



Antioxidant, Brine Shrimp Lethality, and Antiproliferative Properties of Gel and Leaf Extracts of *Aloe schweinfurthii* and *Aloe vera*

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ABSTRACT

Leaf and gel extracts of *Aloe schweinfurthii* and *A. vera* were subjected to *in vitro* antioxidant assay using 2,2-diphenyl-1-picryl hydrazyl (DPPH), brine shrimp lethality bioassay, and cytotoxicity using the MTT assay with two human cancer cell lines: Rd and Hep-2c. Extracts of *A. schweinfurthii* gel had IC₅₀ values of 44.59; *A. vera* gel, 41.48, and *A. vera* leaf, 38.84 $\mu\text{g.mL}^{-1}$, had similar DPPH radical-scavenging properties and were more active than *A. schweinfurthii* leaf. Ascorbic acid had an IC₅₀ of $9.26 \pm 0.14 \mu\text{g.mL}^{-1}$. *Aloe vera* leaf (LC₅₀ = $325 \pm 5.38 \mu\text{g.mL}^{-1}$) was more active than the other three extracts in the BSL assay. The gel extracts of *A. schweinfurthii* (CC₅₀ = $4.06 \mu\text{g.mL}^{-1}$ on Rd and $9.00 \mu\text{g.mL}^{-1}$ on Hep-2c) and *A. vera* (CC₅₀ = $4.31 \mu\text{g.mL}^{-1}$ on Rd, $9.06 \mu\text{g.mL}^{-1}$ on Hep-2c) elicited similar and more potent cytotoxicity comparable to cyclophosphamide, with CC₅₀ = $2.2 \mu\text{g.mL}^{-1}$ on Rd and $2.66 \mu\text{g.mL}^{-1}$ on Hep-2c. Leaf extracts were less active than gel extracts. This study showed that *A. schweinfurthii* gel, *A. vera* gel and leaf had weak DPPH activity which were similar. *Aloe vera* and *A. schweinfurthii* did not elicit potent cytotoxicity in BSL assay. The gels from both *Aloe* species displayed antiproliferative activity on Rd and Hep-2c, two human cell cancer lines.

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Introduction

Globally, cancer-related deaths in the year 2008 were 7.6 million. It is projected that this toll will increase in developing countries, especially in Africa and Asia (10). Approximately 100,000 cases of cancer are reported in Nigeria annually (6).

Humans have depended on plants for food, shelter, clothing, and medicines since antiquity (18). It is well known that over 80% of Africans rely on plant-based medicines for their primary health-care needs, more so because

of the rising cost of modern medicines and their associated side effects (4, 21). The successful use of medicinal plants and products has not only revolutionized modern medicine, it has provided a reliable channel for new medicines. Over 60% of the approved anticancer and 75% anti-infective agents currently in use are derived from plants (2, 3, 5).

Aloe schweinfurthii (Xanthorrhoeaceae) is closely related to *A. vera*. *Aloe* sp. are locally called *Etierin* (Yoruba). *A. vera*, unlike *A. schweinfurthii*, has been studied extensively. *A. schweinfurthii* (commonly referred to as West African *Aloe vera*) is a stemless, succulent, evergreen, perennial flowering plant. It forms a compact rosette of fleshy green leaves with sharply dentate margins. When mature, the leaf can grow up to 60 cm long and often 7–8 cm wide, while the stem can be up to 1 m tall. Traditionally, *A. schweinfurthii* has been used in Nigeria, Burkina Faso, and other West African countries in the treatment of intestinal and uro-genital problems, cough, venereal diseases, infertility in women, rheumatism, and skin infections. In West Africa, the decoctions of the root and leaf are taken to treat liver problems, especially jaundice. It is also used as a laxative, to treat stomachache and for internal parasites (20). The aim of the study is to determine and compare the DPPH radical scavenging, brine shrimp lethality, and antiproliferative properties of *A. schweinfurthii* and *A. vera* gel and leaf extracts.

Materials and methods

Plant collection and authentication

The whole plant of *A. schweinfurthii* was collected in December 2014 from Idanre hill, Ondo State, Nigeria. It was identified and authenticated at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan; *A. vera* whole plant was collected in January 2015 from an *A. vera* garden at Ilorin, identified and authenticated at the Department of Plant Biology Herbarium, University of Ilorin, Ilorin. Voucher samples of *A. schweinfurthii* (FHI/110105) and *A. vera* (UILH/001/1058) were deposited at the herbarium.

Plant preparation and extraction

The leaves of *A. schweinfurthii* were washed, weighed, and cut longitudinally open at the middle with the aid of a clean knife; the gel was obtained by scratching and scooping from the green rind/leaf. The gel was then homogenized in a blender and filtered through a muslin cloth. The filtrate was freeze-dried, the solid mass obtained, weighed, and refrigerated at -4°C until analysis. The resulting green rind/leaves were collected and air-dried under shade and extracted into distilled methanol by maceration at room temperature (RT; $25\text{--}33^{\circ}\text{C}$). The extract was concentrated *in vacuo*, weighed, and refrigerated at -4°C until needed. The same procedure was repeated with mature *A. vera* plant.

Biological assays

In vitro DPPH radical-scavenging assay

The abilities of the four extracts from both plants to scavenge 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radicals were evaluated (16). The extracts (2 mg each) were dissolved in distilled methanol to achieve the concentration of 1000 $\mu\text{g.mL}^{-1}$, and serial dilutions of five concentrations from (6.25 to 200 $\mu\text{g.mL}^{-1}$) were made in a 96-well plate by diluting the extracts solutions (100 μL each) with 150 μL of a solution of DPPH (0.04 $\mu\text{g.mL}^{-1}$) in methanol. The negative control wells contained only DPPH and the solvent (methanol). Ascorbic acid was used as positive control. The microplate was incubated in the dark at RT (25°C) for 30 min. The absorbance of the resulting solution was measured at 517 nm using a UV spectrophotometer (Paul Bucher Analytik und Biotechnologie, Switzerland). The experiment was carried out in three replicates. The results were expressed as percentage inhibition and calculated by the formula

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

where A = the mean optical density of control wells

B = the mean optical density of the wells with plant extracts

Brine shrimp lethality (BSL) assay

Brine shrimp (*Artemia salina*) eggs (obtained from one of us, EOA) were hatched in natural seawater obtained from the Bar Beach, Ikoyi, Lagos, and incubated for 48 h in 3.8 g.L^{-1} seawater. The BSL assay was conducted as described earlier with some modifications (11). The extracts (10 mg each) were diluted to 1000 $\mu\text{g.mL}^{-1}$ by adding the seawater (water-insoluble compounds were dissolved in 50 μL of dimethyl sulfoxide (DMSO) prior to the addition of seawater). Serial dilutions of the extracts were made in 96-well microplates in triplicates. Negative control wells contained 0.5% DMSO in seawater. A 250- μL suspension of nauplii in the extract was added to each well. The plates were incubated at RT (25°C) for 24 h. The number of dead nauplii in each well was counted. The lethality of the extracts on the brine shrimp was classified as follows (14): $\text{LC}_{50} \geq 1000 \mu\text{g.mL}^{-1}$ was “nontoxic,” $\text{LC}_{50} = 500\text{--}1000 \mu\text{g.mL}^{-1}$ was “weakly toxic,” $\text{LC}_{50} = 100\text{--}500 \mu\text{g.mL}^{-1}$ was “moderately toxic,” and $\text{LC}_{50} \leq 100 \mu\text{g.mL}^{-1}$ was “strongly toxic.”

Cytotoxicity assay

Human cancer and normal cell lines culture

Cancer cell lines were obtained from the Centers for Disease Control (CDC), Atlanta, GA, USA and maintained in the WHO Polio Laboratory,

Department of Virology, University of Ibadan, Nigeria. Two human cancer cell lines, Rhabdomyosarcoma (Rd) and laryngeal carcinoma (Hep-2c), and a normal cell line (Vero) from African green monkey kidney were used. These cells were grown in minimum essential medium (MEM), all supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, and 1% penicillin-streptomycin (the antibiotic combination was used to prevent bacterial contamination of cell cultures due to either Gram-positive or Gram-negative bacteria). The MEM was also supplemented with 0.01 mg.mL⁻¹ insulin and 1 mM sodium pyruvate. Cells were incubated in a 5% CO₂ humidified incubator at 37°C and passaged bi-weekly.

The MTT colorimetric assay

The cytotoxicities of the four extracts were determined using the MTT assay (15). Each of the crude extracts was dissolved in DMSO to give a concentration of 10 mg.mL⁻¹. The stock (0.1 mL) was added to 0.9 mL of maintenance medium to obtain a dilution of 1000 µg.mL⁻¹. This was designated as “neat.” Serial 10-fold dilutions of the extracts were made from the “neat” using maintenance medium as diluent to obtain different concentrations. Then 50 µL of each extract dilution was dispensed into 96-well microtiter plates already seeded with a monolayer of Rd, Hep-2c, or Vero cells. The plates were incubated at 37°C in a CO₂ environment and the cells observed microscopically after 72 h.

Supernatants were removed from all wells after 72 h incubation. Then, 25 µL of 2 mg.mL⁻¹ MTT solution in phosphate-buffered saline (PBS) was added to each well and the plates were incubated for an additional 2 h at 37°C. Thereafter 125 µL of DMSO was added into the wells to solubilize the formed formazan crystals. The plates were placed on a shaker for 15 min and absorbance was read at 492 nm on a multiwell spectrophotometer (Paul Bucher Analytik und Biotechnologie, Switzerland). Experiments were done in triplicate.

The percentage cytotoxicity was calculated as

$$\% \text{ cytotoxicity} = \frac{(A - B)}{A} \times 100$$

where A = the mean optical density of untreated cells

B = the mean optical density of cells treated with plant extracts/fractions

The selective indices (SI) of the four extracts and cyclophosphamide were estimated using Vero cells. Data were expressed as the means $\pm$ SEM. Selective index (SI) > 1 is indicative of selectivity.

$$\text{Selective index (SI)} = \frac{\text{CC}_{50} \text{ on Vero cells}}{\text{CC}_{50} \text{ on cancer cells}}$$

Data analysis

Data obtained from the three experiments was analyzed using the Graphpad prism computer program. The concentration with 50% DPPH radical scavenging (IC_{50}), 50% lethality (LC_{50}), or 50% cytotoxicity (CC_{50}) was calculated from a dose–response inhibition curve using nonlinear regression data analysis, expressed as mean $\pm$ SEM of three replicates, and subjected to student's t test ($P \leq 0.05$).

Results

The yield of the leaf extracts was greater (0.91%) for *A. schweinfurthii* than for *A. vera* (0.69%), while the yield of the gel extracts of *A. schweinfurthii* was lower (0.19%) than for *A. vera* (0.24%) (Table 1).

A. schweinfurthii gel ($IC_{50} = 44.59 \pm 0.13 \mu\text{g.mL}^{-1}$), *A. vera* gel ($IC_{50} = 41.48 \pm 0.11 \mu\text{g.mL}^{-1}$), and *A. vera* leaf ($IC_{50} = 38.84 \pm 0.26 \mu\text{g.mL}^{-1}$) had comparable DPPH radical-scavenging properties, unlike the leaf extracts of *A. schweinfurthii* ($IC_{50} = 80.77 \pm 2.15 \mu\text{g.mL}^{-1}$) which had a weaker DPPH radical-scavenging property (Table 1).

The extracts of *A. schweinfurthii* gel ($LC_{50} = 755 \pm 7.41 \mu\text{g.mL}^{-1}$), *A. vera* gel ($LC_{50} = 880 \pm 9.14 \mu\text{g.mL}^{-1}$), and *A. schweinfurthii* leaf ($LC_{50} = 730 \pm 10.20 \mu\text{g.mL}^{-1}$) were “weakly toxic.” *A. vera* leaf extract was “moderately toxic” ($LC_{50} = 325 \pm 5.38 \mu\text{g.mL}^{-1}$). It was over 200% more toxic compared to the three other extracts. The cyclophosphamide had LC_{50} of 98.76 ± 0.15 .

The MTT colorimetric cytotoxicity assay using cancer cell lines exhibited a concentration-dependent cytotoxicity of the extracts (Table 1). The gel extracts of *A. schweinfurthii* ($CC_{50} = 4.06 \pm 3.03 \mu\text{g.mL}^{-1}$) and *A. vera* ($CC_{50} = 4.31 \pm 1.76 \mu\text{g.mL}^{-1}$) had similar cytotoxicity on the Rd. The gel extracts of *A. schweinfurthii* ($CC_{50} = 9.00 \pm 0.06 \mu\text{g.mL}^{-1}$) and *A. vera* ($CC_{50} = 9.06 \pm 2.0 \mu\text{g.mL}^{-1}$) showed similar cytotoxicity on Hep-2c as well. *A. vera* leaf extract displayed higher cytotoxicity on the Rd and Hep-2c cancer cell lines than *A. schweinfurthii* leaf extract (Table 1). *A. schweinfurthii* and *A. vera* gels had SI of 3.45 ± 0.2 and 2.09 ± 0.19 , respectively, on Rd, and *A. schweinfurthii* (SI = 1.89 ± 0.18) had some selectivity for the Hep-2c cancer cell line. *A. vera* gel (SI = 0.9 ± 0.13) and leaf extract (SI = 0.40 ± 0.11) were not selectively toxic to the Hep-2c cancer cells. Cyclophosphamide had SI of 3.91 ± 0.06 and 3.27 ± 0.05 on Rd and Hep-2c, respectively.

Discussion

Aloe gels had lower yields than the leaf extracts. *A. vera* is known to contain about 99% water (19). The gel extracts of *A. schweinfurthii* and *A. vera* had similar DPPH radical-scavenging activity. On the other hand, the leaf extract

Table 1. Antioxidant activity, brine shrimp lethality (BSL), and cytotoxicity activities on Rd and Hep-2c cancer cell lines of *Aloe vera* and *Aloe schweinfurthii* leaf and gel extracts.

Extracts	Percentage (%)	Yield (w/w)	IC ₅₀ (µg.mL ⁻¹)	LC ₅₀ (µg.mL ⁻¹)	CC ₅₀ (µg.mL ⁻¹)			Selective index (SI)	
					Rd	Hep-2c	Vero	Rd	Hep-2c
<i>A. schweinfurthii</i> (G)	0.19		*44.59 ± 0.13	*755 ± 7.41	4.06 ± 3.03	*9.00 ± 0.6	14.00 ± 2.7	3.45 ± 0.2	1.89 ± 0.18
<i>A. vera</i> (G)	0.24		*41.48 ± 1.26	*880 ± 9.14	4.31 ± 1.76	9.06 ± 2.0	9.01 ± 2.3	2.09 ± 0.19	*0.9 ± 0.13
<i>A. schweinfurthii</i> (L)	0.91		*80.77 ± 2.15	*730 ± 10.20	*72.52 ± 6.38	*73.27 ± 3.4	*5.02 ± 0.3	*0.07 ± 0.02	*0.07 ± 0.02
<i>A. vera</i> (L)	0.69		*38.84 ± 0.26	*325 ± 5.38	17.52 ± 4.53	*17.41 ± 0.7	7.02 ± 2.9	*0.41 ± 0.13	*0.40 ± 0.11
Standard drug			9.26 ± 0.14	98.76 ± 0.15	2.23 ± 0.14	2.66 ± 0.8	8.05 ± 0.6	3.91 ± 0.06	3.27 ± 0.05

Note. G = gel and L = leaves, * = significant at (*P* < 0.05), IC₅₀ = 50% inhibitory concentration in DPPH activity, LC₅₀ = 50% lethality in concentration in BSL ASSAY, CC₅₀ = 50% cytotoxic concentration in MTT assay.

of *A. vera* had better DPPH scavenging properties compared to the *A. schweinfurthii* leaf extract. All four extracts had poor DPPH radical-scavenging activities compared to ascorbic acid with an IC_{50} of $9.26 \pm 0.14 \mu\text{g.mL}^{-1}$. The DPPH scavenging property of the leaf and gel of *A. vera* obtained in this study ($IC_{50} = 38.84$ and $41.48 \mu\text{g.mL}^{-1}$, respectively) are comparable with previous report, where *A. vera* whole plant had DPPH radical-scavenging activity with IC_{50} of $58.36 \mu\text{g.mL}^{-1}$ compared to ascorbic acid with $IC_{50} = 11.48 \mu\text{g.mL}^{-1}$ (9). The antioxidant activity of *A. vera* has been attributed to the presence of antioxidant polyphenols (22). The nonflavonoid polyphenols in *A. vera* account for the majority of its total polyphenolic content (12).

The BSL model has been helpful as a preliminary screening model in the drug design and synthesis of cytotoxic compounds (13). *A. schweinfurthii* gel extract was slightly more cytotoxic on the brine shrimp than *A. vera* gel extract, while the *A. vera* leaf extract exhibited higher brine shrimp lethality than the *A. schweinfurthii* leaf extract. The gel and leaf extracts of both *Aloe* species were less toxic compared to cyclophosphamide ($LC_{50} = 98.76 \pm 0.15 \mu\text{g.mL}^{-1}$). Nevertheless, the leaf extracts of *A. vera* and *A. schweinfurthii* appear to be relatively more toxic compared to the gel, and this could suggest why the gels are often used in medicinal practice. The LC_{50} of *A. vera* gel ($880 \mu\text{g.mL}^{-1}$) extract in this study was comparable to a previous report, where the *A. vera* gel was moderately toxic with LC_{50} of $1443 \mu\text{g.mL}^{-1}$ (7).

The gel and leaf extracts of both *Aloe* sp. demonstrated similar cytotoxicity on the two human cancer cell lines, however, the gel extracts of both *Aloe* sp. were more cytotoxic than the leaf extracts. The cytotoxicity of *A. schweinfurthii* gel, *A. vera* gel and leaf extracts on the Rd human cancer cell line were comparable to the positive control/cyclophosphamide ($CC_{50} = 2.23 \pm 0.14 \mu\text{g.mL}^{-1}$). The results of this study are in agreement with an earlier report on cytotoxicity of lyophilized *A. vera* extract ($CC_{50} = 10.45 \pm 0.31 \mu\text{g.mL}^{-1}$) on human hepatocellular carcinoma (Hep-G2) cell line (17). Another study reported that *A. vera* gel had its best cytotoxicity on B16F10 melanoma human cancer cell line at $300 \mu\text{g.mL}^{-1}$ (1). Flavonoids isolated from *A. vera* have also been reported to have cytotoxic activity against a human breast carcinoma cell line, MCF-7, with IC_{50} of $54.97 \pm 0.36 \mu\text{g.mL}^{-1}$ (8).

The major challenge of cancer chemotherapy and novel drug development is the lack of selectivity of a large number of such drugs on noncancerous cells. The normal African green monkey kidney epithelial cell line (Vero) was included in this study to evaluate selectivity. *A. schweinfurthii* gel, *A. vera* gel, *A. vera* leaf extracts, and cyclophosphamide exhibited similar cytotoxicity on the normal cells (Vero). *A. schweinfurthii* leaf extract was more toxic to the normal cell (Vero) than the standard drug (Table 1). Of the extracts evaluated for cytotoxicity using human cancer cell lines, *A. schweinfurthii* gel appears to be safer on normal cells compared to cyclophosphamide, with $CC_{50} = 14.00 \pm 2.7 \mu\text{g.mL}^{-1}$ and $8.00 \pm 0.6 \mu\text{g.mL}^{-1}$, respectively. The safety

of the extracts and reference drug was evaluated by their selective indices (SI) using the Vero cells (normal cell line). Any component with $SI > 1$ when cancer cells are compared to normal cells is considered selective for cancer cells. *A. schweinfurthii* and *A. vera* leaf extract lacked selectivity ($SI = 0.07 \pm 0.02$ for Rd and 0.07 ± 0.02 for Hep-2c) on the two cancer cell lines. *A. vera* gel was also not selectively toxic to Hep-2c ($SI = 0.90 \pm 0.13$). These three extracts with lack of selectivity to the cancer cells may be regarded as unfit for chemotherapy of cancer. It is noteworthy that the standard cyclophosphamide used in cancer chemotherapy displayed the highest selectivity.

Abbreviations

DPPH 2,2-diphenyl-1-picryl hydrazyl radical MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide IC_{50} , LC_{50} , CC_{50} inhibitory, lethal and cytotoxic concentrations at 50%, respectively

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References

1. Chandu, A. N., S. C. Kumar, C. Bhattacharjee, and S. Debnath. 2012. Cytotoxicity study of plant *Aloe vera* (Linn). *Chron. Young Sci.* 3(3):233–235.
2. Cragg, G. M., and D. J. Newman. 2005. Plants as a source of anti-cancer agents. *J. Ethnopharmacol.* 100:72–99.
3. David, J. N., and M. C. Gordon. 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.* 75(3):311–335.
4. Ekor, M. 2014. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Front. Pharmacol.* 4(177):1–10.
5. Elujoba, A. A., O. M. Odeleye, and C. M. Ogunyemi. 2005. Traditional medical development for medical and dental primary health care delivery system in Africa. *Afri. J. Trad. Compl. Alt. Med.* 2(1):46–61.
6. Ferlay, S. H. R., and F. Bray. 2010. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer* 127:2893–2917.
7. Ian, E. C., and R. David. 2008. High performance liquid chromatographic separation and identification of a toxic fraction from *Aloe barbadensis* Miller leaf gel using the *Artemianauplii* bioassay. *Int. J. Toxicol.* 4(2):1–13.
8. Joby, J., S. Sudheesh, K. T. M. Sumesh, J. E. Sony, and V. Jayadevi. 2014. A comparative evaluation of anticancer activities of flavonoids isolated from *Mimosa pudica*, *Aloe vera* and *Phyllanthus niruri* against human breast carcinoma cell line (MCF-7) using MTT assay. *Int. J. Pharm. Pharmaceut. Sci.* 6(2):319–322.
9. Khaing, T. A. 2011. Evaluation of the antifungal and antioxidant activities of the leaf extract of *Aloe vera* (*Aloe barbadensis* Miller). *World Acad. Sci. Eng. Technol.* 54:610–612.

10. Lyerly, H. K., A. P. Abernethy, M. R. Stockler, B. Koczwara, Z. Aziz, and R. Nair. 2010. Need for global partnership in cancer care: Perceptions of cancer care researchers attending the 2010 Australia and Asia Pacific Clinical Oncology Research Development Workshop. *J. Oncol. Pract.* 7:324–329.
11. McLaughlin, J. L. 1991. Crown gall tumors on potato disc and brine shrimp lethality. Two simple bioassays for higher plant screening and fractionation. In K. Hostettmann, Ed.), *Assays for Bioactivity*, vol. 1. Academic Press, London.
12. Nejatizadeh-Barandozi, F. 2013. Antibacterial activities and antioxidant capacity of *Aloe vera*. *Org. Med. Chem. Lett.* 3:5–13.
13. Nazir, S., F. L. Ansari, T. Hussain, K. Mazhar, A. G. Muazzam, et al. 2013. Brine shrimp lethality assay “an effective prescreen”: Microwave-assisted synthesis, BSL toxicity and 3DQSAR studies-based designing, docking and antitumor evaluation of potent chalcones. *Pharmaceut. Biol.* 51(9):1091–1103.
14. Padmaja, R., P. C. Arun, D. Prashanth, M. Deepak, A. Amit, and M. Anjana. 2002. Brine shrimp lethality bioassay of selected Indian medicinal plants. *Fitoterapia* 73:508–510.
15. Raja, S. R., M. G. Dinesh, and T. Jaya. 2011. An *in vitro* study of cytotoxic activity of *Euphorbia hirta* on Hep2 cells of human epithelioma of larynx. *Int. J. Pharm. Pharmaceut. Sci.* 3(3):101–103.
16. Reena, P., P. Yogesh, K. Prasant, and K. Anju. 2015. DPPH free radical scavenging activity of phenolics and flavonoids in some medicinal plants of India. *Int. J. Curr. Microbiol. Appl. Sci.* 4(1):773–780.
17. Shalabi, M., K. Khilo, M. M. Zakaria, M. G. Elsebaei, W. Abdo, and W. Awadin. 2015. Anticancer activity of *Aloe vera* and *Calligonum comosum* extracts separately on hepatocellular carcinoma cells. *Asian Pac. J. Trop. Biomed.* 5(5):375–381.
18. Soladoye, M. O., N. A. Amusa, S. O. Raji-Esan, and A. A. Taiwo. 2010. Ethnobotanical survey of anti-cancer plants in Ogun State, Nigeria. *Ann. Biol. Res.* 1(4):261–273.
19. Surjushe, A., R. Vasani, and D. G. Saple. 2008. *Aloe vera*: A short review. *Ind. J. Dermatol.* 53(4):163–166.
20. Useful Tropical Plants (UTP). 2014.Database 2014, by Ken Fern. Web 22 December 2015. <http://tropical.theferns.info/contact.php>. (accessed October 9, 2015).
21. World Health Organization. 2003.Traditional Medicine World Health Organization. Fact sheet N°134 Revised May 2003.
22. Yun, H., X. Juan, and H. Qiuhui. 2003. Evaluation of antioxidant potential of *Aloe vera* (*Aloe barbadensis* Miller) extracts. *J. Agric. Food Sci. Chem.* 51:7788–7791