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RESEARCH

Myelo-protective Activity of Fractions of *Vitex doniana* Sweet Leaves Extract in Wistar Rats

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ABSTRACT

Myelo-protective activity of fractions of leaves extracts of *Vitex doniana* was investigated. Wistar rats (n=60) were intra-peritoneally induced for myelo-suppression with 3mg/kg bodyweight (b.wt) cyclophosphamide for 7 days. Column chromatography revealed 4 aqueous fractions (AF1, AF2, AF3, and AF4) and 2 methanol fractions (MF1 and MF2). The rats were divided into 6 groups and each group was orally administered with 100mg/kg b.wt of each fraction (group 1 =AF1, group 2=AF2, group 3= AF3, group 4=AF4, group 5= MF1 and group 6=MF2) for 14 days. Blood samples were collected on day 15 for haematological analysis. Data were analyzed using two-way analysis of variance in Graph pad prism version 5.03. Results revealed significantly increased values in AF1:WBC[x 10^9/L]: 5.8 ± 0.4; Neutrophil [%]: 58 ± 2; AF3:WBC[x 10^9/L]: 6.8 ±1.5; Neutrophil [%]: 67±2.5 and MF1: WBC[x 10^9/L]: 6.2±0.5; Neutrophil [%]:65±2, compared to AF2:WBC[x 10^9/L]: 2.4 ±0.4; Neutrophil [%]: 35±3; AF4:WBC[x 10^9/L]: 2.35±0.4; Neutrophil [%]: 40±3; MF2:WBC[x 10^9/L]: 3.5±0.4; Neutrophil [%]: 38±3 and control: WBC[x 10^9/L]: 5.8±0.4; Neutrophil [%]: 60±3, (p<0.001). AF1, AF3 and MF1 also revealed significantly increased haemoglobin, haematocrit and RBC compared to other fractions and control (p<0.05). BMC (x10^6/femur) increased in AF1: 15.30±0.7, AF3: 14.50± 1.5 and MF1: 10.50±1.2 compared to AF2: 3.1±0.9; AF4: 9.2±1.1 and MF2: 10.5±1.5 indicating myelo-protection from fractions AF1, AF3 and MF1.

Key words: Myelo-protective, Fractions, *Vitex doniana,* Wistar rats

Abbreviations: BMC-Bone marrow cells, WBC-White blood cells, AF-Aqueous fraction, MF-Methanol fraction

1. INTRODUCTION

Myelo-suppression is a multi-factorial challenge that influences the treatment of oncology patients (Nichols et al., 1994). Most anti-neoplastic agents are known to cause myelo-supression (Ozkan et al., 2005). It is therefore, essential to introduce means to provide myelo-protective effects. Biological response modifiers have been synthesized to circumvent myelo-suppression. The colony-stimulating factors and interleukins induce cell viability by regulating the proliferation of tumor-killing T lymphocytes and so called natural killer cells (Slanicka et al., 1998; Ido et al., 1992; Kiss et al., 2004). Studies indicate that Mpl ligand (Mpl-L) reduces hematopoietic toxicity much more effectively when administered immediately after a myelo-suppressive insult than when given beforehand or at later times (Neelis et al., 1998; Shibuya et al., 1998; Ulich et al., 1999; Mouthon et al., 1999; Pestina et al., 1999). The involvement of Mpl-L (also known as thrombopoietin or TPO) and its receptor Mpl in early hematopoiesis is consistent with the following observations: (1) Mpl is expressed on the $AA4^{+}Sca^{+}$ subpopulation of murine hematopoietic stem cells (Solar et al., 1998). (2) Mpl-L stimulates the expansion of very immature precursors both in vitro and in vivo (Carver-Moore et al., 1996; Alexander et al., 1996). (3) Mpl and Mpl-L play significant roles in both megakaryocyte maturation and in the maintenance of multi-potential hematopoietic progenitors (Alexander et al., 1996; de Sauvage et al., 1998). Signaling by Mpl-L alone or in combination with other early-acting cytokines, also promotes the survival of primitive multi-potent progenitor cells by blocking apoptosis (Borge et al., 1996; Shimomura et al., 2000). Other approaches such as employing herbal medicine with a view to myelo-protection have equally been pursued (Ufelle et al., 2011). Traditional herbal medicines are naturally-occurring, plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices (Tilburt and Kaptchuk 2008). Government, international agencies and corporations are increasingly investing in traditional herbal medicine research (Tilburt and Kaptchuk 2008). In Nigeria, traditional and herbal healing systems play an important role in health care delivery, and about 70-80% of the population depends on traditional healers for most of their ailments (Akah et al., 1998).

Vitex comprises about 150 species and is pan-tropical with a few species in temperate regions (Burkill, 2000). Approximately 60 species occur in tropical Africa of which *Vitex doniana* is most widespread (Burkill, 2000). Leaves opposite; digitately compound with (3-) 5 (-7) leaflets; stipules absent; petiole 5-20cm long; petiolules up to 2.5cm long; leaflets obovate to elliptical, 4 - 25cm x 2.5 – 10.5cm, notched to rounded or shortly acuminate at apex, entire leathery, nearly glabrous (Burkill, 2000). *Vitex doniana* (Verbernaceae) commonly known as black plum has other vernacular names such as West African plum (En), *Prunier Noir, Koro (Fr), Cetona (Po), Mfudu, Mfuru, Mfuu (Sw*), (Mbuya et al., 1994). In the South Western Nigeria, it is called '*Ori-nla'* (Kilani, 2006), while among the Igbos of South Eastern Nigeria; it is called '*Uchakiri*'. *Vitex doniana* is widespread deciduous forest tree largely found in coastal woodlands and savannah, but also in wetter areas at lower altitudes (Mbuya et al., 1994). It is found in deciduous woodlands (especially *Brachystegia*), secondary forests and dry forests.

There are numerous medicinal uses for this tree (Mbuya et al., 1994). Earlier workers have reported the use of the fruits and leaves for medicinal purposes (Sofwora, 1993; Babalola, 1993). *Vitex doniana* is used to improve fertility and to treat leprosy, dysentery, jaundice, control of post partum bleeding after birth and anaemia (Ladeji et al., 2005). It is also used to treat gastroenteritis, diarrhea and gonorrhea (Ladeji et al., 2005). The juice may be squeezed into the eye to treat eye troubles (Ladeji et al., 2005). The use of *Vitex doniana* suggests that it may also possess anti-microbial activity (Kilani, 2006). Recent studies indicate that various crude extracts of *Vitex doniana* leaves possesses myelo-protective properties in cyclophosphamide-induced myelotoxicity with the greater protection coming from the aqueous extract (Ufelle et al., 2011). This may be very useful in cancer therapy and will go a long way to prevent the myelo-suppression (anaemia) exhibited by cancer drugs such as cyclophosphamide (Ufelle et al., 2011).

In the present study, haemoglobin, haematocrit, leucocytes and bone marrow cell counts will be performed on cyclophosphamide-induced myelo-suppressed Wistar rats after oral administration of various fractions of the extracts. Gas Chromatography Mass Spectrometry (GCMS) will be performed on the fractions to identify the compounds responsible for the myelo-protective activity. Phytochemical analysis will be performed on the extract fractions to reveal their phytochemical constituents.

2. MATERIALS AND METHODS

Plant Materials and Study Animals: The leaves of *Vitex doniana* sweet were obtained from its natural habitat in Udi area of Enugu State Nigeria between the month of April and May 2009 and were authenticated by a taxonomist at the Botany department; University of Nigeria Nsukka and a voucher specimen were kept in the herbarum for future reference. The herbarum reference Number of *Vitex doniana* is E. Nig-Enugu: Udi, Latilo FHI 27617 (Keay et al., 1964). Wistar rats (n=60) weighing between 100 to 200 grams, aged 2 to 3 months were purchased and housed in the Animal House of College of Medicine, University of Nigeria Enugu Campus. They were allowed to acclimatize for two weeks and fed with standard pellets (Guinea feed[@] Nigeria PLC). They had access to water and feed *ad libitum.* The rats were kept at a temperature of 28-32^{o}C with dark light (13:11) cycle. They were kept in stainless steel wire mesh cages which were perforated beneath for the exit of the rats' feaces to prevent coprophagy. All the rats were handled in the study according to International guidelines for handling experimental animals by American Physiological Society (APS).

Experimental Design: Six fractions; (4 aqueous fractions [AF1, AF2, AF3, and AF4] and 2 methanol fractions [MF1 and MF2]) were obtained from the column chromatography. GC-MS was done on the 6 fractions to reveal the compound names, molecular structures and molecular weights. Phytochemical analysis was also done on the 6 fractions to reveal the phytochemical constituents (Ioan, 1984). Wistar rats (n=60) were divided into 6 groups according to the number of fractions. Each Wistar rat was IP-induced for myelo-suppression with 3mg/kg b.wt of CP

for 7 days before oral administration of the fractions. Each fraction (100mg/kg b.wt) was orally administered to Wistar rats in each group for 14 days.

Sample collection: Blood samples were collected from all the rats via the retro-bulber plexus of the medial canthus vein on day 15 into *K3-EDTA* anti-coagulated bottles for haematological analysis and bone marrow collected from the femur of rats into a medium containing 2% FCS for bone marrow cell counts.

Analytic Methods: Column Chromatography was done by the method of Still, (Still et al., 1978). Gas Chromatography-Mass Spectrometry (GC-MS) done by the method of Alon and Amirav, (Alon and Amirav 2006), using GC-MS-QP 2010 PLUS ®SHIMADZU JAPAN: This was used to separate both the aqueous and methanolic leaf extract of *V.doniana* after column chromatography and to name the different compounds with their structures. Acute Toxicity Test: (Median Lethal Dose, LD_{50}) was performed on mice according to the procedure described by Lorke, (Lorke, 1983). Data were analyzed using two-way analysis of variance in Graph pad prism version 5.03.

3. RESULTS

Column chromatography of the extracts revealed 4 aqueous fractions (AF1, AF2, AF3, and AF4) and 2 methanol fractions (MF1 and MF2). Gas Chromatography Mass Spectrometry (GC-MS) of the extracts fractions revealed 30 compounds with their molecular weights and formula. The aqueous fractions revealed 19 compounds (AF1=5, AF2=4, AF3=5, AF4=5) while the methanol fractions revealed 11 compounds (MF1=5, and MF2=6). Acute toxicity test revealed median lethal dose, LD_{50} of 3000mg/kg b.wt. The phytochemical analysis revealed flavonoids, alkaloids, tannins, proteins, carbohydrates, reducing sugars and steroids. Haematological analysis revealed significantly increased values in AF1:WBC(x 10^9/L): 5.8 ± 0.4; Neutrophil (%): 58 ± 2; AF3:WBC (x 10^9/L): 6.8 ± 1.5; Neutrophil (%) : 67 ± 2.5 and MF1: WBC(x 10^9/L): 6.2 ± 0.5; Neutrophil (%):65 ± 2, compared to AF2:WBC(x 10^9/L): 2.4 ± 0.4; Neutrophil (%): 35 ± 3; AF4:WBC(x 10^9/L): 2.35 ± 0.4; Neutrophil (%): 40 ± 3; MF2:WBC (x 10^9/L): 3.5 ± 0.4; Neutrophil (%): 38 ± 3 and control: WBC (x 10^9/L): 5.8 ± 0.4; Neutrophil (%) : 60 ± 3, (p<0.001). Fractions AF1, AF3 and MF1 also revealed significant increase in haemoglobin, haematocrit and red blood cell count compared to the other fractions and control (p<0.05). BMC (x10^6/femur) increased in AF1: 15.30 ± 0.7, AF3: 14.50 ± 1.5 and MF1: 10.50 ± 1.2 compared to AF2: 3.1 ± 0.9, 4: 9.2 ± 1.1 and MF2 : 10.5 ± 1.5 indicating more myelo-protection from fractions AF1, AF3 and MF1.

4. DISCUSSION

Myelo-protective activity is observed by judging the changes in leucocytes and bone marrow of Cyclophosphamide-induced myelo-suppressed animal model. The major determinant of myelo-suppression occurrence is the intensity of the cytotoxic drugs given to the patient, but other variables may influence the probability of such an event (Chabner, 1993). Chemotherapy affects not only cancer cells, but other rapidly dividing cells in the body as well which includes the cells in the bone marrow that go on to become red blood cells, white blood cells and platelets (Chabner, 1993).

Cyclophosphamide belongs to the nitrogen mustard subclass of alkylating agents under cytotoxic drugs used in the treatment of lymphomas, some forms of leukaemia and some solid tumors (Shanafelt et al., 2007). Cyclophosphamide converts the base nitrogen mustard into a non-toxic "transport form". This transport form was a pro-drug, subsequently actively transported into the cancer cells. Once in the cells, the pro-drug was enzymatic ally converted into the active, toxic form. The first clinical trials were published at the end of the 1950s (Brock, 1989). It is a "prodrug"; it is converted in the liver to active forms that have chemotherapeutic activity. It was used in this study to induce myelo-suppression in Wistar rats.

The fractions of the *Vitex doniana* Sweet leaves extract were investigated for myelo-protective activity in myelo-suppressed Wistar rats. Out of the 6 fractions revealed by column chromatography (AF1, AF2, AF3, AF4, MF1 and MF2), 3 of them (AF1, AF3 and MF1) demonstrated myelo-protective effects in Cyclophosphamide-induced myelo-suppressed Wistar rats by causing significant increase in haematological and BMC values when compared with AF2, AF4, MF2 and control. The observed myelo-protective effects may probably be due to the molecular weight of the compounds in the fractions, the solvents used in the extraction and phytochemical contents of the extract. Fractions AF1, AF3 and MF1have demonstrated more myelo-protective activity than others. The smaller molecular weight compounds in fractions AF1, AF3 and MF1 may have caused easier penetration of the fractions into cells and caused the observed myelo-protective activity. The aqueous fractions demonstrated more myelo-protection than the methanol fractions probably due to water based solvent of the aqueous fractions which might make it more absorbable than the alcohol based solvent.

The observed increase in haematological and BMC values may also be attributed to the phytochemical constituents of the fractions which include flavonoids, alkaloids, tannins, proteins, carbohydrates, reducing sugars and steroids. Flavonoids and tannins are phenolic compounds, and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Potterat, 1997). Similarly, terpenoids, as vitamins, act as regulators of metabolism and play a protective role as antioxidants (Soetan, 2008). The antioxidant property of the leaf extract may be a strong contributing factor to the applications of the plants in the management and treatment of various diseases (Potterat, 1997). Antioxidants prevent oxidative stress, caused by free radicals, which damage cells and vital bio-molecules (Potterat, 1997). They terminate chain reactions triggered by free radicals by removing free radical intermediates and inhibit other oxidation reactions (Sies, 1997).The fractions that demonstrated more myelo-protection have more concentrations of flavonoid which is a strong antioxidant. This antioxidant may have contributed to cell growth in form of increase in haematological parameters of myelo-suppressed Wistar rats thereby

preventing cell apoptosis. The fractions with less myelo-protection have more concentrations of tannin which is an astringent that causes cell shrinkage thereby leading to cell apoptosis. Studies indicate that *p53* mediates apoptosis of hematopoietic progenitors exposed to DNA-damaging drugs or γ-irradiation (Wlodarski et al., 1998**;** Toefili et al., 1998). Furthermore, *p53* appears to be a key regulator of the proliferation of hematopoietic progenitors, as *p53* status influences both long- and short-term repopulation following bone marrow transplantation. However, *p53* does not appear to affect the lineage determination or differentiation of committed progenitor/precursor cells (Wlodarski et al., 1998).

Other phytochemical constituents of the fractions that demonstrated more myelo-protection (AF1, AF3 and MF1) include saponins, proteins, reducing sugars and steroids than the others. These constituents may have contributed to the observed myelo-protections by inhibiting *p53* dependent apoptosis, especially the flavonoids which was present in all the fractions but with higher concentrations been recorded in the fractions that demonstrated myelo-protection. Saponins are a class of chemical compounds, one of many secondary metabolites found in natural sources, with saponins found in particular abundance in various plant species (Hostettmann and Marston 1995; Riguera, 1997). Specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative (Hostettmann and Marston 1995; Riguera, 1997). There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals, (Asl and Hossein 2008). There is evidence of the presence of saponins in traditional medicine preparations, where oral administrations might be expected to lead to hydrolysis of glycoside from terpenoid (and obviation of any toxicity associated with the intact molecule), (Asl and Hossein 2008).

Tannin (vegetable tannin), as opposed to a modern synthetic tannin) is an astringent, bitter plant polyphenolic compound that either binds and precipitates or shrinks proteins and various other organic compounds including amino acids and alkaloids (Bajaj, 1988). The anti-inflammatory effect of tannins helps control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders (Bajaj, 1988). Tannins can also be effective in protecting the kidneys. Tannins have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhaging, fatigue, skin ulcers and as a cicatrizant on gangrenous wounds (Akiyama et al., 2001). Tannins can cause regression of tumors that are already present in tissue, but if used excessively over time, they can cause tumors in healthy tissue. Tannins are used indirectly as molluscicides to interrupt the transmission cycle of schistosomiasis. They have also been reported to have anti-viral effects. When incubated with red grape juice and red wines with a high content of condensed tannins, the poliovirus, herpes simplex virus, and various enteric viruses are inactivated (Bajaj, 1988). Tannins are sometimes used to treat poisons from poison oak or from bee stings, causing instant relief Bajaj, 1988). Tannins have shown potential antiviral (Lu et al., 2004), anti-bacterial (Akiyama et al., 2001) and anti-parasitic effects (Kolodziej and Kiderlen 2005). It is believed that tannins isolated from the stem bark of *Myracrodruon urundeuva* are of neuro-protective functions capable of reversing 6-hydroxydopamine induced toxicity. The plant has shown promising futures for therapeutic use, which may be of benefit to neuro disease patients. It was discovered that the tannins isolated from the stem bark also have the anti-inflammatory and antiulcer potency on rodents, showing a strong antioxidant property for possible therapeutic applications (Souza et al., 2006).

Proteins and steroids are also present in the extract fractions and may have contributed to the observed myelo-protective activity. Proteins are important to the structure and function of all living cells and viruses. Due to the anti-inflammatory nature of steroids, they suppress the action of some white blood cells in the immune system.

These phytochemicals and compounds identified in the extract fractions of *Vitex doniana* leaves makes it pharmacologically active and confirmed it as a medically useful herb according to previous researchers especially in the treatment of anaemia (Ladeji et al., 2005).

5. CONCLUSION

This study has demonstrated that fractions of *Vitex doniana* sweet leaves extracts possess myelo-protective properties with the greater protections coming from fractions with smaller molecular weights (AF1, AF3 and MF1) in myelo-suppressed Wistar rats. It can therefore be concluded that these fractions of *Vitex doniana* possess properties that promote the survival of primitive multi-potent progenitor cells by blocking cell apoptosis in a condition of myelo-suppression. This may be very useful in cancer therapy and will help to prevent the myelo-suppression exhibited by cancer drugs such as cyclophosphamide.

SUMMARY OF RESEARCH

1. Fractions of *Vitex doniana* sweet leaves extract possess myelo-protective properties.
2. The fractions that exhibit more myelo-protection have smaller molecular weight compounds which may be responsible for easier penetration into cells to act.
3. More of the aqueous fractions demonstrated myelo-protective activity than the methanol fractions probably due to water based solvent of the aqueous fractions which might make it more absorbable than the alcohol based solvent.

FUTURE ISSUES

However further pharmacochemical work is recommended to be done on the leaves extract of *V.doniana* to be reputed as a good drug like other existing synthesized biological response modifiers against myelo-suppression induced by cytotoxic drugs.

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REFERENCES

1. Nichols CR, Fox E P, Roth BJ, Williams SD, Loehrer PJ, Einhorn, LH. Incidence of neutropenic fever in patients treated with standard – dose combination chemotherapy for small cell lung cancer and the cost impart of treatment with granulocyte colony stimulating factor. *J. Clin Oncol,* 1994, 12(6), 1245-1250.
2. Ozkan K, Turkkan E, Ender K, Mutlu D, Murat A, Nalan B, Abdulmecit Y, Osman M. 5-Fluorouracil, epirubicin and cisplatin in the treatment of metastatic gastric carcinoma: a retrospective analysis of 68 Patients. *Indian J. Cancer,* 2005, 42(2), 85-88.
3. Slanicka Krieger, M; Nissen, C; Manz, C.Y; Toksoz, D; Lyman, S.D; Wodnar-Filipowicz, A. The membrane – bound isoform of stem cell factor synergizes with soluble flt 3 ligand in supporting early hematopoietic cells in long – term cultures of normal and aplastic anaemia bone marrow, *Experimental Hematology,* 1998, 26 (5): 365-373.
4. Ido M, Harada M, Furuichi H, Matsuoka N, and Nakano K, Sohmura Y. Interleukin 1 induced sequential myelorestoration: dynamic relation between granulopoiesis and progenitor cell recovery in myelosuppressed mice; *Experimental Hematology,* 1992, 20 (2): 161-166.
5. Kiss, C; Benko, I; Kovacs, P. Leukemic cells and the cytokine patchwork *Pediatrics Blood Cancer* 2004, 42 (2): 113 – 121.
6. Neelis KJ, Visser TP, Dimjati W. A single dose of thrombopoietin shortly after myelo-suppressive total body irradiation prevents pancytopenia in mice by promoting short-term multilineage spleen-repopulating cells at the transient expense of bone marrow-repopulating cells. *Blood,* 1998, *92:1586-1597.*
7. Shibuya K, Akahori H, Takahashi K, Tahara E, Kato T, Miyazaki H. Multi-lineage hematopoietic recovery by a single injection of pegylated recombinant human megakaryocyte growth and development factor in myelo-suppressed mice. *Blood, 1998, 91:37-45.*
8. Ulich TR, del Castillo J, Senaldi G, Harley C, Molineux G. PEG-rHuMGDF promotes multilineage hematopoietic recovery in myelosuppressed mice. *Experimental Hematology,* 1999, *27: 1776-1781.*
9. Mouthon MA, Van der Meeren A, Gaugler MH. Thrombopoietin promotes hematopoietic recovery and survival after high-dose whole body irradiation. *Int J Radiat Oncol Biol Phys, 1999, 43: 867-875.*
10. Pestina TI, Abushullaih B, Jackson CW. A single injection of pegylated recombinant mouse megakaryocyte growth and development factor (PEG-rmMGDF) prevents death in lethally myelosuppressed mice by inhibiting p53-dependent apoptosis. *Experimental Hematology, 1999, 27[abstract]:241.*
11. Solar GP, Kerr WG, Zeigler FC. Role of c-mpl in early hematopoiesis; *Blood, 1998, 92: 4 - 10.*
12. Carver-Moore K, Broxmeyer HE, Luoh SM. Low levels of erythroid and myeloid progenitors in TPO- and in c-mpl-deficient mice. *Blood, 1996,* 88:803-809.
13. Alexander WS, Roberts AW, Nicola NA, Li R, Metcalf D. Deficiencies in progenitor cells of multiple hematopoietic lineages and defective megakaryocytopoiesis in mice lacking the thrombopoietin receptor c-mpl. *Blood, 1996, 87:2162-2170.*
14. de Sauvage FJ, Villeval JL, Shivdasani RA. Regulation of megakaryocytopoiesis and platelet production: lessons from animal models. *Journal of Laboratory and Clinical Medicine, 1998, 131: 496-501.*
15. Borge OJ, Ramsfjell V, Veiby OP, Murphy MJ, Look S, Jacobsen SEW. Hrombopoietin, but not erythropoietin promotes viability and inhibits apoptosis of multi-potent murine hematopoietic progenitor cells in vitro. *Blood, 1996, 8:2859-2870.*
16. Shimomura T, Yonemura Y, Miyazoe T. Thrombopoietin stimulates murine lineage Negative Sca-1$^+$, c-Kit$^+$, CD34$^-$ cells: comparative study with stem cell factor or interleukin-3.*International Journal of Hematology,* 2000, *71:33-39*
17. Ufelle, Silas A, Ernest O Ukaejiofo, Samuel I Ghasi, Chigozie N Okwuosa, Emeka E Neboh. Myelo-protective activity of aqueous and methanolic leaf extracts of vitex doniana in cyclophosphamide-induced myelo-suppression in wistar rats*. International Journal of Biological and Medical Research, 2011,* 2(1): 409-414.
18. Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health: an ethical analysis; *Bulletin of the World Health Organization*, 2008, 86:594-599.
19. Akah, P.A; Orisakwe, O.E; Gamaniel, K.S and Shittu, A. Evaluation of Nigerian Traditional medicines: Effects of some Nigerian folk remedies on peptic ulcer. *Journal of Ethno pharmacology, 1998, 62: 123-127*
20. Burkill H.M. The useful plants of West Tropical Africa, Families S-z, Addenda. Royal Botanic Gardens, Kew, Richmond, United Kingdom, 2nd Edition, 2000, vol. 5: pp.686.
21. Mbuya, L.P; Msanga, H.P; Rurro, C.K; Birnie, A and Tengnas, B. Useful trees and shrubs for Tanzania: identification, propagation and management for agricultural and pastorial communities. Technical Hand book 6. Regional soil; 1994, PP.10-15.
22. Kilani, A.M. Antibacterial assessment of whole stem bark of *Vitex doniana* against some enterobactriaceae. *African Journal of Biotechnology,* 2006, 5, No (10); 958 – 959
23. Sofwora, A. Medicinal plants and traditional medicine in Africa; Spectrum Books limited. 2nd Edition Sunshine House, Ibadan, Nigeria: Spectrum Book Limited; Screening Plants for Bioactive Agents, 1993, pp. 134 -156.
24. Babalola, E.O. The persistence of African Traditional Medicine in Contemporary Nigeria Society, *African Marburgensia, 1993, 26:4*
25. Ladeji, Olusola; Udoh, F.V; Okoye, Z. S .C. Activity of aqueous Extract of the bark of *vitex doniana* on uterine muscle response to drugs; *Phyto-therapy Research, 2005,* 19: (9); 804- 806.
26. Keay, R.W.J, Onochie, C.F.A, and Stanfield, O.P. NIGERIA TREES. Department of Forest Reserve Ibadan, 1964, Volume 2: 495
27. Ioan, Ciulei. Methodology for Analysis of Vegetable Drugs. Faculty of Pharma, Eucharest Romania, 1984, PP 20-30.
28. Still WC, Kahn M, Mitra A. Chromatography; *Journal of Organic Chemistry*, 1978*, 43(14)*, 2923-2925. doi:10.1021/jo00408a04
29. Alon, T and Amirav, A. "Isotope Abundance Analysis Method and Software for Improved Sample Identification with the Supersonic GC-MS", *Rapid Communications in Mass-Spectrometry, 2006,* 20: 2579–2588.
30. Lorke, D.A. A new approach to practical acute toxicity testing. *Archives in Toxicology*, 1983, 53: 275-289.
31. Chabner, B. Anticancer drugs, In Cancer: Principles and Practice of Oncology; De Vita VT Jr, Hellman S, Rosenberg SA (eds): Philadelphia, PA,Lippincott, 1993, pp 325-417
32. Shanafelt TD, Lin T, Geyer SM. "Pentostatin, cyclophosphamide, and rituximab regimen in older patients with chronic lymphocytic leukemia". *Cancer*, 2007, 109 (11): 2291–2298.
33. 33. Brock, N. Oxazaphosphorine cytostatics: past-present-future. Seventh Cain Memorial Award lecture; *Cancer Research, 1989,* 49 (1): 1–7.
34. Potterat, O. "Antioxidants and free radical scavengers of natural origin; "*Current Organic Chemistry*, 1997, vol. 1 no. 4, pp. 415–440.
35. Soetan, K.O. "Pharmacological and other beneficial effects of anti-Nutritional factors in Plants - a review," *African Journal of Biotechnology*, 2008, vol. 7, no. 25, pp. 4713–4721.
36. Sies, H. "Oxidative stress: oxidants and antioxidants," *Experimental Physiology*, 1997, vol. 82, no. 2, pp. 291–295

37. Wlodarski P, Wasik M, Ratajczak MZ. Role of p53 in hematopoietic recovery after cytotoxic treatment; *Blood, 1998, 91:2998-3006.*
38. Toefili L, Rutella S, Putzulu R. *Expression of p53, Bcl-2, and Bax in Cd34+ cells recovering after chemotherapy. Blood, 1998, 92[letter]:4880-4881.*
39. Hostettmann, K. and Marston, A. *Saponins*. Cambridge: Cambridge University Press, 1995, PP. 3 ISBN 0-521-32970-1. OCLC 29670810
40. Riguera, Ricardo. "Isolating bioactive compounds from marine organisms". *Journal of Marine Biotechnology* 1997, 5 (4): 187–193. http://www.springerlink.com/content/m9ccl rm1y0e5ge5/
41. Asl Marjan Nassiri and Hossein Hosseinzadeh. "Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds". *Phytotherapy Research*, 2008, 22 (6): 709– 724.
42. Bajaj, Y. P. S. *Medicinal and aromatic plants*. Biotechnology in Agriculture and Forestry, 24. Berlin: Springer-Verlag. ISBN, 1988, 0-387-56008-4
43. Lu L Liu SW, Jiang SB, Wu SG. "Tannin inhibits HIV-1 entry by targeting gp41". *ACTA Pharmacol. Sin.* 2004, 25 (2): 213–218.
44. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. "Antibacterial action of several tannins against Staphylococcus aureus". *Journal of Antimicrobial Chemotherapy, 2001,* 48 (4): 487– 491
45. Kolodziej H and Kiderlen AF. "Antileishmanial activity and immune modulatory affects of tannins and related compounds on Leishmania parasitised RAW 264.7 cells" *Phytochemistry*, 2005, 66 (17): 2056–2071.
46. Souza, S. M. C.; Aquino, LC; Milach Jr, AC; Bandeira, MA; Nobre, ME; Viana, GS. "Antiinflammatory and antiulcer properties of tannins from Myracrodruon urundeuva Allemão (Anacardiaceae) in Rodents" *Phytotherapy Research* (John Wiley & Sons), 2006, 21 (3): 220–225.
47. Ladeji, Olusola; Udoh, F.V; Okoye, Z. S .C. Activity of aqueous Extract of the bark of *vitex doniana* on uterine muscle response to drugs; *Phytotherapy Research, 2005,* 19: (9); 804- 806.

Table 1

Gas chromatography-mass spectrometry of Aqueous Fractions (AF) and Methanol Fractions (MF) of *V.doniana* leaves extracts.

AF1	AF2	AF3	AF4	MF1	MF2
1.Compound Name: 9-Tetradeceiial, (Z) - S$ (Z)-9-Tetradecenal $$ Z-9-Tetradecenal $$ Z-9-Teadecenol $$ (9Z)-9-Tetradecenal # Z-9-Tetadecenal $$ **Formula:** C I 4H260 CAS: 53939-27-8 **Molecular Weight: 210** **2.Compound Name**: Octadecanioc acid SS Stearic acid n-Octadecanoic acid Humko Industrene R SS Hydrofol Acid 150 SS Hystrene S-97Hysrene T-70 **Formula**: CI 8H3602 CAS: 57- I 4 **Molecular Weight: 284** **3.Compound Name**: Octadecanoic acid $$ Stearic acid $S n-Octadecanoic acid $$ Humko 1ndustrene R $$ Hydrofol Acid 50 S$ Hystiene 5-97 $$ Hystrene T-7 **Formula:** C18H3602 CAS: 57-I -4 **Molecular Weight: 284** **4.Compound Name**: Cyclopentadecanone, 2-hydroxy- $S 2 Hydroxycyclopentadecanone # $$ **Formula:** C15H28O2	**1.Compound Name**: cis-9-Hexadecena S$ 9-Hexadecetial, (Z) - $S (Z)-9-Hexadecenal $$ Z-9-Hexadecenal $$ (9Z)-9-Hexadecenal # $$ **Forrnula**: Cl 6H300 CAS: 562 19-04-6 **Molecular Weight: 238** **2.Compound Name**: 9-Octadecenoic acid, I, 2, 3-propanetriyl ester, (E, E, E) - $$ 2, 3-Bis [(9E)-9-octadecenoyloxy) propyl (9E)-9-octadecenoate # $ **Formula:** C57H 10406 CAS: 537-39-3 **Molecular Weight: 884** **3.Compound Name**: cis-9-Hexadecenal $$ 9-1-lexadecenal, (Z) - $ (Z)-9-Hexadecenal $ Z-9-Hexadecenal $$ (9Z)-9-Hexadecenal # $ **Formula**: C I 6H300 CAS: 562 19-04-6 **Molecular Weight: 238** **4.Compound Name**: Oleic Acid S 9-Octadecenoic acid (Z) - $$ delta (Sup9)-cis-Oleic acid $$ cis-.dJta. (Sup9)-Octadecenoic acid S$ cis-Oleic Acid $$	**1.Compound Name**: 8-Hexadecenal, 14 methyl-, (Z)- $ 14-Methyl-8-hexadecenal Z (8Z)-14-Methyl-8-hexadecenal # S$ **Formula**: C I 7H320 CAS: 6O6O953-2 **Molecular Weight: 252** **2.Compound Name**: cis-9-Hexadecenal S$ 9-Hexadecena [, (Z) - $$ (Z)-9-Hexadecenai $$ Z-9-Hexadecenal $$ (9Z)-9-Headecenal # $$ **Formula:** C16H300 CAS: 56219-04-6 **Molecular Weight: 238** **3.Compound Name**: cis-9-Hexadecenal S 9-Hexadecenal, (Z) - $$ (Z)-9-Hexadecen S$ Z-9-Hexadecenal S (9Z)-9-Hexadecenal **Formula**: C' I 6H300 CAS: 562I 9-04-6 **Molecular Weight: 238** **4.Compound Name**: 9-Octadecenoic acid (Z)-, 2-hydroxy-l-(hydro<ymethyl) ethyl esEer $$ Oleiii, 2-mono- $.beta.-Monoolein $S Gycerol 2-monooleate $$ 2-Moii **Formula**: C21H4004 CAS: 3443-84-3 **Molecular Weight: 356** **5. Compound Name**:	**1.Compound Name:** 8-Hexadecenal, 14-methyl-, (Z)- $ 14-Methyl-8-hexadecenal Z (8Z)-14-Methyl-8-hexadecenal # S$ **Formula:** C I 7H320 CAS: 6O6O953-2 **Molecular Weight: 252** **2.Compound Name**: cis-9-Hexadecenal S$ 9-Hexadecena [, (Z) - $$ (Z)-9-Hexadecenai $$ Z-9-Hexadecenal $$ (9Z)-9-Headecenal # $$ **Formula**: C16H300 CAS: 56219-04-6 **Molecular Weight: 238** **3.Compound Name**: cis-9-Hexadecenal S 9-Hexadecenal, (Z) - $$ (Z)-9-Hexadecen S$ Z-9-Hexadecenal S (9Z)-9-Hexadecenal **Formula**: C' I 6H300 CAS: 562I 9-04-6 **Molecular Weight: 238** **4.Compound Name**: 9-Octadecenoic acid (Z)-, 2-hydroxy-l-(hydro<ymethyl) ethyl esEer $$ Oleiii, 2-mono-$.beta.-Monoolein $S Gycerol 2-monooleate $$ 2-Moii	**1.Compound Name**: Octadecanoic acid $$ Stearic acid $$ n-Octadecanoic acid $$ Humko Industrene R $$ Hydrofol Acid 150 S$ Hystrene S-97 $ Hystrene T-70 $ **Formula:** C 1 8H3602 CAS: 57-1 1-4 **Molecular Weight: 284** **2.Compound Name**: Cyclopenradecanone, 2-hydroxy- $$ 2-Hydroxycyclopentadecanone # $ **Formula**: C1 5H2802 CAS: 4727-l 8-8 **Molecular Weight: 240** **3.Compound Name**: 7-Tetiadecenal, (Z) - $$ Z-7-Tetradecenal $ (7Z)-7-Tetradecenal # **Formula**: C'1 4H260 CAS: 651 28-96-3 **Molecular Weight; 2 10** **4.Compound Name**: Octadecanoic acid $$ Stearic acid $S n-Octadecanoic acid $$ Humko Industrene R $$ Hydrolol Acid 150 $ Hystrene S-97 S$ Hystrene T-70 **Formula**: C I 8H3602 CAS: 57- I 1-4 **Molecular Weight: 284** **5.Compound Name**: 7 -Tetradecenal, (Z) - $$ Z-7 -Tetradecenal $S (7 Z)-7 -Tetradecenal # $$ Formula: Q1 4H260	**1.Compound Name**: n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid $ Pentadecanecauboxylic acid £$ I – Pentadecanecarboxylic **Formula**: CI 6H3202 CAS: 57-1 0-3 **Molecular Weight: 256** **2.Compound Name**: 6-Ocradecenoic acid, (Z) - $$ (6Z)-O-Octadecenoic acid # **Formula**: C I 8H3402 CAS: 593-39-5 **Molecular Weight: 282** **3.Compound Name**: 9, 12-Octadecadienoyl chloride, (Z, Z) - $$ Linoleoyl chloride $$ Lineoleoyl chloride $ Linoleic acid chloride $$ (9El2E)-9, l2-Octadecadienoyl **Formula:** Cl 8H3 I ClO CAS: 7459-33-8 **Molecular Weight: 298** **4.Compound Name**: II -Octadecenoic acid, methyl ester, (Z)- $$ cis-I I -Octadecenoic acid methyl ester $$ Methyl cis-octadec- I I -enoate S$ cis-Vaccenic acid methyl **Formula**: Cl9136O2 CAS: 1937-63-9 **Molecular Weight: 296** **5.Compound Name**: 9-Octadecenoic acid, 1, 2, 3-propanetriyl ester, (E, E, E) - $$ 2, 3-Bis [(9E)-9-octadecenoyloxy] propyl (9E)-9-octaciecenoate # $ **Formula:** C57H 10406

CAS: 4727-18-8 **Molecular Weight 240** **5.Compound Name**: Octadecanoic acid S$ Stearic acid n-Octadecanoic acid S$ Humko lndustrene R S Hydrolbi Acid ISO $ Hysirene S-07 Hystrene T-7O **Formula**. C 8H3602 CAS: 57- 1-4 **Molecular weight: 284**	cis-9-Octad **Formula**: C1 8H3402 CAS: 112-80-1 **Molecular Weight: 282**	Oleic Acid S$ 9-Octadecenoic acid (Z) - $S .delta. (Sup9)-Cis-Oleic acid $$ cis-.delta. (Sup9)-Octadecenoic acid S$ cis-Oleic Acid S$ cis-9-Octad93 **Formula**: C1 8H3402 CAS: 112-80-1 **Molecular Weight: 282**	**Formula**: C21H4004 CAS: 3443-84-3 **Molecular Weight: 356** **5. Compound Name**: Oleic Acid S$ 9-Octadecenoic acid (Z) - $S .delta. (Sup9)-Cis-Oleic acid $$ cis-.delta. (Sup9)-Octadecenoic acid S$ cis-Oleic Acid S$ cis-9-Octad93 **Formula:** C1 8H3402 CAS: 112-80-1 **Molecular Weight: 282**	CAS: 65 128-96-3 **Molecular Weight: 2 10**	CAS: 537-39-3 **Molecular Weight: 884** **6.Compound Name**: Hexadecanoic acid, 1 - [(2-aminoethoxy) hydroxyphosphinyl] oxyjmethy)]-1, 2-elhanediyl ester $$ Palmitin, I, 2-di-, 2-aminoethyl hydrogen phos **Formula**: C37H74N08P CAS: 3026-45-7 **Molecular Weight: 691**

Table 2

Phytochemical analysis of the fractions of *Vitex doniana* Sweet leaves Extracts

Constitutents/fraction	AF1	AF2	AF3	AF4	MF1	MF2
Flavonoids	+++	+	++	+	++	+
Antraquinone Glycosides	-	-	-	-	-	-
Anthracene glycosides	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-
Saponins	++	+	+	+	+	-
Tannins	++	-	-	-	+	-
Resins	-	-	-	-	-	-
Proteins	++	+	+	+	+	-
Carbohydrate	-	-	-	-	+	-
Reducing sugars	+	-	+	-	+	+
Hydrolysis test for Glycosides	-	-	-	-	-	-
Cyano-genetic Glycosides	-	-	-	-	-	-
Fat and oils	-	-	-	-	-	-
Steroids	++	+	+	+	++	+
Terpenoids	-	-	-	-	+	-
Acidic compounds	neutral	neutral	neutral	neutral	neutral	neutral
Cardiac glycoside	-	-	-	-	-	-

Key: - Absent, + Present, ++ moderately present, +++ abundantly present

Table 3

The mean ±SD of haematological parameters of Cyclophosphamide induced myelo-suppressed Wistar rats orally administered with various aqueous fractionated extract components of *Vitex doniana* Sweet Leaves and control.

Fractions/ Variables	AF1	AF2	AF3	AF4	Control
Haemoglobin (g/dL)	11.85 ±0.5	7.5 ±0.62	13.9 ±2*	9.70 ±0.62	11.5 ±0.42
Haematocrit (L/L)	0.35±0.01	0.22 ±0.01	0.42±0.01*	0.29 ±0.01	0.34±0.01
RBC ($x10^{12}$/l)	5.50 ±0.85	3.05 ±0.2*	7.03 ±0.5*	4.57 ±0.22	5.73±1.5
MCV (Fl)	60.00±0.83	63.46 ±2.2	59.74±2.04	63.46 ±2.2	59.34±0.25
MCH (Pg)	21.36±0.55	21.23 ±0.7	19.77±0.45	21.23 ±0.7	20.07±0.45
MCHC (g/l)	35.61±1.12	33.45±0.53	33.10±0.43	33.45±0.53	33.82±1.50
Reticulocyte (%)	1.2±0.02	2.45±0.02	1.6±0.50	2.26±0.02	1.5±0.52
Total WBC($\times 10^9$/L)	5.80 ±0.4	2.35 ±0.5	6.8 ±1.5	2.35 ±0.4	5.5 ±0.3
Platelet ($\times 10^9$/L)	128 ± 4	105 ± 10	135 ± 2	122 ± 10	130 ± 5
Neutrophil (%)	58±2	35 ± 3	67 ± 2	40 ± 3	56 ± 3
Lymphocyte (%)	39±3	62 ± 2	29 ± 0.8	57 ± 2	40 ± 2
Monocyte (%)	2 ± 0.5	1 ± 0.5	2 ± 0.5	1 ± 0.5	2 ± 1
Eosinophil (%)	1± 0.5	2 ± 1	2 ± 0.5	2 ± 1	2 ± 0.5

Key: * p<0.05, ** p<0.001 (Significant)

Table 4

The mean ±SD of haematological parameters of Cyclophosphamide induced myelo-suppressed Wistar rats orally administered with methanol fractionated extract components of *Vitex doniana* Sweet leaves and control.

Fractions Variables	MF1-administered Rats	MF2-administered Rats	Control Rats
Haemoglobin (g/dL)	13.8 ± 1.5*	10.5 ± 0.85	11.5 ± 0.42
Haematocrit (L/L)	0.40 ± 0.02*	0.31 ± 0.02	0.34±0.08
RBC ($x10^{12}$/l)	6.88 ± 0.72*	4.90 ± 0.65	5.73± 1.50
MCV (Fl)	58.14 ±1.04	52.54 ±1.85	59.34± 0.25
MCH (Pg)	20.06 ± 0.87	17.80 ±0.76	20.07± 0.45
MCHC (g/l)	34.50 ±1.43	33.87 ±0.50	33.82± 1.50
Reticulocyte (%)	0.8 ±0.05*	2.5±0.3	2.5± 0.5
Total WBC ($\times 10^9$/L)	6.2 ± 0.5	3.5 ± 0.4	5.8 ± 0.3
Platelet ($\times 10^9$/L)	156 ± 15	145 ± 10	130 ± 5
Neutrophil (%)	65 ± 2	38 ± 3*	60 ± 3
Lymphocyte (%)	32 ± 3	59 ± 2*	37 ± 2
Monocyte (%)	2 ± 0.5	1 ± 0.5	2 ± 1
Eosinophil (%)	1 ± 0.33	2 ± 0.5	1 ± 0.5

Key: * p<0.05, ** p<0.001 (Significant)

Table 5

The mean ± SD of Bone Marrow cell count of Cyclophosphamide induced myelo-suppressed Wistar rats orally administered with fractionated extracts and control.

Cellularity	AF1	AF2	AF3	AF4	MF1	MF2	Control
Day 15 $x10^6$/femur	15.30±0.8*	3.10±0.9**	14.50±1.5*	9.20 ±1.1*	13.80±1*	10.50±1.2	10.50±1.5

Key: * $p<0.05$, ** $p<0.001$ (Significant)