Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 01, pp.11269-11273, January, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND ACUTE ORAL TOXICITY OF AQUEOUS EXTRACTS OF *VERNONIA AMYGDALINA* (DELILE) AND *NAUCLEA LATIFOLIA* (SMITH)

[1*]Olanrewaju, Comfort A., [1]Idris, Halima S., [2]Okwute, Simon K., [1]Olayanju, Segun

[1]Department of Biological Sciences, University of Abuja, Nigeria
[2]Department of Chemistry and Industrial Chemistry, University of Abuja, Nigeria

ARTICLE INFO

Article History:
Received 21st October, 2014
Received in revised form
24th November, 2014
Accepted 05th December, 2014
Published online 23rd January, 2015

Key words:
Phytochemical screening,
Acute Oral Toxicity,
Mortality,
Pack Cell Volume and
White Blood Cell counts.

ABSTRACT

The phytochemical screenings and acute oral toxicity of the aqueous extracts of *Vernonia amygdalina* and *Nauclea latifolia* were carried out. The leaf extract of *V. amygdalina* had the highest yields of alkaloids, flavonoids and saponins with 16.00%, 9% and 9% respectively. Tannins yield was found to be highest in *N. latifolia* where $6.70x10^{-5}$mol/dm^3 was recorded in its stem extract. An evaluation of the acute oral toxicity of aqueous extracts of these plants showed no mortality of any of the rats at 1000 and 2000mg/kg after 72 hours. The LD_{50} of the two plants extracts were greater than 3,000mg/kg except the *V. amygdalina* stem bark which was 2449.49mg/kg. Treatment with *V. amygdalina* and *N. latifolia* produced no significant changes (P= 0.999 and P= 0.798) in Pack Cell Volume and White Blood Cell counts respectively. The t-test analysis showed that there was significant difference (P= 0.0005) in the weight in relation to the different extracts concentrations. The possession of the active secondary metabolites potentiates their medicinal values and at lower concentrations per body weight (<2000mg/kg) the plants extracts can be used for a prolonged period with little or no adverse effect.

INTRODUCTION

Scientific evaluation of medicinal plants is important to the discovery of novel drugs and also helps to assess toxicity risks associated with the use of herbal preparations and other conventional drugs of plant origin. There are about over 5000 species of plants on earth but only 1 – 10% has been studied for their potential medicinal values (Verpoorte, 2000). Many plants contain elements or active principles, obviously natural chemical molecules, which fight out pathogenic agents/factors both in animals and plants. *Nauclea latifolia* ,"Egbo egbesi" in Yoruba, "Ubuluinu" in Igbo and "Tabasiya" in Hausa is a Rubiaceae commonly known as pin cushion tree (family: Rubiaceae). It is a straggling shrub or small tree native to tropical Africa and Asia. The plant is also used in the treatment of ailments like malaria (Kokwaro, 1976; Akabue and Mittal, 1982; Boye, 1990), gastrointestinal tract disorders (Maduabunyi, 1995), sleeping sickness (Kerharo, 1974), prolong menstrual flow (Elujoba, 1995), hypertension (Akabue and Mittal, 1982) and as a chewing stick (Asubiojo *et al.*, 1982). *Vernonia amygdalina,* popularly known as bitter leaf, "ewuro" in Yoruba, "onugbu" in Igbo and "chuwar-doki" in Hausa, is an Asteraceae. It is a shrub or small tree of 2 – 5 m with petiolate leaf of about 6 mm diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste (Anonymous, 1999).

Corresponding author: Olanrewaju, Comfort, A.
Department of Biological Sciences, University of Abuja, Nigeria.

Philipson *et al*. (1993) reported the antiplasmodial effects of sesquiterpene and steroidal constituents of *V. amygdalina* and are also effective against *Plasmodium falciparium* in vitro. The plant has acquired special relevance recently, having been proved in human medicine to possess potent antimalarial and antihelmintic properties (Abosi and Raseoka, 2003) as well as antitumorigenic properties (Izevbigie *et al*., 2004). Pharmacological studies have also shown that the leaf extracts has both hypoglycaemic and hypolipidaemic properties in experimental animals and so could be used in managing diabetes mellitus (Akah and Okafor, 1992). The phytochemical analysis of some constituents and acute oral toxicity test was carried out on these two plants: *Nauclea latifolia* and *Vernonia amygdalina*

MATERIALS AND METHODS

Plant materials and preparation

Fresh leaves and stem barks of *Vernonia amygdalina* and *Nauclea latifolia* were harvested in bushes around the University of Abuja, mini campus, Gwagwalada. Plant identification was carried out at the Biological garden of the Department of Biological Sciences of the University and at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja and a specimen of each of the plants was deposited there. Identification numbers of the plant specimens are as follows: *Vernonia amygdalina*-

NIPRD/H/6555 and *Nauclea latifolia*- NIPRD/H/6559. Extraction and concentration of plant extracts was done according to Cuellar Cuellar and Okori (2010). The dried extracts were stored in small plastic containers (20ml capacity). The resulting extracts were reconstituted in distilled water to give the required doses of 1000 and 2000 mg/kg body weight respectively. The weights of the extracts were determined and the percentages of extractives were calculated as follows

$$\frac{\text{Weight of dried extract}}{\text{Weight of dried powdered plant}} \times 100$$

The results were then recorded. The dried extracts were stored in small plastic containers (20ml capacity). The resulting extracts were reconstituted in distilled water to give the required doses of 1000, 2000 and 3000 mg/kg body weight respectively.

Qualitative phytochemical analysis

Chemical tests were carried out on the aqueous extracts and on the powdered specimens using standard procedures to identify the various classes of chemical constituents (Sofowora, 1993; Treas and Evans, 1989 and Harborne, 1973).

Quantitative phytochemical analysis

Alkaloids, flavonoids, saponnins and tannins were quantitatively determined using standard methods (Harborne, 1973; Boham and Kocipai, 1974; Obadoni and Ochuko, 2001 and Van Burden and Robinson, 1981).

Acute oral toxicity test

The acute oral toxicity test was carried out using the acute oral toxic class method according to OECD/OCDE (2001). The animals were kept according to National Research Council (1996) guidelines. Thirty nine Wister albino rats of both sexes between 100-200 grams, bought from the Nigeria Institute for Trypanosomiasis Research, Kaduna were used for the experiment. Acclimatization period of 7 days was used. Prior to dosing, 50 rats were weighed and then deprived of food for approximately 17 hours. During the fasting period, the rats were examined for health and weighed (initial). Individual body weights of the animals were recorded prior to extract administration (initial) and again on day 7 and 14.

Grouping and extract administration

Thirty-nine (39) healthy animals were selected out of the 50 fasted rats and grouped into thirteen groups of 3 rats each. The groups were labeled A, B, C, D, E, F, G, H, I, J, K, L and M. Groups A, B and C were given 3000,2000 and 1000mg/kg of *Nauclea latifolia* aqueous leaf extract respectively. Groups D, E and F were given 3000, 2000 and 1000mg/kg of *N. latifolia* aqueous stem bark extract respectively. Groups G, H and I were given 3000, 2000 and 1000mg/kg *Vernonia amygdalina* aqueous leaf extracts respectively. Groups J, K and L were given 3000, 2000 and 1000mg/kg of *V. amygdalina* aqueous stem bark extract respectively. Each of the extract was dissolve in 2ml of distilled water before administration. Group M served as the control group and was given 2ml of distilled water only. The extracts were administered orally using intragastric cannula (feeding tube) attached to a 5mls syringe. After administration each animal was returned to its designated cage. Feed was replaced approximately three and a half hours after dosing. The day of administration was considered day zero of the study. The animals were observed for mortality, signs of gross toxicity and behavioural changes at 1 and 3 hours post-dosing and once daily thereafter for 14 days. All rats were euthanized via chloroform inhalation on day 14.

Collection of blood for haematological examination

Blood samples were collected through heart puncture of each chloroform anaesthetized rat into different EDTA bottles. The blood samples were analyzed for packed cell volume (PCV) and white blood cell (WBC) counts.

Statistical analysis

The LD_{50} was determined using OECD/OCDE (2001) analysis for limit dose test and Locke (1983) calculation i.e LD50 = the square root of a X b (where a = least dose that killed a rat and b = highest dose that did not kill any rat). Microsoft Excel was used to analyze data obtained from this study. The weights and blood parameters of experimental animals were expressed as mean and standard error. The significance of the effects of the plant extracts on the weight and blood parameters were analyzed using chi-square and t-test analyses respectively.

RESULTS AND DISCUSSION

The percentage yield of the aqueous extracts of the leaf and stem bark extracts of *N. latifolia* and *V. amygdalina* are shown on Table 1.

Table 1. Plant parts and percentage yield of the aqueous extracts

Plants	Plant parts/ yield (%)	
	Leaf	Stem bark
Vernonia amygdalina	6.1	5.2
Nauclea latifolia	6.4	7.3

Investigation of some phytochemical constituents of the aqueous extracts of *Vernonia amygdalina* and *Nauclea latifolia* revealed the presence of saponins, sterols, tannins, alkaloids, phenols, terpenes, resins, flavonoids and carbohydrates Table 2. These compounds are known to be biologically active. These secondary metabolites exert antimicrobial and antiparasitic activities through different mechanisms. These phytochemicals were widely reported as quencher of free radicals in the biological system and amelioration of various diseases associated with free radicals. Flavonoids exhibits a wide range of biological activities such as antimicrobial, anti-inflammatory, antiangiogenic, analgesic, antiallergic effects, cytostatic, and antioxidant properties (Hodek *et al*., 2002). Aliyu *et al*. (2009) reported that phenolic compounds are the major group of compounds that acts as primary antioxidant because it can reacts with oxygen free radicals such as hydroxyl, superoxide anion radicals and lipid peroxyl radicals. There is high correlation between antioxidant activity and phenolic compounds (Odabasoglu *et al*., 2004).

Table 2. Phytochemical (quantitative) analysis of the plants

Metabolites	Leaves		Stem bark	
	V. amygdalina	*N. latifolia*	*V. amygdalina*	*N. latifolia*
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	-	+	-
Phenols	+	-	+	-
Terpenes	+	+	+	+
Sterols	+	+	+	+
Resins	-	+	-	+
Anthraquinones	-	-	-	-
Carbohydrates	+	+	+	+

Key: Present= + Absent= -

Table 3. Number of death after 72 hours of toxicity test

Extracts	Group(concentration)	Number of rats	Number of dead rats
N.latifolia leaves	A (3,000 mg/kg)	3	1
	B (2,000mg/kg)	3	0
	C (1,000mg/kg)	3	0
N. latifolia stem bark	D (3,000mg/kg)	3	0
	E (2,000mg/kg)	3	0
	F (1,000mg/kg)	3	0
V.amygdalina leaves	G (3,000mg/kg)	3	0
	H(2,000mg/kg)	3	0
	I (1,000mg/kg)	3	0
V. amygdalina stem bark	J (3,000mg/kg)	3	2
	K (2,000mg/kg)	3	0
	L (1,000mg/kg)	3	0
Control	M (Distlled water)	3	0

Table 4. Mean body weights of rats before and after toxicity test

Extracts	Groups (concentration)	Initial Weight (g) ± S.E	Final Weight (g) ± S.E
N.latifolia leaves	A (3000mg/kg)	121.67 ± 4.41	128.33 ± 4.41
	B (2000mg/kg)	166.67 ± 8.82	160.00 ± 15.28
	C (1000mg/kg)	166.67 ± 16.92	193.33 ± 3.33
N. latifolia stem bark	D (3000mg/kg)	118.33 ± 10.93	135.00 ± 7.64
	E (2000mg/kg)	112.67 ± 3.71	133.33 ± 3.33
	F (1000mg/kg)	121.67 ± 6.01	131.67 ± 8.33
V.amygdalina leaves	G (3000mg/kg)	148.33 ± 7.26	168.33 ± 9.28
	H (2000mg/kg)	133.33 ± 8.82	141.67 ± 1.67
	I (1000mg/kg)	130.00 ± 27.54	143.33 ± 3.33
V. amygdalina stem bark	J (3000mg/kg)	130.00 ± 12.58	131.67 ± 12.02
	K (2000mg/kg)	128.33 ± 15.90	166.67 ± 18.56
	L (1000mg/kg)	135.00 ± 15.26	160.00 ± 15.26
Control	M (distilled water)	123.33 ± 17.40	143.33 ±20.28

KEY- S.E = Standard Error

Table 5. Haematological parameters of the rats 14 days after toxicity test

Bld Parameters	Extracts	Control	Treated (mean ± S.E.)		
			3,000 mg/kg	2,000 mg/kg	1,000 mg/kg
PCV (%)		37 ± 0.5			
	NL leaves		37.8 ± 0.43	37.9 ± 0.50	38.2 ± 0.20
	NL stem bk		37.1 ± 0.33	37.3 ± 0.02	38.5 ± 0.30
	VA leaves		40.2 ± 0.52	40.8 ± 0.60	39.5 ± 0.58
	VA stem bk		39.4 ± 0.21	39 ± 0.61	38.9 ± 0.62
WBC ($Cx10^3/mm^3$)		9.05 ± 0.21			
	NL leaves		9.37 ± 0.20	9.36 ± 0.15	9.28 ± 0.67
	NL stem bk		9.38 ± 0.33	9.36 ± 0.21	9.45 ± 0.41
	VA leaves		8.70 ± 0.31	8.65 ± 0.35	8.60 ± 0.33
	VA stem bk		8.34 ± 0.10	8.35 ± 0.21	8.30 ± 0.50

Bever (1980) listed glycosides, flavonoids, and tannins as active hypoglycemic compounds. The leaf extract of *V. amygdalina* had the highest yields of alkaloids, flavonoids and saponins with 16.00%, 9% and 9% respectively.

Tannins yield was found to be highest in *N. latifolia* where $6.70x10^{-5}mol/dm^3$ was recorded in its stem extract Figures 1a and b. Three rats died in the first 72hrs after extract administration.

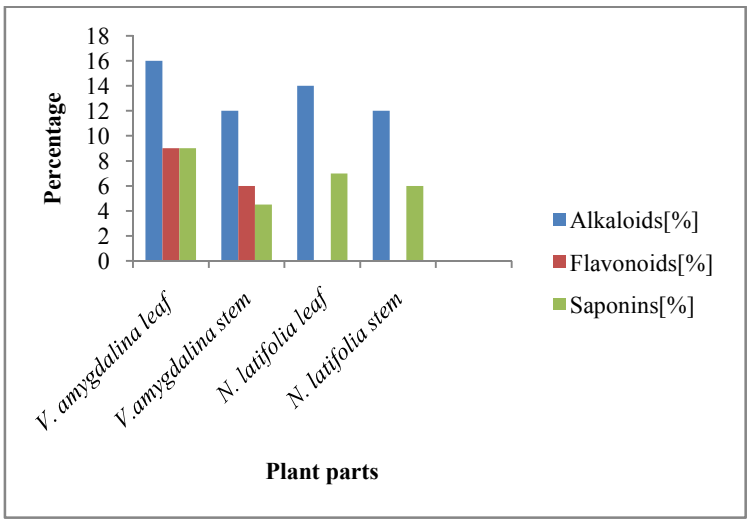

Figure 1a. Quantitative phytochemical analysis of the plant extract

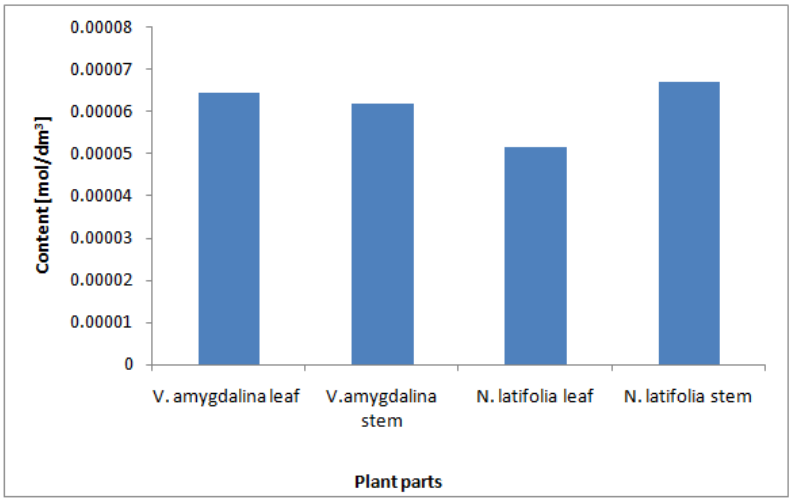

Figure 1b. Quantity of tannins in the plant parts

One from the group given 3,000mg/kg of *N. latifolia* leaf extract (group A) and two from those given 3,000mg/kg *V. amygdalina* stem bark (group J). The LD_{50} were determined and observed to be 2, 449.49mg/kg in rats administered with stem bark extract of *N. latifolia* and leaf extract of *V. amygdalina*. LD_{50} was observed to be higher than 3,000mg/kg in all other plant parts (those administered with *V. amygdalina* stem bark and *N. latifolia* leaf) since no rat death was recorded at this dosage. The percentage of death in groups A and J are 33.33% and 66.67% respectively. The number of death within 72 hours is shown in Table 3. The nontoxic nature of the aqueous extracts of *V. amygdalina* and *N. latifolia* and the oral safety at selected dose levels was confirmed where the administration of the drugs does not lead to mortality of any of the rats at 2,000mg/kg. According to Clarke and Clarke (1997), any compound or drug with the oral LD_{50} estimate greater than 1000 mg/kg could be considered to be of low toxicity and safe. This supports that the aqueous extracts were found to be safe up to the dose level of 2,000 mg/kg body weight except in *Vernonia* stem bark and *N. latifolia* leaf with LD_{50} of 2,449.49 mg/kg.

However LD_{50} has not been considered as a biological constant because many variables such as animal species, strain, age, gender, diet, bedding, ambient temperature, caging conditions, and time of the day can all affect the LD_{50} value obtained. Hence, there are considerable uncertainties in extrapolating the LD_{50} value obtained for a species to other species (Zbinden and Roversi, 1981). Body weight of all treated animals were unaffected by the treatments Table 4. However, there were appreciable increase in body weight in groups C, D, E, G, K and L. Group J have the lowest change in weight. It was observed that two rats in this group died within 72 hours of extract administration. Groups with high increase in weight were those given 2000mg/kg and 1000mg/kg. The student t-test analysis showed that there was significant difference (P= 0.0005) in the weight in relation to the different extracts concentrations. Observation from the blood parameters analyzed (PCV and WBC) showed that there were no wide deviations from that of the control group. However, there were increase in PCV in groups given *V. amygdalina* leaf and stem bark extracts. The increase was highest in groups given 3000mg/kg extract (Groups G and J). WBC were higher in groups given *N. latifolia* leaf and stem bark extracts when compared to that of the control group but lower in rats given

V. amygdalina leaf and stem bark extracts. The Chi square analysis on treatment with *V. amygdalina* and *N.latifolia* produced no significant changes (P= 0.999 and P= 0.798) in pack cell volume (PCV) and white blood cell counts (WBC counts) respectively. The PCV and WBC of rats at the 14th day after toxicity test are shown on Table 5. The haematological parameters of the treated were found to be better than the ones from the untreated control group. Since no major alterations were found in the hematological parameters, the results of this study have established that the oral administration of both plants were safe up to 2,000 mg/kg.

Conclusion

Body weight of both the treated and untreated animals rose progressively as the duration increased. However, there is significant difference in the animal weights in relation to the different extracts concentrations. This study therefore suggested that the aqueous leaf extract of *V. amygdalina* and stem bark extract of *N. latifolia* may be safe, even when taken for a prolonged period and that of the *V. amygdalina* stem bark and *N. latifolia* leaf may not be safe at a dose higher than 2449.49mg /kg body weight if taken for a prolonged period.

REFERENCES

Abosi, A. O. and Raseroka, B. H. 2003. In vivo antimalarial activity of *Vernonia amygdalina. British Journal of Medical Sciences,* 60, 89-91.

Akabue, P. and Mittal, G. C. 1982. Clinical Evaluation of a Traditional Herbal Practice in Nigeria:Preliminary Rep. *Journal of Ethnopharmacology*, 6(3), 355-359.

Akah, P. A. and Okafor, C. I. 1992. Hypoglycaemic effect of *Vernonia amygdalina* Del in experimental rabbits. *Plant Medicinal Research*, 1, 6-10.

Aliyu, A. B., Ibrahim, M. A., Musa, A. M., Ibrahim, H., Abdullkadirm I. E. and Oyewale, A. O. 2009. Evalaution of antioxidant activity of leaves extract of *Bauhinia refurscens* Lam. *Journal of Medicinal Plant Research,* 3, 563-567.

Anonymous. 1999. *Vernonia amygdalina*. http://bkb-china.com/fidelity/bitter.htm

Asubiojo, O. I., Guinn, V. P. and Okunuga, A. 1982. Multi-element Analysis of Nigerian Chewing Sticks by Instrumental Neuron Activation Analysis. *Journal of Radiology and Analytical Chemistry*, 74, 149 -156.

Bever, B. O. 1980. Oral hypoglycaemic plants in West Africa. *Journal of Ethanopharmacology*, 2, 119-127.

Boham, B. A. and Kocipai, A. C. 1974. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinum vaticulatum* and *V.calycinum*. *Pacific Sci*.48:458- 463.

Boye, G. L. 1990. Studies on Antimalarial Action of *Cryptolepiss anguinolenta* Extract. Proc. Int. Syp. On East-West Med. Seoul, Korea, 243 – 251.

Clarke, E. G. and Clarke, M. L. 1997. Landers veterinary toxicology in London. London Bailliere Tindall. P15

Cuellar Cuellar, A. and Okori, D. 2010. Preliminary photochemical and antimicrobial evaluation of the fresh and dried whole plant extracts from *Commelina benghalensis*. *Rev. Colombiana cienc. Anim.*, 2 (1), 104-116.

Elujoba, A. A. A. 1995. Female Infertility in the Hands of Traditional Birth Attendants in South-West Nigeria. *Fitoterapia,* 66 (3), 239 – 248.

Harborne, J. B. 1973. Phytochemical methods. London. Chapman and Hall, Ltd. 49-188.

Hodek, P., Trefil, P. and Stiborova, M. 2002. Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. *Chem Biol Interact*, 139, 1-21.

Izevbigie, E.B., Bryant, J.L. and Walker, A. 2004. A novel natural inhibitor of extracellular signal-regulated kinases and human breast cancer cell growth. *Experimental Biology and Medicine*, 229, 163-169.

Kerharo, J. 1974. Historic and Ethnopharmacognosic Review on the Belief and Traditional Practices in the Treatment of Sleeping Sickness in West Africa. *Bulletin of the Society Med. Afr. Noire Lang. FR*, 19, 400 – 420.

Kokwaro, J. O. 1976. Medicinal Plants of East Africa. East African Literature Bureau, Nairobi. 37.

Madubunyi, I. I. 1995. Anti- Hepatotoxic and Trypanocidal Activities of the Ethanolic Extract of *Nauclea latifolia* Root Bark. *J. Herbs Spices Med Plants*, 3 (2), 23 – 53.

National Research Council 1996. Institute of Laboratory Animals Resources Commission in Life Sciences. National Academy Press, Washington, DC., USA. 7.

Obadoni, B. O. and Ochuko, P. O. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences* 86, 203- 208.

Odabasoglu, F., Aslan, A., Cakir A and Suleyman, H. K. (2004). Comparision of antioxidant activity and total phenolic content of 3lichen species. *Phytother Research,* 18, 938-41.

OECD/OCDE 2001. Acute Toxic Class Method. OECD 423 Guidelines for the Testing of Chemicals. 14.

Phillipson, J.D., Wright, C.W. and Kirby, G.C. 1993. Phytochemistry of some plants used in traditional medicine for the treatment of protozoal diseases. International Symposium of the Phytochemical Society of Europe. Abstract Book. University of Lausanne, Lausanne, Switzerland. 3.

Sofowora, A. 1993. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 289.

Tona, L., Cimanga, R.K., Mesia, K., Musuamba, C.T., de Bruyne, T., Apers, S., Hernans, N., Van Miert, S., Pieters, L., Totte, J. and Vlientinck, A.J. 2004. In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. *Journal of Ethnopharmacology*, 93, 27-32.

Trease, G. E. and Evans, W. C. 1989. Pharmacognosy. (11thed.) Brailliar Tiridel Can.Macmillian publishers. 144-148

Van Burden, T. P. and Robinson, W. C. 1981. Formation of complexes between protein and tannin acid. *Journal of Agriculture and Food chemistry* 1, 77

Verpoorte R. 2000. In *Metabolic Engineering of Plant Secondary Metabolism.* eds Verpoorte R, Alfermann AW (Kluwer Academic Publishers, Dordrecht, The Netherlands), 1–29.

Zbinden, G. and Roversi, F. 1981. Significance of the LD50 test for the toxicological evaluation of chemical substances. *Arch Toxicology,* 47, 77-99.

****