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Full Length Research Paper

In vivo biochemical assessment of aqueous extracts of *Vernonia amygdalina* (Bitter leaf)

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This research was carried out on the aqueous extracts of bitter leaf (*Vernonia amygdalina*) to evaluate its phytochemical, proximate and antioxidant composition and its effects *in vivo* on diabetes and obesity biomarkers, antioxidant and hematological profiles. The phytochemical screening of the bitter leaf extract showed a high concentration of flavonoids as the most abundant phytochemical present. Daily administration of extract to rats led to a slight decrease in the lipid profile of the test rats relative to control and no significant difference in the liver function, kidney function, glucose level and hematological profile of test rats relative to control. Antioxidant assay showed high levels of total antioxidant activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity that was concentration dependent. *In vivo* antioxidant enzyme assay showed an appreciable increase in the level of the antioxidants, glutathione (GSH), superoxide dismutase (SOD), catalase and malondialdehyde (MDA) of the test rats as compared to control. This suggests improved functionality of the antioxidant system of the test rats probably due to the effect of the phytochemical antioxidants in the extract. It is concluded that aqueous extract of *V. amygdalina* can be consumed as food or as an herbal medicine without plausible toxicity to body organs and tissues.

Key words: Bitter leaf, medicinal plants, lipid profile, hematology, antioxidants.

INTRODUCTION

Vernonia amygdalina is commonly called bitter leaf because of its bitter taste. It is a member of the Asteraceae family and a small ever-green shrub that grows all over Africa. It is reported to be a medicinal plant for diabetes and fever (Crellin et al., 1989). Bitter herbs are reportedly good for the body as they help tone the vital organs of the body like the kidney and liver. Ethnomedically, the leaves are consumed either as a vegetable (macerated leaves in soup) or aqueous extracts as tonics for the treatment of various illnesses (Igile et al., 1995). In the wild, chimpanzees have been observed to ingest the leaves when suffering from parasitic infections (Huffman et al., 1993). The roots of *V. amygdalina* have been used for gingivitis and toothache due to its proven antimicrobial activity (Ademola and Eloff, 2011). In North America, of the 17 species of *Vernonia* all have the same effective properties as a blood purifier, uterus toner and helps also to prevent atherosclerosis (Erasto et al., 2007; Nwanjo, 2005).

Many herbalists and naturopathic doctors recommend aqueous extracts for their patients as treatment for anemia, nausea, diabetes, loss of appetite, dysentery and other gastro intestinal track problems. *V. amygdalina* extracts have also been reported to help suppress, delay, or kill cancerous cells (Kupchan et al., 1969).

This study was designed to investigate the biochemical assessment of bitter leaf extract in the management of conditions like diabetes and obesity. This is because the bitter leaves have been reported to be used ethnomedically to manage these conditions. Various experiments were done to analyze the chemical composition of bitter leaf aqueous extract and carry out *in*

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Table 1. Qualitative phytochemical screening of bitter leaf.

Phytochemical	Result
Tannins	+
Phlobatanin	-
Saponin	+
Flavonoids	+
Cardiac glycosides	+
Alkaloid	+

Phytochemical screening results showed that phytochemicals: tannin, flavonoids, cardiac glycosides, saponins, and alkaloids were present in bitter leaf, while phlobatanin was absent.

vivo studies using the rat model to determine the effects of the extract on hematological, lipid, glucose and antioxidant profiles.

MATERIALS AND METHODS

Chemicals

All chemicals and biochemicals used were of analytical grade, purchased from Sigma Chemical Company, USA and used without further purification.

Collection of plant

Fresh bitter leaves were collected at Yaba Market, Lagos State. The leaves were authenticated by Dr. Kadiri, Department of Botany, University of Lagos and the samples were kept at their herbarium.

Extraction and preparation of extract

The extraction of the bitter leaf sample was done by hot infusion.180 ml of hot water was added to 2 g of plant sample. This was left to stand for 20 min and then filtered. The filtrate was then administered orally to the rats.

Phytochemical screening and quantitative estimation of chemical constituency

Chemical tests were carried out on the bitter leaf extract to identify and quantify its constituents; tannins, flavonoids, alkaloids, saponins, cardiac glycosides and steroids, using standard procedures as described by Rios and Recio (2005), Okwu and Okwu (2004), Sofowora (1993), Evans (1996), and Harbone (1973). A diluted solution was made by mixing 2 ml of sample with 20 ml water.

Antioxidant assay of bitter leaf

0.2 g of the bitter leaf tonic was weighed and diluted with 100 ml of water. From this solution, 20 ml solution with concentrations of 100, 75, 50 and 25 were obtained and labeled appropriately. Tests for ferric reducing power capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, antioxidant enzymes, lipid peroxidation and total protein were carried out using previously described methods (Aiyegoro and Okoh, 2009; Edeoga et al., 2005).

Animal study

Forty albino female rats (180 to 200 g) purchased at Animal House, College of Medicine, University of Lagos, Idi Araba, Lagos were used for this experiment. The rats were allowed to acclimatize for a period of fourteen days during which they were fed *ad-libitum* with standard rodents feed (rat chow) and tap water. Then, the rats were equally divided (7 rats/group) into two groups; the test and the control group. The weights of the rats in the test group were measured and used to calculate the dosage of bitter leaf extract, 1 ml/kg/day, to be administered to each rat throughout the three weeks of treatment. Administration was done via the oral route with the aid of oral cannula and syringe. After three weeks of administration, blood was collected from the rats into labelled; lithium-heparin, fluoride and ethylenediaminetetraacetic acid (EDTA) bottles via retro-orbital sinus technique. The rats were then sacrificed using cervical dislocation and their livers were collected into plain bottles and placed in ice. The blood samples and livers were then analyzed for various parameters.

Biochemical assays

The sera of the rats were analyzed using Randox diagnostic kits to assess the liver function, kidney function, lipid profile and glucose level of the rats according to standard protocols as described by Burtis et al. (2011).

Hematological assay

A complete blood count was carried out on the blood of the experimental rats using an Automated Analyzer to measure the levels of white blood cell (WBC), red blood cell (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), neutrophils and lymphocytes, according to standard protocols by Burtis et al. (2011).

Statistical analyses

Data from the various studies are presented as mean ± standard error of mean (SEM). The results were analyzed for statistical significance using Microsoft Excel© software systems (2007). Students'-test and Satterwhaites' method of one way analysis of variance were used to compare mean values between groups. $p<0.05$ was taken to indicate a statistical significance.

RESULTS AND DISCUSSION

The bitter leaf *(V. amygdalina)* extract was analyzed for its phytochemical and nutrient composition and the presence of the alkaloids, tannins, saponins, flavonoids, and cardiac glycosides were detected, with flavonoids as the most preponderant (Table 1 and Figures 1 and 2). Flavonoids have been reported to possess antioxidant, anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities (Edeoga et al., 2005). Their highly antioxidant property present in the extract may act in

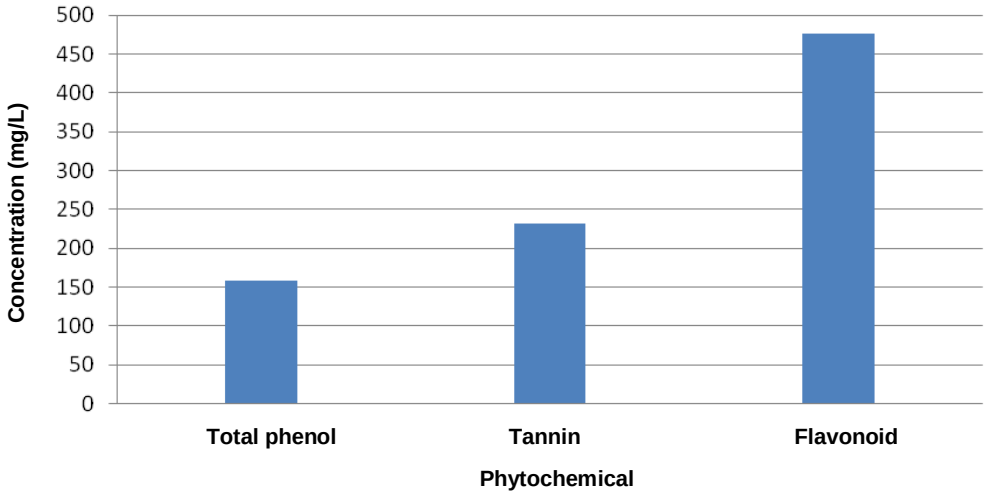

Figure 1. Quantitative estimation of concentration of phytochemicals in bitter leaf. The result shows that flavonoids were present in higher concentration than any other phytochemical.

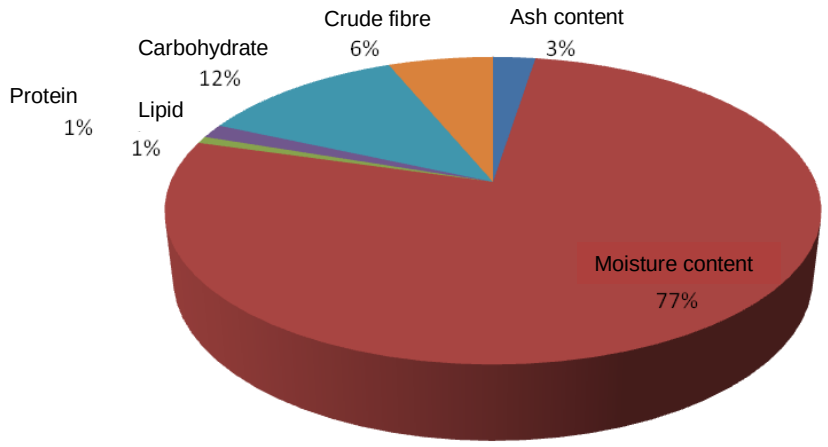

Figure 2. Proximate nutrient composition of bitter leaf.

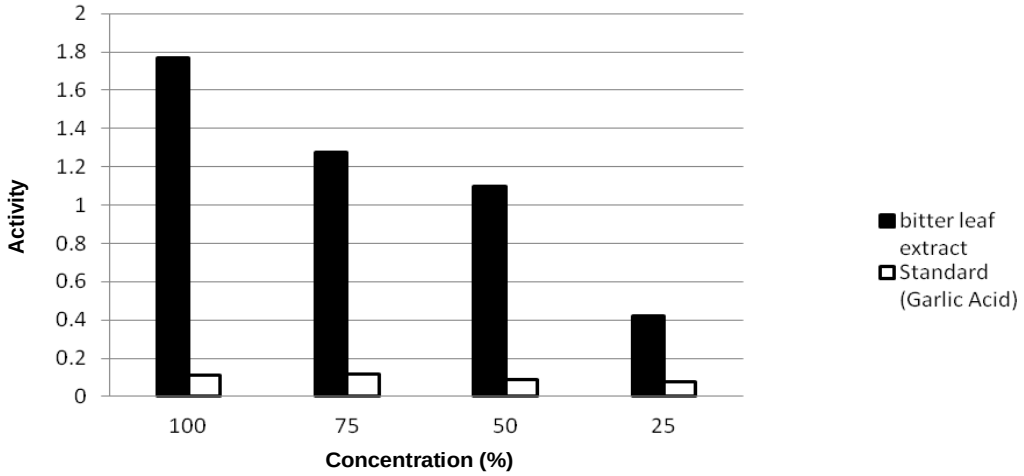

Figure 3. Total reducing power of bitter leaf extract. Determined against that of a known standard at varying concentrations, the reducing power of bitter leaf was very high as compared to the standard at all concentrations.

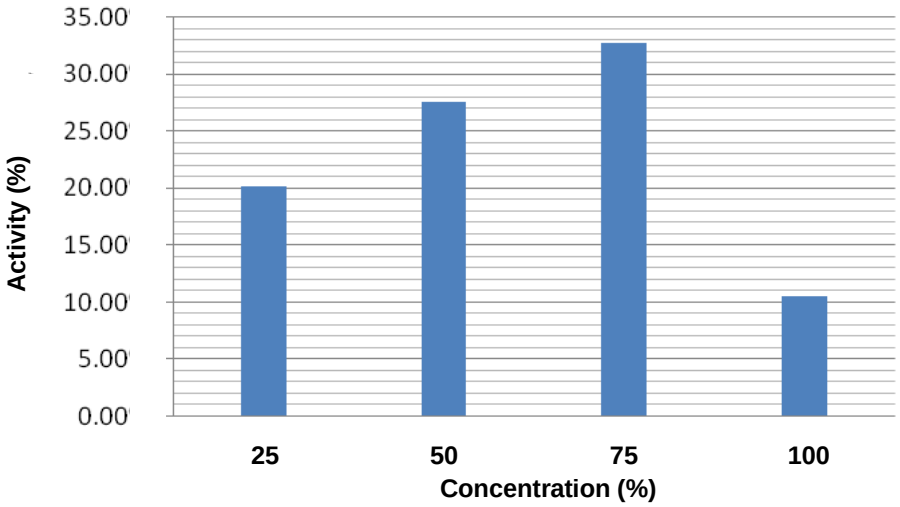

Figure 4. DPPH free radical scavenging activity of bitter leaf extract. From the result, it was noticed that the DPPH free radical scavenging activity of bitter leaf increases with increasing concentration but reduced at the highest concentration.

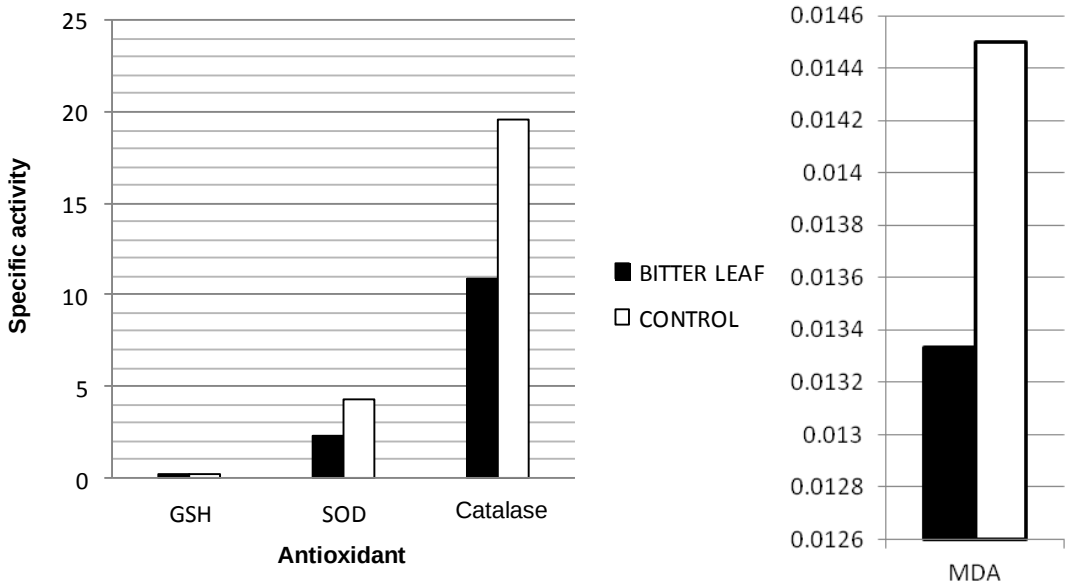

Figure 5. (a) Specific activity of SOD and catalase and GSH in rat liver; (b) Concentration of MDA in rat liver. The results showed that the levels of SOD, CAT, GSH and MDA were lower as compared to the control.

synergy with other phytochemicals present to produce the medicinal benefits inherent in the bitter leaf extract (Akpulu et al., 1994; Boham and Kocipai-Abyazan, 1974).

Antioxidant assay showed high levels of total antioxidant activity and DPPH free radical scavenging activity that was concentration dependent (Figures 3 and 4). *In vivo* antioxidant enzyme assay showed an increase in the level of the antioxidants, glutathione (GSH), superoxide dismutase (SOD), catalase and malondialdehyde (MDA) of the test rats as compared to control (Figure 5) suggesting a possible prevention of lipid peroxidation in tested rats. Unlike a previous report (Opata and Izevbigie, 2006), this suggests improved functionality of the antioxidant system of the test rats probably due to the effect of the phytochemical antioxidants in the extract.

The lipid profile (which involves levels of total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and very-low density lipoprotein (VLDL)) serves as diagnostic indices in conditions such as chronic

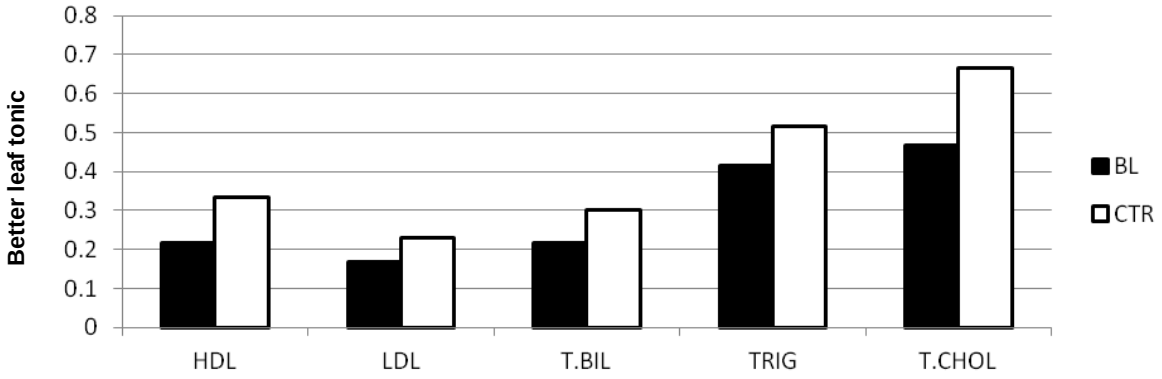

Figure 7. Effect of bitter leaf (BL) tonic on the lipid profile of rats. HDL: High density lipoprotein; LDL: low density lipoprotein; T.BIL: total bilirubin; TRIG: triglycerides; T.CHOL: total cholesterol.

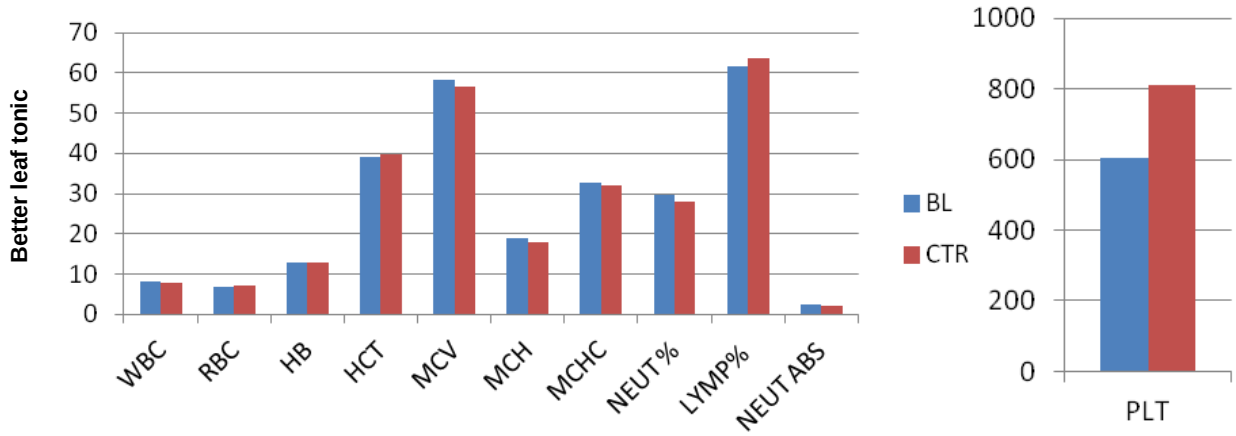

Figure 8. Effect of bitter leaf (BL) tonic on hematological parameters of rats. There was no significant difference at $p<0.05$.WBC: White blood cell; RBC: red blood cell; HB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelets; NEUT: neutrophils; LYMP: lymphocytes; NEUT ABS: neutrophils absorbance.

obstructive jaundice, hepatitis, coronary heart disease and atherosclerosis. Hyperlipidaemia is one of the risk factors for coronary heart disease while cholesterol is the major lipid constituent of atherosclerotic plague. In support of previous findings (Erasto et al., 2007), extracts of *V. amygdalina* gave a slight decrease in the lipid profile of the test rats relative to control and no significant difference in the liver function, kidney function, glucose level and hematological profile of test rats relative to control (Figures 6 to 8).

The present research shows that there were no significant changes or toxicity potential in most of the investigated parameters following the administration of the aqueous extract of *V. amygdalina*, lending credence to existing reports that *V. amygdalina* is useful in the ethnotherapy of diabetes mellitus (Nwanjo, 2005).

Conclusion

From our study, it can be concluded that aqueous extract of *V. amygdalina* is safe for consumption as food or as herbal medicine without plausible toxicity to body organs and tissues.

REFERENCES

Ademola IO, Eloff JN (2011). Anthelminthic activity of acetone extract and fractions of *Vernonia amygdalina* against *Haemonchus contortus* eggs and larvae. Trop. Anim. Health Prod. 43(2):521-527.

Aiyegoro OA, Okoh AI (2009). Phytochemical Screening and Polyphenolic Antioxidant Activity of Aqueous Crude Leaf Extract of *Helichrysum pedunculatum.* Int. J. Mol. Sci. 10:4990-5001.

Akpulu IN, Dada JD, Odama EL, Galadima O (1994). Anti Bacterial activity of aqueous extracts of some Nigerian medicinal plants. Nig. J. Bot. 7:45-48.

Boham BA, Kocipai-Abyazan R (1974). Flavonoids and condensed tannins from leaves of *Hawallan vaccinium vaticultum* and *V. calycinium*. J. Pacif. Sci. 48:458-463.

Burtis CA, Ashwood ER, Bruns DE (2011). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th Edition. Saunders. pp. 1-2238.

Crellin JK, Philport J, Tommie B (1989). A Reference Guide to Medicinal Plants: Herbal Medicine, Past and Present, Duke

University Press, p. 265.
Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical Constituents of some Nigerian Medicinal Plants. Afr. J. Biotechnol. 4:685-688.
Erasto P, Grierson DS, Afolayan AJ (2007). Evaluation of Antioxidant activity and the fatty acid profile of the leaves of *Vernonia amygdalina* growing in South Africa. Food Chem. 104:636-642.
Evans WC (1996). Trease and Evans' Pharmacognosy. UK: WB Saunders Company LTD., 14th edition p. 1098.
Harbone JB (1973). Phytochemical methods, London, Chapman and Hall, Ltd. p. 188.
Huffman MA, Gotoh, S, Izutsu D, Koshimizu K, Kalunde MS (1993). Further observations on the use of the medicinal plant, *Vernonia amygdalina* (Del) by a wild chimpanzee, its possible effect on parasite load, and its phytochemistry. Afr. Study Monogr. 14(4):227-240.
Igile GO, Olesezk W, Burda S, Jurzysta M (1995). Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. J. Agric. Food Chem. 43:2162-2166.
Kupchan SM, Hemingway RJ, Karim A, Werner D (1969). Tumor inhibitor XLVII Vernodalin and vernomygdin, two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina* J. Org. Chem. 34(12):3908.
Nwanjo HU (2005). Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. Nig. J. Physiol. Sci. 20(1-2):2, 30-42.
Okwu DE, Okwu ME (2004). Chemical composition of Spondias mombia Linn plant parts. J. Sust. Agric. Environ. 6: 140-147.
Opata MM, Izevbigie EB (2006). Aqueous *V. amygdalina* Extracts Alter MCF-7 Cell Membrane Permeability and Efflux. Int. J. Environ. Res. Pub. Health 3(2):174-179.
Rios JL, Recio MC (2005). Medicinal plants and Antimicrobial activity. J. Ethnopharmacol. 100:80.
Sofowora A (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria p. 346.