokawa K. Measurement of immunoreactive endothelin-1 in plasma of a patient with malignant hemangioendothelioma. Nippon Hifuka Gakkai Zasshi. 1990; 100(14):1453-1456.
42. Nelson J, Bagnato A, Battistini B, Nisen P. The endothelin axis: emerging role in cancer. Nat. Rev. Cancer. 2003; 3(2):110-116.
43. Okwu DE. Phytochemical and vitamin content of indigenous spices of South Eastern Nigeria. J Sustain Agric Environ. 2004; 6:30-34.
44. Parzefall W. Minireview on the toxicity of dietary acrylamide. Food Chem. Toxicol. 2008; 46:1360-1364.
45. Phan TT, Allen J, Hughes MA, Cherry G, Wojnarowska F, Upregulation of adhesion complex proteins and fibronectin by human keratinocytes treated with an aqueous extract from the leaves of Chromolaena odorata (Eupolin). Eur. J Dermatol. 2000; 10(7):522-526.
46. Pidgeon GP, Barr MP, Harmey JH, Foley DA, Bouchier-Hayes DJ. Vascular endothelial growth factor (VEGF) upregulates BCL-2 and inhibits apoptosis in human and murine mammary adenocarcinoma cells. Brit. J Cancer. 2001; 85:273-278.
47. Pokorný J. In Food Preservation Techniques (P. Zeuthen, L. Bøgh-Sørensen, eds.) Woodhead Publishing, Cambridge, UK, 2000, 33-48.
48. Rangwala SM, Lazar MA. Peroxisome proliferator-activated receptor γ in diabetes and metabolism. Trends Pharm. Sc. 2004; 25:331-336.
49. Salah W, Miller NJ, Pagauga G, Tijburg A, Bolwell GP, Rice E, Evans C. Polyphenolic flavonols as scavenger of

aqueous phase radicals and chain breaking antioxidants. Arch. Biochem. Biol. 1995; 2:339-346.
50. Schoonjans K, Staels B, Auwerx J. The peroxisome proliferator-activated receptor (PPARs) and their effects on lipid metabolism and adipocyte differentiation. Biochim. Biophys. Acta. 1996; 1302:93-109.
51. Senger DR, Van dewater L, Brown LF. Vascular permeability factor (VPF, VEGF) in tumor biology. Cancer Metastasis Rev. 1993; 12:303-324.
52. Stalikas CD. Phenolic acids and flavonoids: Occurrence and analytical methods. In: Free radicals and antioxidant protocols, methods in molecular biology. Uppu, R.M., Murthy, S.N., Pryor, W.A. and Parinandi, N.L. eds. Humana Press, New York. 2010, 74.
53. Thang PT, See P, Lee ST, Chan SY. Anti-oxidant effects of the extracts from the leaves of Chromolaena odorata on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine−xanthine oxidase induced damage. Burns. 2001; 27:319-327.
54. Thomasset SC, Berry DP, Garcea G, Marczylo T, Steward WP, Gescher AJ. Dietary polyphenolic phytochemicals – promising cancer chemopreventive agents in humans? A review of their clinical properties. Int. J Cancer. 2007; 120:451-458.
55. Vijayaraghavan K, Ali SM, Maruthi R. Studies on phytochemical screening and antioxidant activity of Chromolaena Odorata and Annona squamosa. International J Innov Res Sc Eng Technol. 2013; 2(12):7315-7321.
56. Wang C, Harrell JC, Iwanaga R, Jedlicka P, Ford HL. Vascular endothelial growth factor C promotes breast cancer progression via a novel antioxidant mechanism that involves regulation of superoxide dismutase 3. Breast Cancer Res. 2014; 16:462.
57. Yanagisawa M, Inoue A, Ishikawa T, Kasuva Y, Kimura S, Kumagaye S *et al*. Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. Proc. Natl. Acad. Sc. U.S.A. 1988a; 85(18):6964-6967.
58. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y *et al.* A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature. 1988b; 332(6163):411-415.